Crystal Structure of Rhodopsin: A G Protein–Coupled Receptor

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Heterotrimeric guanine nucleotide–binding protein (G protein)–coupled receptors (GPCRs) respond to a variety of different external stimuli and actuate G proteins. GPCRs share many structural features, including a bundle of seven transmembrane α-helices connected by six loops of varying lengths. We determined the structure of rhodopsin from diffraction data extending to 2.8 Ångstroms resolution. The highly organized structure in the extracellular region, including a conserved disulfide bridge, forms a basis for the arrangement of the seven-helix transmembrane motif. The ground-state chromophore, 11-cis-retinal, holds the transmembrane region of the protein in the inactive conformation. Interactions of the chromophore with a cluster of key residues determine the wavelength of the maximum absorption. Changes in these interactions among rhodopsins facilitate color discrimination. Identification of a set of residues that mediate interactions between the transmembrane helices and the cytoplasmic surface, where G-protein activation occurs, also suggests a possible structural change upon photoactivation.

GPCRs activate signaling pathways in response to stimuli such as Ca2+1, amine, hormones, peptides, and even large proteins (2–5). GPCRs share a conserved transmembrane structure comprising seven α-helices. Binding of specific ligands to the extracellular or transmembrane domains causes conformational changes that act as a switch to relay the signal to G proteins that in turn evoke further intracellular responses (4).

Rhodopsins are a member of the largest subfamily, constituting ~90% of all GPCRs. These are activated by light and turn on the signaling pathway that leads to vision. Mutations in the rhodopsin gene lead to human retinal pathologies (5). Rhodopsin is composed of the protein opsin (~40 kD in size) covalently linked to 11-cis-retinal (a derivative of vitamin A) through Lys296 (6, 7). Absorption of a photon by the 11-cis-retinal causes its isomerization to all-trans-retinal (8), leading to a conformational change of the protein moiety, including the cytoplasmic surface. The photolyzed chromophore only transiently activates opsin, before the all-trans-retinal is hydrolyzed and dissociated from the opsin. Rhodopsin is regenerated by newly synthesized 11-cis-retinal delivered from adjacent retinal epithelial cells. The absorption of a single photon results in the activation of hundreds of G-protein molecules with extraordinary reproducibility (9), whereas the 11-cis-retinal–bound rhodopsin has extremely low activity. These two properties allow the human scotopic visual system to detect as few as five photons (10).

A low-resolution structure of frog rhodopsin that reveals the organization of the seven transmembrane helices has been determined by cryo-electron microscopy (11), and biochemical and theoretical studies have given further insights into rhodopsin structure and function (12–18). Here, we describe the three-dimensional (3D) crystal structure of rhodopsin at 2.8 Å. This gives further insights into the mechanisms of receptor activation, and the source of specific ligand and G-protein interactions.

Structure determination: Overall fold and molecular contacts. To obtain structural information for rhodopsin in the ground state, diffraction data for bovine rhodopsin crystallized from mixed micelles (19) were collected to 2.8 Å after mercury soaking. Phasing information was obtained employing multi-wavelength anomalous diffraction (MAD) methods (20). The least twinned data were selected after the collection of several MAD data sets. Initial calculations were performed without correction for twinning for 3.3 Å data set. The majority of other crystals examined were nonisomorphous and highly merohedrally twinned (20).

Rhodopsin molecules are packed in the crystal lattice to form an array of helical tubes (Fig. 1). The two molecules in the asymmetric unit are related by a noncrystallographic twofold axis between the two H-I helices. The current model of bovine rhodopsin (Fig. 2) includes all 194 residues that make up seven transmembrane helices; these are 35 to 64 for H-I, 71 to 100 for H-II, 107 to 139 for H-III, 151 to 173 for H-IV, 200 to 225 for H-V, 247 to 277 for H-VI, and 285 to 306 for H-VII (Fig. 2, A through C), 74 of 348

Fig. 1. Molecular packing viewed along the a axis of the crystal. A unit cell is superposed. One of the asymmetric units containing two rhodopsin molecules is drawn with thick lines. The NCS axis is nearly parallel to the crystallographic a axis.
residues from the extracellular region: 1 to 34 for NH₂-terminal tail, 101 to 106 for E-I, 174 to 199 for E-II, and 278 to 285 for E-III; 70 residues in the cytoplasmic region; and 65 to 70 for C-I, 140 to 150 for C-II, 226 to 235 and 240 to 246 for C-III, and 307 to 327 and 334 to 348 for the COOH-terminal region.

