Rhodopsin with 11-cis-Locked Chromophore Is Capable of Forming an Active State Photoproduct*

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The visual pigment rhodopsin is characterized by an 11-cis retinal chromophore bound to Lys-296 via a protonated Schiff base. Following light absorption the C11=C12 double bond isomerizes to trans configuration and triggers protein conformational alterations. These alterations lead to the formation of an active intermediate (Meta II), which binds and activates the visual G protein, transducin. We have examined by UV-visible and Fourier transform IR spectroscopy the photochemistry of a rhodopsin analogue with an 11-cis-locked chromophore, where cis to trans isomerization around the C11=C12 double bond is prevented by a 6-member ring structure (Rh 6.10). Despite this lock, the pigment was found capable of forming an active photoproduct with a characteristic protein conformation similar to that of native Meta II. This intermediate is further characterized by a protonated Schiff base and protonated Glu-113, as well as by its ability to bind a transducin-derived peptide previously shown to interact efficiently with native Meta II. The yield of this active photointermediate is pH-dependent and decreases with increasing pH. This study shows that with the C11=C12 double bond being locked, isomerization around the C6=C10 or the C13=C14 double bonds may well lead to an activation of the receptor. Additionally, prolonged illumination at pH 7.5 produces a new photoproduct absorbing at 385 nm, which, however, does not exhibit the characteristic active protein conformation.

Rhodopsin, a seven-transmembrane helical protein, is composed of 348 amino acids and a ligand, 11-cis retinal, which covalently binds to the protein through a protonated Schiff base linkage to the ε-amino group of Lys-296 in the center of helix 7 (1). Glu-113 at helix 3 serves as the counterion of the protonated Schiff base (2–4). The recently resolved crystal structure of rhodopsin (5) provides detailed structural information on the interactions between the retinal chromophore and its surrounding residues, which contribute to the red-shifted absorption maximum of the chromophore (λmax 500 nm) relative to protonated retinal Schiff base in methanol solution (λmax 440 nm) and to the high pK of the protonated Schiff base (6).

Following light absorption, the retinal chromophore isomerizes from 11-cis to trans configuration in 200 fs (7). A series of photointermediates are produced that can be trapped below characteristic transition temperatures (8, 9): bathorhodopsin (λmax 543 nm, T < −140 °C), lumirhodopsin (λmax 497 nm, T < −40 °C), and metarhodopsin I (Meta I, λmax 478 nm) above −40 °C, which equilibrates with metarhodopsin II (Meta II, λmax 380 nm). Meta II is the active species, capable of activating transducin, the visual G protein. The equilibrium between Meta I and Meta II is affected by temperature, pressure, pH, and glycerol (9), as well as by ions (10, 11).

Meta II formation is associated with a movement of helix 6 relative to helix 3 (12, 13), a translocation of a proton from the Schiff base to its counterion, Glu-113, and recruitment of a proton from the bulk solution (14, 15). Using photoaffinity techniques at low temperature and trapping of different intermediates (16), it was demonstrated that in the dark state as well as in the bathorhodopsin intermediate, the β-ionone ring is located in the vicinity of Trp-265 in the center of helix 6, whereas in the lumirhodopsin, Meta I, and Meta II intermediates, the ring is located in the vicinity of Ala-169 in helix 4. This movement, a flip-over of the chromophoric ring, may trigger conformational changes in the cytoplasmic membrane loops, which interact with transducin.

The important role that 11-cis double bond isomerization plays in initiating the photochemically induced reaction was demonstrated by studies of a series of 11-cis locked artificial pigments. Picosecond time-resolved spectroscopy of 11-cis locked rhodopsin bearing a 5-member ring did not show any ground state photoproduct following illumination except for a long-lived excited state intermediate (17). Excitation of an 11-cis-locked rhodopsin with a 7-member ring produced a photorhodopsin-like species, whereas locked rhodopsin with an 8-member ring exhibited both photorhodopsin- and bathorhodopsin-like products (18). These results were interpreted by the gradual increase in the rotational flexibility along the C11=C12 double bond from a 5- to 8-member ring. To further shed light on the primary event of rhodopsin light activation, the photochemistry of locked pigments was studied under steady state irradiation. Locked rhodopsin bearing a 5-member ring at −196 °C following either orange or blue light irradiation did...
not produce a bathorhodopsin-like species, whereas at 0 °C a hypsichromic product was observed (19). Analysis of the retinal components by HPLC did not detect new isomers in the photoproduct. In addition, this pigment could not activate cyclic-GMP phosphodiesterase. Illumination of a locked pigment bearing a 7-member ring did not change its absorption spectrum at 4 °C, and yet it produced 12% of other isomers (20). All of these results led to the conclusion that C_{11}−C_{12} double bond isomerization is a prerequisite for the phototransduction process in rhodopsin. The photocascade of a 6-member ring 11-cis-locked pigment was examined as well (21, 22). Irradiation of this artificial pigment led to a gradual blue shift of the absorption maximum, with a broad shoulder around 380 nm and a loss of ~40% of the visible absorbance. The light-induced transducin activation was about 10% that of Meta II in native rhodopsin. The reason for this marginal activity was unclear.

