

# Structure and Function of G-Protein-Coupled Receptors: Lessons from the Crystal Structure of Rhodopsin

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## Introduction

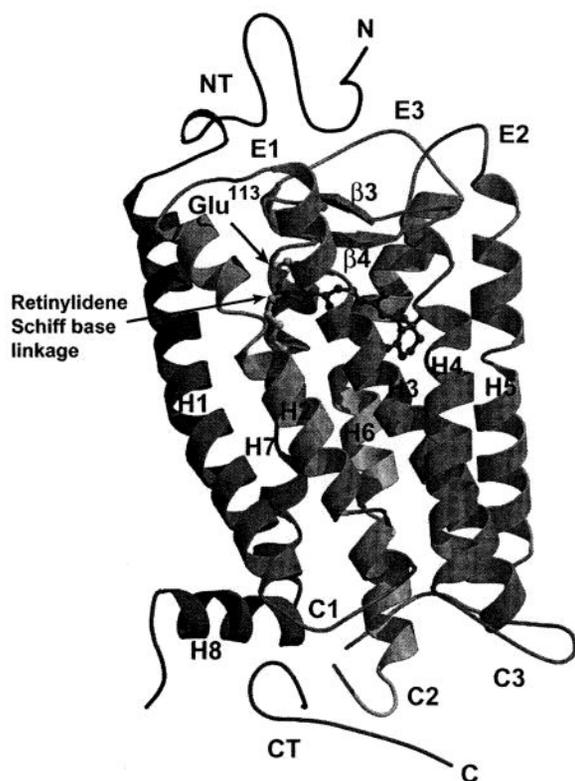
The crystal structure of rhodopsin was recently solved at 2.8-Å resolution. As a prototypical seven-helical G-protein-coupled receptor (GPCR), rhodopsin has provided significant insights toward defining structure–activity relationships among other related receptors. In particular, many advances in understanding the molecular mechanism of receptor activation and how an active receptor catalyzes the exchange of guanine nucleotides on heterotrimeric G proteins have been suggested from biochemical and biophysical studies of rhodopsin and expressed rhodopsin mutants. The report of a high-resolution crystal structure of rhodopsin now provides new opportunities to understand how GPCRs work. For example, the ligand-binding pocket of rhodopsin is remarkably compact, and several apparent chromophore–protein interactions were not predicted from extensive mutagenesis or spectroscopic studies. The transmembrane helices are interrupted or kinked at multiple sites. An extensive network of interhelical interactions stabilizes the ground state of the receptor. The helix movement model of receptor activation, which might apply to all GPCRs in the rhodopsin family, is supported by several structural elements that suggest how light-induced conformational changes in the

ligand-binding pocket are transmitted to the cytoplasmic surface. Future high-resolution structural studies of rhodopsin and other GPCRs will form a basis to elucidate the detailed molecular mechanism of GPCR-mediated signal transduction.

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## Introduction to Rhodopsin: a Prototypical G-Protein-Coupled Receptor

Rhodopsin (Rho) is a highly specialized G-protein-coupled receptor (GPCR) that detects photons in the rod photoreceptor cell. Within the superfamily of GPCRs that couple to heterotrimeric G proteins, Rho defines the so-called family A GPCRs, which share primary structural homology [1–3]. Rho shares a number of structural features with other GPCRs, including seven transmembrane segments (H1 to H7) (Fig. 1). In visual pigments, a Lys residue that acts as the linkage site for the chromophore is conserved within H7 in all pigments, and a carboxylic acid residue that serves as the counterion to the protonated, positively charged Schiff base is conserved within H3. The position analogous to the Schiff base counterion is one helix turn away from the position of an Asp residue conserved in biogenic amine receptors that serves as



**Figure 1** A molecular graphics ribbon diagram of Rho prepared from the 2.8-Å crystal structure coordinates (PDB 1f88). The amino terminus (N) and extracellular surface is toward the top of the figure and the carboxyl terminus (C) and intracellular surface is toward the bottom. Seven transmembrane segments (H1 to H7), which are characteristic of GPCRs, are labeled. The RET chromophore is shown in magenta, and the side chain of Glu-113 and the retinylidene Schiff base linkage are shown to highlight the orientation of the chromophore in the binding pocket. The Schiff base imine nitrogen is labeled. The Rho crystal structure does not resolve a small segment of the C3 loop linking H5 and H6 or a longer segment of the carboxyl-terminal tail distal to H8. The  $\alpha$ -helical transmembrane segments are tilted with respect to the presumed plane of the membrane bilayer, and they contain significant kinks and irregularities.

the counterion to the cationic amine ligands. A pair of highly conserved Cys residues is found on the extracellular surface of the receptor and forms a disulfide bond. A Glu(Asp)/Arg/Tyr(Trp) tripeptide sequence is found at the cytoplasmic border of H3. This sequence is conserved in family A GPCRs and has been shown to be involved in G-protein interaction [4,5].