Thus, a total of 338 amino acids in our model corresponds to 97.1% of the whole opsin molecule, although the last 15-amino acid COOH-terminal segment is modeled as Ala residues. In addition, the 11-cis-retinal chromophore connected to Lys²⁰⁶, a part of two oligosaccharides at Asn⁴, Asn¹³ (27), two Zn ions, three Hg ions, and some water molecules are also included per monomer in the current structural model.

Comparison to other receptors. Our current experimental model of rhodopsin offers a structural template for other GPCRs, including the assignment of secondary structural elements and the location of highly conserved amino acids. The molecular size of bovine rhodopsin, 348 amino acids, is intermediate among the members of the family and thus can feature most of the essential parts of functional importance in G-protein activation. The lengths of the seven transmembrane helices and of the three extracellular loops are expected to be nearly the same for most of the family members, as can be seen in the sequence of β-adrenergic receptor (Web fig. 1) (22). Variation in other regions probably reflects the specificity of each receptor for either its ligand or its G protein.

Because most of the vertebrate visual pigments share similar size distributions for all of the domains, structure-function relationships deduced from the current model are likely to be directly applicable to the members of this subfamily. The structure of bovine rhodopsin, represented schematically in Fig. 3, contains many features found in most GPCRs, and clearly demonstrates many differences between GPCRs and bacterial retinal-binding proteins (23–25). Although the mass of transmembrane bundles of rhodopsin and bacterial rhodopsins does not differ significantly, the arrangements of seven helices are found to be different. The structure of rhodopsin disclosed larger and more organized extramembrane regions than that of bacteriorhodopsin, demonstrating the functional differences between these two retinal-binding proteins.

Extracellular region. Regions in the extracellular domain of rhodopsin (NH₂-terminal and interhelical loops E-I, E-II, and E-III) associate to form a compact structure (Fig. 2 and Fig. 4A). The NH₂-terminal tail of rhodopsin contains five distorted strands. The NH₂-terminus is located just below loop E-I, with the side chain of Asp²⁸⁵ close to that of Asn². The first two antiparallel strands, Gly⁵ to Pro¹², form a typical β-sheet fold (B1 and B2) running almost parallel to the expected plane of the membrane. Strands three to five (S3–S5) form a right triangle from Phe¹³ to Pro¹⁴, with the third strand running just below E-II, almost parallel to the long axis of the molecule. S4 connects Ser¹⁴–Asn¹⁵ in the NH₂-terminal region of the molecule with Pro¹⁵, located close to E-I. S5 from Pro¹⁷ to Pro⁴⁸ runs along the surface of the membrane covering the extracellular (intradsal) space between H-I and H-II. Oligosaccharides at Asn² and Asn¹³ extend from the domain and are not included in any interactions. Mutations of Pro⁴⁸ or Glu⁷⁸ cause the eye disease retinitis pigmentosa (5). These side chains are located close together in a region between the S4 and S5 strands and are also close to the side chain of Tyr¹⁰² from the E-I loop. Thus, these residues may maintain the proper orientation between E-I and the NH₂-terminal domain. The NH₂-terminal domain may also contact the E-I-III loop in the region of Pro¹².