The four isomers of this 6-member ring 11-cis-locked retinal were separated and reconstituted with opsins (Scheme 1) (21). During regeneration, the 9,11-dicis and the 11,13-dicis species isomerized thermally in the dark around the C_{13}=C_{14} double bond to the 9,11,13-tricis and the 11-cis species, respectively. The two 9-trans as well as the two 9-cis isomers, therefore, gave identical pigments absorbing at 510 and 494 nm, respectively. Following continuous irradiation, each of the resulting pigments produced a photoproduct mixture characterized by a similar absorption (λ_{max} 498 nm). The activity of the pigment, under high light intensities, varied from 6 to 15% of that of native Meta II. These pigments as well as their corresponding photoproducts were reexamined recently by HPLC analyses of detergent-solubilized samples and FTIR spectroscopy of membrane samples at neutral pH (23). The study concluded that photoisomerization indeed took place but that the protein moiety did not undergo conformational alterations. Molecular modeling suggested that movement of the β-ionone ring in these pigments is restricted, and therefore it was suggested that movement of the β-ionone ring is crucial for rhodopsin activation. This study further classified two of the four isomers as 11-cis-like and the other two as all-trans-like by their affinities for 11-cis- and all-trans-retinol dehydrogenase, respectively (Scheme 1). Importantly, the two 11-cis-like isomers, 11-cis and the 9,11,13-tricis, were the same as previously found to form the dark states of the pigments (21).

In an attempt to investigate whether the 6-member ring locked pigment (Rh_{6,10}) is capable of producing an active conformation following light absorption, we have studied the photchochemistry of Rh_{6,10} by UV-visible spectroscopy at different pH levels. Furthermore, FTIR spectroscopy was employed to monitor protein conformational changes following light absorption. It was revealed that the photochemistry of Rh_{6,10} is pH-dependent. An active Meta II-like intermediate with a protonated Schiff base is produced, particularly at low pH, while at high pH, presumably photoequilibration processes between the 11-cis-like inactive isomers dominate the photochemistry. In an accompanying article (24) we further investigate the thermal decay behavior of the photoproducts of Rh_{6,10}, revealing an interesting slow photocycling behavior of this visual pigment analog.

**EXPERIMENTAL PROCEDURES**

*Synthesis of 11-cis-locked Retinal (Rh_{6,10})*—The mixture of locked retinal isomers was synthesized as reported previously (21, 25). Briefly, β-ionone reacted with 3-methoxy-2-cyclohexene in the presence of lithium diisopropylamide at −60 °C followed by neutralization, reduction with lithium disobutylaluminum hydride, hydrolysis, and water elimination. Horner-Emmons condensation between the ketone with ethyl (diethylphosphono)acetate followed by reduction and oxidation afforded the final mixture of isomers identified by MS and NMR.

*Pigment Preparation*—Opsin was prepared from rhodopsin in washed disk membranes from cattle retinae according to standard procedures (26, 27) and regenerated with the synthetic chromophores overnight on ice (28). The isomeric composition of the chromophores was ~50:50 9-cis/9-trans, unless stated differently. Extraction of excess retinal and retinal oxime was achieved by two washes with 10 mM heptakis(2,6-di-O-methyl)-β-cycloexetrin (Aldrich) and three washes with distilled water (29). Pigment concentration was determined using an absorption coefficient of 41,200 s·cm⁻¹·mol⁻¹ (22).