While both the E-I and E-III loops run along the periphery of the molecule, a part of E-II folds deeply into the center of rhodopsin. From the extracellular end of H-I-V, a long strand from Gly¹⁷⁴ to Met³⁸ crosses the molecule along the membrane surface. The terminal two residues, Met³⁸ and Gln¹⁸⁴, have extended side chains. The former points to a hydrophobic pocket around H-I while the latter is surrounded by hydrophilic groups, including a water molecule located close to peptide carbonyl of Pro¹⁸⁰ and OH group of Tyr¹⁸⁵. Residues in the middle of this strand, Arg¹⁷⁷ to Glu¹⁸¹ (B3), form an antiparallel β-sheet with residues, Ser¹⁸⁰ to Asp¹⁸⁵ (B4), which is deeper inside the molecule than B3. B4 is just below the 11-cis-retinal and is a part of the chromophore-binding pocket.
Cys\textsuperscript{187} forms a disulfide bond with Cys\textsuperscript{110} at the extracellular end of H-III. This disulfide is conserved in most GPCRs. Residues Tyr\textsuperscript{191} to Asp\textsuperscript{206} from E-II form a loop region at the periphery of the molecule, like E-I and E-III. The peptide carbonyl of Tyr\textsuperscript{191} in E-II and the side-chain amide of Gln\textsuperscript{279}, which is at the beginning of E-III, are close to each other, while Asn\textsuperscript{180} is near to Trp\textsuperscript{177}, which is one of the initial residues of E-II, thus in proximity to the extracellular end of H-IV. This arrangement places E-II in extensive contact with the extracellular regions and also with retinal.

Transmembrane helices. From a cryo-EM study, the helical bundle of rhodopsin appears to have different sided faces on the two ends, suggesting that it could be asymmetric along the axis perpendicular to the membrane surface (11). However, examination of the cross section of the bundle at the two surfaces indicates that these are nearly equal (Fig. 2). As expected, helices H-I, H-IV, H-VI, and H-VII are bent at Pro residues, although it is not significant in H-I, while in H-IV, it causes distortion only around the extracellular end. H-V, with Pro\textsuperscript{215} in the middle, is almost straight. There is a significant bend at Pro\textsuperscript{267} in H-VI. H-VII exhibits irregular helicity, mainly around Lys\textsuperscript{268} to which retinal is covalently attached. H-II is also kinked around Gly\textsuperscript{26} and Gly\textsuperscript{90}, so that in this region it is closer to H-III than to H-I, placing Gly\textsuperscript{90} close to the residue that interacts with the Schiff base, Glu\textsuperscript{112}. This location of Gly\textsuperscript{90} is consistent with the previous studies showing that replacement of this residue by Asp causes night blindness (26), probably because of destabilization of the salt-bridge between Glu\textsuperscript{112} and the Schiff base (27, 28).

The cytoplasmic ends of H-II and H-IV are near each other, but they diverge in the region of Trp\textsuperscript{161}, one of the residues that are highly conserved among GPCRs. This residue is near the point where H-III penetrates toward H-V between H-II and H-IV. Gly\textsuperscript{220} and Gly\textsuperscript{121} do not distort H-III, but the region, Glu\textsuperscript{130}-Arg\textsuperscript{135} Tyr\textsuperscript{136}, does exhibit a slight deviation from regular helical structure. This cytoplasmic terminal region is surrounded mostly by hydrophobic residues from H-II (Pro\textsuperscript{271}, Leu\textsuperscript{272}), C-II (Phe\textsuperscript{143}), H-V (Leu\textsuperscript{226}, Val\textsuperscript{225}), and H-VI (Val\textsuperscript{220}, Met\textsuperscript{225}), forming the binding site for a G protein. H-IV and H-V exhibit irregular helicity in the cytoplasmic region and at His\textsuperscript{311}, respectively. The phenolic ring of Tyr\textsuperscript{222}, which is also highly conserved among GPCRs, partially covers the interhelical region between H-V and H-VI near the lipid interface. The cytoplasmic end of H-VI extends past the putative membrane surface to Thr\textsuperscript{255}. Three basic residues, Lys\textsuperscript{245}, Lys\textsuperscript{248}, and Arg\textsuperscript{252}, located near the cytoplasmic end of H-VI, extend from the helical bundle, making this region of C-III highly basic. In H-VII, two phenyl rings of Phe\textsuperscript{253} and Phe\textsuperscript{294} interact with Leu\textsuperscript{209} of H-I and Cys\textsuperscript{264} of H-VI, respectively. This interaction with H-VI is likely to be particularly important because it is facilitated by distortion of H-VI in the region of Ile\textsuperscript{262}. H-VII is considerably elongated in the region from Ala\textsuperscript{265} to Tyr\textsuperscript{280}. This region includes Ala\textsuperscript{266}, whose peptide carbonyl can hydrogen bond with the side chains of Asn\textsuperscript{239} in H-I and Asp\textsuperscript{83} in H-II. Details of this region are shown in Fig. 5C. A highly conserved NPXY motif (29) in GPCRs follows this region in a regular helical structure.