**FTIR Spectroscopy**—FTIR spectroscopy was performed with a Bruker IFS 28 spectrometer with a mercury-cadmium-tellurium detector on sandwich samples with 1 nmol of pigment as described previously (30). IR spectra, recorded in blocks of 512 scans with a spectral resolution of 4 cm⁻¹ and an acquisition time of 1 min, were corrected for temporal base-line drifts. As buffers we used 20 μl of 200 mM citrate, MES, and BTP (bis-tris propane), in overlapping ranges, containing 200 mM NaCl. Samples were photolyzed for 120 s (150-watt tungsten lamp) with a ~530 nm long-pass filter, and half-time for reaching a photostationary state was 20 s at pH 5.0.

Binding of a high affinity peptide analogue derived from transducin was monitored in sandwich samples prepared with 0.5 nmol of pigment and 20 nmol of peptide 23 (ac-VLEDLKSCLG) (31).

UV-visible Spectroscopy—For UV-visible spectroscopy, sandwich samples identical to the infrared samples were used in a PerkinElmer Lambda 17 spectrophotometer equipped with a temperature-controlled sample holder. Illumination was similar to that used in the IR experiments. Alternatively, a Hewlett Packard 8452A diode array spectrophotometer was used.

**RESULTS**

*Characterization of Photoproducts at pH 7.5 and 5.0*—Sandwich samples, often used for IR spectroscopy, contain fully hydrated pigment films. For rhodopsin in native washed membranes, the use of sandwich samples offers the possibility of performing both IR and UV-visible spectroscopy with the same sample type under conditions close to those found in membrane suspensions. In particular, the Meta I/Meta II equilibrium of rhodopsin in membranes is unchanged in this sample type (11). Sandwich samples of Rh_{6,10} (consisting of a 50:50 mixture of the 9-trans and 9-cis isomers; Scheme 1) gave, following irradiation for ~2 min at pH 7.5, a blue-shifted intermediate (495 nm) characterized by a smaller extinction coefficient, in keeping with previously described results (21, 22). Irradiation for ~2 min at pH 5.0 gave a product absorbing similar to that obtained at pH 7.5, yet with a more pronounced blue shift (Fig. 1A). In both cases, the position of the absorption maximum indicates that the retinal Schiff base remains protonated during formation of these photoproduct states.

To further characterize the intermediates obtained at pH 7.5 and 5.0, we carried out FTIR measurements. IR spectrum of the photoproduct obtained at pH 7.5 shows, except for a strong negative chromophore band at 1205 cm⁻¹, only small features (Fig. 1C), indicating only very modest conformational changes of the pigment during photolysis at this pH, in agreement with previously published data (23). At pH 5.0, however, we observed an entirely different photoproduct, similar to that of native Meta II (see Fig. 2A), with large conformational changes of the pigment during photolysis at this pH, in agreement with previously published data (23). At pH 5.0, however, we observed an entirely different photoproduct, similar to that of native Meta II (see Fig. 2A), with large conformational changes of the pigment during photolysis at this pH, in agreement with previously published data (23).
changes, as is evident from bands appearing in the difference spectrum in the amide I and amide II ranges of ~1650 and 1550 cm⁻¹, respectively (Fig. 1B). Also, in the absorption range of protonated carboxylic acids above 1700 cm⁻¹, large bands in the difference spectrum can be observed, indicating considerable environmental changes of the involved groups.

The contributions of the different isomers to the difference spectrum obtained at pH 7.5 was examined by using pigments regenerated with only the 9-cis and the 9-trans isomers, respectively, instead of a 50:50 mixture (Fig. 1D). The spectra show clear differences, reflecting the two different isomers, 9,11,13-tricis (D) and 11-cis (Scheme 1), in the respective dark states (21). Under continuous illumination the different chromophoric isomers may fully interchange, and in the photostationary equilibrium (after 2 min), the photoproduct isomeric composition therefore becomes largely independent of the initial composition. By subtracting the two spectra, the photoproducts cancel out each other, and we were able to obtain a difference spectrum corresponding to a transition between the two dark states only, shown in Fig. 1E in black. A spectrum with a very similar band structure was obtained after subtraction of residual contributions of the Meta II-like spectrum obtained at pH 5.0 (Fig. 1B) from the pH 7.5 spectrum of the 50:50 mixture (Fig. 1C), the result of which is shown as well in Fig. 1E in gray. We can therefore conclude that, besides the small contribution of the low-pH photoproduct, the pH 7.5 spectrum is determined largely by a photoequilibration process between the two isomers that constitute the dark states of the pigment. This process also contributes to the spectrum obtained at pH 5.0, as evident from a similar analysis with pure isomers (not shown).
but is superimposed there by the large difference bands of the Meta II-like photoproduct. The same photoequilibration process is described in more detail in the accompanying article (24), where we analyze the thermal relaxation behavior of the Rh6.10 photoproducts and show that Rh6.10 can be photolyzed repeatedly to undergo identical excitation/relaxation cycles on the time scale of minutes to hours.