11-cis-Retinal chromophore. From the experimental electron density, the conformation of the retinal chromophore in the Schiff base linkage with Lys\textsuperscript{296} is 6\textsuperscript{a}-cis, 11-cis, 12\textsuperscript{a}-trans, anti C\textsuperscript{2}N (Fig. 6). The density for the 11\textsuperscript{b}-ionone ring exhibits a larger bulge indicating the position of the two methyl groups connected to C\textsuperscript{11} and a smaller bulge for the single methyl at C\textsuperscript{2}. Two small bulges along the polyene chain indicate the positions of the C\textsuperscript{11} and C\textsuperscript{13}-methyl groups. The refined structure of the retinylidine group is consistent with resonance Raman spectroscopy (30, 31), Nuclear magnetic resonance (NMR) (32), and chemical analysis (33). The density of the polyene chain merges with that of the side chain of Lys\textsuperscript{296}, indicating the presence of a Schiff base linkage. The retinylidine group is located closer to the extracellular side in the putative lipid bilayer, as suggested previously (34).

The position of the 13\textsuperscript{b}-ionone ring is mainly covered by the cytoplasmic side by the residues in H-III and H-VI, Glu\textsuperscript{122}, Phe\textsuperscript{261}, and Trp\textsuperscript{262} (Fig. 6, C and D). The indole ring of Trp\textsuperscript{262} points down to the retinylidine group near the 11\textsuperscript{b}-ionone ring, and also comes close to its C\textsubscript{13}-methyl group with a distance of 3.8 Å. Because deletion of this methyl group is known to cause partial constitutive activity of rhodopsin in the dark (35), loss of its interaction with Trp\textsuperscript{262} may be a possible mechanism of this activity. From the 11\textsuperscript{b}-ionone ring to C\textsubscript{11}, the retinylidine group runs almost parallel to H-III, which provides many of the side chains for the binding pocket, Glu\textsuperscript{113}, Gly\textsuperscript{114}, Ala\textsuperscript{117}, Thr\textsuperscript{118}, Gly\textsuperscript{120}, and Gly\textsuperscript{121}, mainly around the polyene chain. The side chain of Thr\textsuperscript{118}, in addition to Tyr\textsuperscript{256} and Ile\textsuperscript{189} from the extracellular side, appears to determine the position of the C\textsubscript{11}-methyl of the retinylidine group. Side chains mostly from H-V and H-VI, Met\textsuperscript{207}, His\textsuperscript{211}, Phe\textsuperscript{212}, Tyr\textsuperscript{288}, and Ala\textsuperscript{266} also surround the 13\textsuperscript{b}-ionone ring. The proximity of Phe\textsuperscript{261} and Ala\textsuperscript{266} to the retinylidine group is consistent with information showing that these are responsible for the absorption difference between red and green pigments in humans (36). Arrangement of the four residues from H-VI appears

![Fig. 3. Two-dimensional model of bovine rhodopsin adopted after Hargrave's (1, 29). Some of the key residues are shown in filled circles, while residues not modeled in the current structure are shown in gray circles.](https://www.sciencemag.org/content沈科学杂志的内容)
Fig. 4. Cα-traces showing the packing of the polyprotein, with some key residues described in the text, on the (A) intracellular side and (B) the cytoplasmic side of the molecule. Colors of the side chains are red for acidic, blue for basic, yellow for polar, and gray for nonpolar group.

Fig. 5. Structural details for four regions in rhodopsin. (A) The E-II loop near the disulfide bridge connecting Cys110 and Cys187, viewed from extracellular side. (B) The C-IV cytoplasmic loop from Lys311 to Leu321, forming a short amphphilic helix (H-VIII). (C) Interhelical hydrogen bonds mediated by a highly conserved Asn55, connecting H-I, H-II, and H-VII, and by Asn86 for H-II, H-III, and H-IV. (D) The tripeptide region, Glu134-Arg135-Tyr136, known as a (D/E)R(Y/W) motif located near the cytoplasmic end of H-III.

OH group is also close to that of Tyr268 in H-VI. Since mutation of this residue does not affect the absorption but reduces the ability to activate transducin (38), it may participate in the transition to the active form of rhodopsin through interaction with Tyr268.

The arrangement around the Schiff base is of particular interest in terms of understanding the mechanism of the primary process in photocatalysis of rhodopsin. The direction of the side chain of Lys296, almost along the long axis of rhodopsin, is supported by two hydrophobic side chains in H-I, Met64 and Leu72, and by a nearby peptide bond between Phe265 and Phe264. This region is stabilized through the two phenyl rings interacting with other helices. Since it is difficult to determine exactly from the current structure how the protonated Schiff base linkage is stabilized in the protein environment, our model cannot discriminate whether any water molecules participate in making a complex counterion (39) or not. The distances between the carboxylate oxygen atoms of Glu113 and the Schiff base nitrogen are 3.3 Å and 3.5 Å. Also, the OH group of Thr292 comes close to one of the oxygen atoms of Glu113 with a distance of 3.4 Å. Any other residues, including the nearby Thr292 and Thr285, are too far from the Schiff base region to contribute to stabilization of its protonated state. Further improvements in resolution will provide more detailed views of this region.

Cyttoplasmic surface. The structure around the C-I loop exhibits a rigid organization (Fig. 4B). Of the three basic side chains in this region, Lys67 projects toward the solvent, whereas Lys66 and Arg69 point toward lipid-facing region. Another basic side chain of His65 sits closely to C-IV loop (H-VIII). The side chain of Lys67 appears to interact with a part of COOH-terminal tail region, which runs nearly parallel to C-I. The extreme COOH-terminal residues are the most exposed part of rhodopsin molecule and could be involved in vectorial transport of
rhodopsin to rod outer segment.

We assign the region from Cys<sup>410</sup> to Glu<sup>509</sup> as the C-II loop. This loop exhibits an L-shaped structure, when viewed parallel to membrane plane, with a barrel (Met<sup>449</sup> to Phe<sup>469</sup>) almost along the main axis of rhodopsin. Four polar side chains in this loop (Lys<sup>431</sup>, Ser<sup>444</sup>, Asn<sup>455</sup>, and Arg<sup>457</sup>) form a distinct cytoplasmic border from the transmembrane region. The height of these side chains is roughly comparable to that of the cytoplasmic border of C-III loop. Thus, the current model can assign a border corresponding to the major cytoplasmic part of rhodopsin. The extra membranous extension from H-VI, tentatively assigned from Thr<sup>242</sup> to Ala<sup>246</sup>, still exhibits helical structure with no obvious break. In contrast, the cytoplasmatic extension of H-V breaks around Leu<sup>239</sup>, followed by an S-shaped flat loop structure almost along the surface of membrane. This connection from H-V to H-VI, the C-III loop, reaches close to the lipid-facing side of H-VI at Ala<sup>335</sup>, without covering the cytoplasmatic surface of the helical bundle of rhodopsin. Thus, although our model demonstrates a highly flexible nature of this region and still lacks the tetrapeptide from Gin<sup>248</sup> to Glu<sup>250</sup>, it is obvious that C-III does not fold over the helical region at all. On the other hand, two polar side chains of Ser<sup>349</sup> and Thr<sup>342</sup> in C-III comes close to a part of COOH-terminal tail around Ser<sup>334</sup>, making a cluster of OH groups in this region. It should be also noted that the C-III loop is known to vary considerably among related GPCRs, so the flexibility and variability of this region may be critical for functionality and specificity in G-protein activation.