The 11-cis-locked Pigment Forms a Photoproduct with an Active State Conformation—A more detailed comparison of the FTIR spectrum of the low-pH photoproduct of Rh6.10 with that of Meta II of native rhodopsin reveals difference bands that are very similar in part. In Fig. 2A, we show the difference spectra of both transitions. As the Schiff base in the low-pH photoproduct remains protonated, for a better comparison with native rhodopsin, we show the transition of native rhodopsin to a Meta II state with a protonated Schiff base. Such a state can be obtained in the presence of solute anions without changing the active state conformation of Meta II, as reported previously (32). In the absorption range of protonated carboxylic acids above 1700 cm\(^{-1}\), where there are characteristic marker bands of Meta II, both spectra show very similar difference bands, indicating similar changes in hydrogen bonding for the membrane-embedded residues, Glu-122 and Asp-83 (33, 34). The positive band at 1709 cm\(^{-1}\) is slightly down-shifted compared with the corresponding band of Meta II of native rhodopsin, which was shown to reflect Glu-113 of the chromophore binding pocket becoming protonated during the transition to Meta II (35). Similar band structures around 1560 cm\(^{-1}\) are observed as well in the amide I range, whereas there are deviations in the amide II range around 1550 cm\(^{-1}\). The latter, however, is superimposed with the strong ethylenic stretch modes of the respective chromophores. Also, in the fingerprint range between 1100 and 1300 cm\(^{-1}\), we observed difference bands characteristic of the respective retinal chromophores.

To compare the content of the low-pH Meta II-like photoproduct of Rh6.10 with Meta II of native rhodopsin, we determined the amplitude of the Meta II marker band of Asp-83 at \(-1768/1747 \text{ cm}^{-1}\) (33, 34) as a function of pH. We normalized these values with the total amount of pigment present in the single samples, which was determined from the absolute amide II band intensity at 1550 cm\(^{-1}\) in the absorption spectra. These values were ratioed against the corresponding value of full Meta II of native rhodopsin. The data obtained are plotted in Fig. 2B and may serve as a rough estimate for the amount of Rh6.10 pigment converted to the active state conformation by photolysis. At the low-pH end, about 60% of the pigment adopts a Meta II-like conformation. The reduced yield compared with native rhodopsin is due in part to the strong overlap of the absorption bands, allowing only the establishment of a photo-equilibrium between dark and photoproduct states in Rh6.10 and also presumably to the only partial regeneration yield during pigment preparation. With increasing pH, the amount of the active state photoproduct decreases such that there is less than 10% of this species at pH 7.5 (Fig. 2B). Importantly, this considerable decrease may not be a direct consequence of a pH-dependent Meta I/Meta II equilibrium, which is established in native rhodopsin with a \(pK\) of 7.8 at 20 °C, as it is not counterbalanced by the formation of substantial amounts of a Meta I species, at least not up to pH 8.5. Instead it reflects a rather general decrease of the total photoproduct yield with increasing pH in favor of the inactive dark states.

The structural similarity of the Rh6.10 photoproduct obtained at pH 5.0 to the active state of native rhodopsin suggests a similar activity of the low-pH photoproduct toward the visual G protein, transducin. As direct coupling to and activation of transducin is hampered by the low pH required for formation of the addressed photoproduct, this proposition was examined by testing its ability to interact with a specific peptide analogue derived from the transducin \(\alpha\)-subunit C terminus, which is known to be involved in receptor-G protein coupling (36). This analogue, peptide 23 (ac-VLEDLKSCGLP), was previously shown to bind specifically with high affinity to the active state Meta II of native rhodopsin (31). Also, in the case of the Meta II-like photoproduct of Rh6.10, we observe characteristic changes between the FTIR difference spectra obtained in the presence and absence of peptide (Fig. 3A). The double difference spectrum, “with peptide – w/o peptide,” gives the peptide binding spectrum, which is very similar to that obtained for native Meta II (Fig. 3A) and is in full agreement with previously published peptide binding spectra (37, 38). Furthermore, we tested the ability of the peptide to stabilize the Meta II-like photoproduct at the expense of the dark states in the photo-equilibrium during illumination (extra-Meta II effect). At pH 7.5, where there is only a marginal contribution of the Meta II-like species to the photoproduct, we observed an ~4-fold increase of this active state species in the presence of the peptide (Fig. 3B).