The helical structure of the C-IV loop is of particular interest in the cytoplasmic region, considering previous studies of a variety of synthetic peptides and their effects on the activation of G proteins. Direct evidence for interaction of this region with the G-protein transducin has been provided using a synthetic peptide from Asn<sup>310</sup> to Leu<sup>321</sup> of bovine rhodopsin (40). The short helix is clearly distinct from H-VII and, via Met<sup>309</sup> to Lys<sup>314</sup> linker, lies nearly perpendicular to H-VII. It is also the region that follows the NPXY motif as a part of a conserved block of residues up to Cys<sup>322</sup>. The presence of a helix for this region was demonstrated for a corresponding peptide of turkey β-adrenergic receptor by solution NMR spectroscopy in a nonpolar solvent (41). It has also been supposed that a group of peptides called mastoparan, which assume an amphipathic helical structure and have activity on G proteins, mimic the structure of receptors in this region (42). From the rhodopsin structure, it appears that this short stretch of amino acids is located in a hydrophobic environment, which could induce α-helical structure. The distribution of side chains along this helix also exhibits an amphipathic pattern; the charged/polar groups cluster on one side while hydrophobic ones are on the other, suggesting that the latter, Phe<sup>313</sup>, Met<sup>317</sup> and Leu<sup>321</sup>, are buried in hydrophobic core of the receptor (Fig. 5B). Phe<sup>313</sup> and Arg<sup>314</sup> are the most conserved residues in this region, suggesting that the arrangement of this short helix in rhodopsin may be functionally important.

Although we do not include any lipid-like structure in the current model, the side chains of Cys<sup>322</sup> and Cys<sup>325</sup> project to the outside of rhodopsin, consistent with the probable attachment of palmitic acid residues (43). The helical structure appears to be terminated by Gly<sup>321</sup> and the following COOH-terminal tail changes the direction. Although current model lacks residues from 328 to 333, the positions of 327 and 334 suggest that this missing part runs covering the short H-VII helix from the solvent region. As a whole, COOH-terminal tail of rhodopsin occupies the space over only a part of the helical bundle, H-I and H-VII. Surface potential of cytoplasmic and extracellular surfaces is shown in Web fig. 2 (23).

Intramolecular interactions and activation. The transmembrane region of rhodopsin is stabilized by a number of interhelical hy-
hydrogen bonds and hydrophobic interactions, and most of them are mediated by highly
conserved residues in GPCRs. One of the residues that exhibit the highest conservation
is Asn in I-H. Its side chain is responsible for two interhelical hydrogen bonds to Asp in H-II and to the peptide carbonyl of Ala (Fig. 5C). Asp is in turn connected via a water molecule to the peptide carbonyl of Gly in H-III. Another region that mediates constraints for three helices includes Asn of H-II, which is hydrogen-bonded to OH groups of Ser and Thr of H-III and Thr of H-IV. Helices H-III, H-IV and H-V can be also linked through interaction among Gly, Met, and His.

The tripeptide Glu-Arg-Tyr is part of a highly conserved D/E/R(Y/W) motif found in GPCRs (Fig. 5D). These residues participate in several hydrogen bonds with surrounding residues. The carboxylate of Glu forms salt-bridge with guanidium of the next Arg. Arg is also connected to Glu and Thr in H-VI, Val, Val, to Val, and also closely located to partially cover the cytoplasmic side of Glu and Arg. These could be one of the critical constraints keeping rhodopsin in the inactive conformation. This region has high B-values, however, and the side chains may assume different orientations.

H-VII of most of the GPCRs in the rhodopsin family contains an NPXXY sequence near the cytoplasmic end, but the functional importance of this motif remains unclear. The side chains of the two polar residues in this region, Asn and Tyr, and Tyr in bovine rhodopsin, project inside the molecule. The OH group of Tyr is close to Asn, which is also highly conserved among GPCRs, suggesting the presence of additional interhelical hydrogen-bonding constraints between H-VII and H-II. Although the distance between Asn and Asp is too long to make a hydrogen bond, it appears possible that the water near Asp interacts with the side chain of Asn. In this case, this water mediates a contact among H-II, H-III, and H-VII.

The energy of light is utilized for photoisomerization of the 11-cis-retinal chromophore to an all-trans-configuration. This change in conformation would cause multiple effects, including movement of β-ionone toward H-III and/or displacement of Schiff base C17/C13 methyl regions, ultimately switching the receptor to active conformation, metarhodopsin II (27, 28, 44, 45). Our model of bovine rhodopsin confirms that these effects can change the environment of the salt-bridge between the Schiff base and Gly, resulting in its neutralization (46). Displacement of H-III will result in changing the environment of the ERY motif and its reorientation. Our rhodopsin model also

Fig. 6. The environment of the 11-cis-retinal chromophore. (A) Experimental electron density of 3.3 Å resolution with the final model of 3.3 Å data set using MAD phases after NCS averaging and solvent flattening with DM (20) for the retinal chromophore. Blue for 2IF, red for 1IF, map (1o). (B) Electron density for the retinal chromophore with the current model refined against the 2.8 Å data set. Blue for 2IF, red for 1IF, map (1o) and red for omit 1IF, map (5o) phases calculated using the current model. (C) Schematic showing the side chains surrounding the 11-cis-retinylidene group, viewed from cytoplasmic side. Ala interacts with β-ionone ring of all-trans-retinal in photo-activated states (58). When the intrinsic 11-cis-retinal was substituted by all-trans-retinal in the crystal structure, the β-ionone ring can reach Ala residue. (D) Schematic presenting the residues within 4.5 Å distance from retinal molecule. Blue labels indicate the distances between Schiff base nitrogen atom and charged or polar atoms within 4.5 Å.
suggests that interaction between \( \beta \)-ionone ring and H-III occurs at Glu122, which is one of the residues that determine the rate of metarhodopsin II decay (47). Because Glu122 interacts with His21 in rhodopsin, the proposed movement of H-III caused by the \( \beta \)-ionone ring can affect the interaction between these residues in the transition to metarhodopsin II. In addition, the change around the Schiff base region can affect the interaction between the Cys13-methyl group of retinal and Thr856. The photoreactivation may also cause breakage of some of the three interhelical constraints mediated by Ala126, Asn127, and Tyr128, and hydrophobic constraints via Phe294 to the highly kinked region in H-VI. As a result, rearrangement of the helical bundle may be triggered, and finally lead to the movements of H-III and/or H-VI (48). Our proposed mechanism stresses importance of the chromophore in the activation process, in agreement with the physiology of photoreceptor cells. Ideally, for a complete picture of the activation process, high-resolution structures of intermediates of photolyzed rhodopsin will be necessary.

**Summary.** The GPCR family is one of the largest and most diverse groups of proteins encoded by 1 to 3% of the genes present in our genome. They are involved in many physiological processes and are attractive targets for pharmacological intervention to modify these processes in normal and pathological states. The crystal structure of rhodopsin reveals a highly organized helical-transmembrane bundle with 11-cis-retinal as a key cofactor involved in maintaining rhodopsin in the ground state. A set of residues that interacts with the 11-cis-retinal chromophore produces the environment that results in an absorption shift of the chromophore to a longer wavelength. The structure provides insight into the spectral tuning of related receptors, cone pigments. The structure also gives information on the molecular mechanism of GPCR activation. A conserved set of residues on the cytoplasmic surface, where G-protein activation occurs, likely undergone a conformational change upon photoactivation of the chromophore that leads to rhodopsin activation and signal transduction.

**References and Notes**


20. The tetragonal crystals of rhodopsin have been obtained by using purified protein from bovine rod outer segment (ROS) membranes (19, 49), and derived by x-ray crystallography. Our present understanding of crystallography, however, is limited to the initial steps, involving the determination of the diffraction data. The space group is P4_22 or P4_22 (19) for native crystal, and they are indexed as a=b=290.5 Å, a=90°, b=90°, and c=5.3 Å. The two-dimensional unit cell contains the information about the crystal, the symmetry, and the unit cell dimensions. The crystal is indexed in the space group P4_22 (19) for native crystal, and the space group is P4_22 (19) for the initial steps.


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