Prolonged Illumination at pH 7.0—As described above, illumination of Rh6.10 at neutral pH produces a blue-shifted intermediate absorbing at about 495 nm. We observed that further illumination decreases this band and produces a new photoproduct absorbing at 385 nm (Fig. 4A). The formation of the latter intermediate is clearly evident by comparing the difference spectra of the short and long illuminations (Fig. 4B). To characterize the newly formed 385 nm absorbing intermediate we carried out FTIR measurements. It is evident that the short (2 min) and long (additional 5 min) illuminations produce different photoproducts (Fig. 4C) and that the intermediate absorbing at 385 nm does not exhibit the characteristic protein

![Fig. 3. Binding and selective stabilization of the Meta II-like photoproduct of Rh6.10 by a G\(_{\alpha}\)-derived peptide analog. A, photoproduct minus initial state” difference spectra of Rh6.10 in the absence (gray) and presence (black) of peptide 23 at pH 5.0. The lower spectrum in A shows the corresponding peptide binding spectrum (black), obtained by subtracting the corresponding difference spectra, with peptide – w/o peptide. For comparison we also show the respective binding spectrum for native rhodopsin (gray). B, selective stabilization of the Meta II-like photoproduct of Rh6.10 (extra-Meta II effect) at pH 7.5 in the presence of peptide 23. The spectra are to scale.](image-url)
Activation of 11-cis-locked Rhodopsin

**Fig. 4. Characterization of a 385 nm photoproduct formed at neutral pH under continuous illumination.** A, UV-visible spectra of the dark state of Rh6.10 at pH 7.0 and 20 °C, as well as the products obtained after 2 and 7 min of continuous illumination. B, differences of the spectra in A corresponding to the spectral changes occurring during the first 2 min and during the subsequent 5 min of illumination. C, corresponding spectra in the IR.

Conformational alterations observed in native rhodopsin Meta II or in the Rh6.10 Meta II-like photoproduct.

**DISCUSSION**

Artificial pigments, in which isomerization around the critical double bond was prevented, were studied in both bacteriorhodopsin and bovine rhodopsin. It was demonstrated that in bacteriorhodopsin locking of the C13=C14 double bond eliminated proton pumping activities as well as the regular photocycle (39–42). These studies concluded that the C13=C14 double bond is the only bond that can isomerize and that its locking did not open any detectable new avenue for a different isomerization. Therefore, the protein catalyzes the C13=C14 isomerization and, in addition, inhibits isomerization around double bonds other than C13=C14. In this respect we note that studies with locked retinals were performed also on rhodopsin of *Chlamydomonas reinhardtii*. Early studies have suggested that activity can be achieved without isomerization of any double bond (43). However, further studies demonstrated that activity probably required isomerization of the C13=C14 double bond because locking this bond abolished activity (44).

The situation might be different in bovine rhodopsin, which, as a visual pigment, regularly experiences isomerization around the cis C11=C12 double bond. For example, it is established that the binding site can accommodate the 9-cis retinal isomer, which forms isorhodopsin and isomerizes to all-trans following light absorption (8). The isomerization to all-trans initiates the formation of photochemically induced intermediates, which eventually lead to biological activity. In rhodopsin, locking one double bond may possibly lead to isomerization of another one. However, various locked rhodopsin artificial pigments did not exhibit photochemically induced intermediates or biological activity. Recent studies with the 11-cis-locked analogue Rh6.10 (containing a 6-member ring) demonstrated that isomerization around other double bonds does take place following light absorption (23). Thus, it is plausible that the specific conformation of the 6-member ring chromophore in the binding site and/or specific chromophore-protein interactions enable such an isomerization process. These studies, however, have also indicated that the usual transducin activation did not occur at neutral pH (21–23).

Our present studies show that Rh6.10 membranes have a strongly pH-dependent photochemistry. At neutral pH, the major photochemical process is merely a photoequilibration between the two 11-cis-like isomers that form the dark state of the pigment, 11-cis and 9,11,13-tricis (21). This photoequilibration process (Fig. 1, C–E) is therefore not associated with major protein conformational alterations, as also confirmed by FTIR measurements and in keeping with the lack of significant transducin activity reported previously. The situation is completely different at pH 5.0, where in addition to this photoequilibration process, about half of the pigment is converted to an active Meta II-like photoprotein with a protonated Schiff base (Fig 1B). Because the Meta II-like photointermediate of Rh6.10 at pH 5.0 is characterized by a protonated Schiff base, we can conclude that the pH of the protonated Schiff base in this intermediate is above 5. It was recently demonstrated that the pH of the protonated Schiff base in the native rhodopsin Meta II is −2 and that it is considerably elevated by interaction with anions (32). The elevated pH observed in the Rh6.10 Meta II-like intermediate indicates an altered Schiff base environment (probably more hydrophilic) relative to that in native Meta II, thereby forming a “complex” counterion to the protonated Schiff base. Glu-113, the counterion to the protonated Schiff base in the dark state, becomes protonated during the activation of Rh6.10 similar to native rhodopsin, despite the fact that the Schiff base does not deprotonate. The coupling between the pH values of Glu-113 and the Schiff base is therefore much weaker in the activated receptor state compared with the dark state, and protonation of Glu-113 in the activated receptor seems to be determined by the protein conformational changes rather than by the protonation state of the Schiff base. This scenario has been suggested recently (14) and was confirmed in the meantime with native rhodopsin and a variety of rhodopsin mutants and analogues, which may form an activated state without deprotonation of the Schiff base (32).

At neutral pH, extended illumination additionally produces a strongly blue-shifted intermediate absorbing at 385 nm, in agreement with previous studies (21, 22). FTIR measurements, however, clearly indicate that formation of this intermediate is not associated with protein changes characteristic of the active Meta II species, and furthermore, this intermediate does not bind peptide 23. The nature of this intermediate should be the subject of future studies.

The question arises as to the mechanism by which an active protein conformation is produced to a substantial extent at pH 5.0 but only marginally at pH 7.5. According to their affinities for 11-cis- and all-trans-retinol dehydrogenase, the four isomers of our 11-cis-locked retinal were classified as 11-cis-like and all-trans-like, respectively (23); in the dark state of the pigment in membranes, the isomeric composition of the chromophores is shifted completely to the side of the 11-cis-like isomers (21). It therefore seems plausible that the active state conformation at low pH is achieved by accumulating all-trans-like isomers during illumination. As the pH is raised, the amount of this Meta II-like species decreases rapidly to a small residual level of about 10%, and yet no pH-dependent Meta I-like intermediate could be detected as a counterpart, which, in analogy to native Meta I, should combine an all-trans-like chromophore with a still inactive protein conformation. Instead, the chromophore composition seems to be dominated...
largely by the 11-cis-like isomers, and accumulation of all-trans-like isomers seems to be inhibited at neutral to alkaline pH. Possible mechanisms for such an inhibition are discussed in more detail in the accompanying article (24).

A previous study indicated substantial formation of all-trans-like isomers also at neutral pH (23), which seems to be in conflict with our results. For those experiments the pigment was solubilized in detergent, which has a dramatic effect both on the Meta I/Meta II equilibrium of native rhodopsin (45) and on the flexibility of the chromophore binding pocket to accommodate different isomers (46).

Our results clearly show that isomerization around the C11=C12 double bond of retinal is not per se a prerequisite for formation of the signaling state in rhodopsin. An all-trans-like, activating chromophore may be produced as well by isomerization around other double bonds, and locking the C11=C12 double bond in a cis configuration therefore does not necessarily prevent activation. The active and inactive states of the receptor protein can instead be formed with whole families of retinal ligands stabilizing either of the two states with varying efficiency, and even in the absence of ligands, a pH-dependent equilibrium between the active and inactive conformations of opsins can be observed (30). Nevertheless, nature uses the 11-cis to all-trans isomerization process of retinal exclusively in all known visual pigments. This is, however, not because it is the only possible way to convert the receptor protein from an inactive to a signaling state but rather because it is the most efficient way to perform photoreception. The 11-cis to all-trans pathway combines a high quantum yield of photoisomerization with extremely low dark isomerization rates (47, 48), thus allowing an exquisite sensitivity of the visual system under low light conditions.

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