### Molecular Structure of Rhodopsin

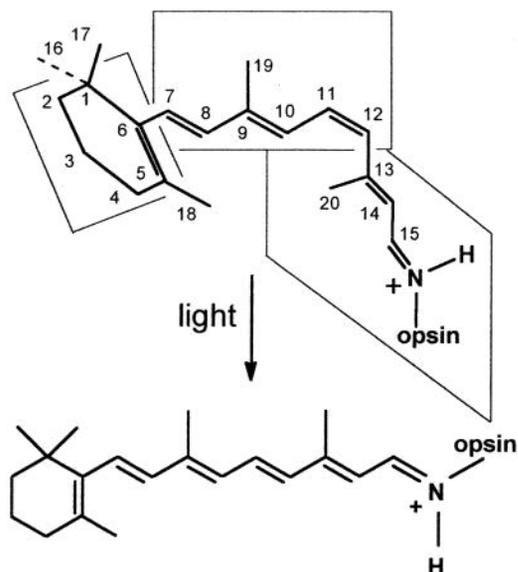
The extracellular surface domain of Rho is comprised of the amino-terminal tail (NT) and three interhelical loops (E1, E2, and E3) (Fig. 1) [6]. There is significant secondary structure in the extracellular domain and several intra- and inter-domain interactions. The E2 loop is extremely interesting in that it is folded deeply into the core of the membrane-embedded region of Rho. In addition to contacts with

the chromophore (11-*cis*-retinol), E2 forms extensive contacts with other extracellular regions. The  $\beta 3$  and  $\beta 4$  strands, which arise from E2, run anti-parallel. The  $\beta 4$  strand is situated more deeply within the membrane-embedded region of Rho than the  $\beta 3$  strand. The  $\beta 4$  strand is adjacent to the chromophore and forms the extracellular boundary, or roof, of the ligand-binding pocket. A disulfide bond between Cys-110 and Cys-187, which forms the extracellular end of H3, is highly conserved among all class A GPCRs.

More than one-half of the 348 amino acid residues in Rho make up the seven transmembrane segments (H1 to H7) included in the membrane-embedded domain. The crystal structure of this domain is remarkable for a number of kinks and distortions of the individual transmembrane segments, which are otherwise generally  $\alpha$ -helical in secondary structure. Many of these distortions from idealized secondary structure were not accounted for in molecular graphics models of Rho based on projection density maps obtained from cryoelectron microscopy [7]. H7 is the most highly distorted of the seven transmembrane helical segments. There are kinks at two Pro residues, Pro-291 and Pro-303. In addition, the helix is irregular around the region of residue Lys-296, which is the chromophore attachment site. Pro-303 is a part of the highly conserved Asn/Pro/X/X/Tyr motif (Asn-302/Pro-303/Val-304/Ile-305/Tyr-306 in Rho).

The membrane-embedded domain of Rho is also characterized by the presence of several intramolecular interactions that may be important in stabilizing the ground state structure of the receptor. One of the hallmarks of the molecular physiology of Rho is that it is essentially silent biochemically in the dark. The bound chromophore serves as a potent pharmacological inverse agonist to minimize activity. The Rho structure reveals numerous potentially stabilizing intramolecular interactions, some mediated by the chromophore and others arising mainly from interhelical interactions that do not involve the chromophore-binding pocket directly. For example, a complex H-bond network appears to link H6 and H7. The key interaction here is between Met-257 and Asn-302. The precise functional importance of the highly conserved Asn/Pro/X/X/Tyr motif (Asn-302/Pro-303/Val-304/Ile-305/Tyr-306 in Rho) is unclear. However, one key structural role is to mediate several interhelical interactions. The side chains of Asn-302 and Tyr-306 project toward the center of the helical bundle. The hydroxyl group of Tyr-306 is close to Asn-73 (cytoplasmic border of H2), which is also highly conserved. A key structural water molecule may facilitate an H-bond interaction between Asn-302 and Asp-83 (H2). A recent mutagenesis study of the human platelet-activating factor receptor showed that replacement of amino acids at the positions equivalent to Asp-78 and Asn-302 in Rho with residues that could not H bond prevented agonist-dependent receptor internalization and G-protein activation [8].

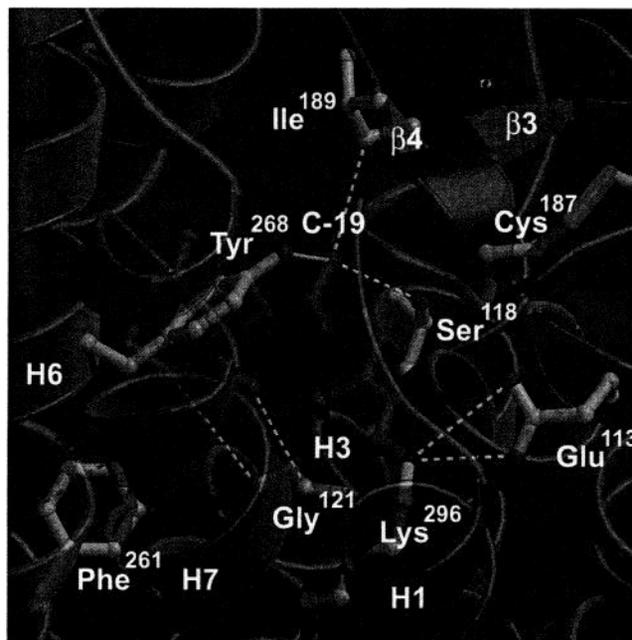
The 11-*cis*-retinol chromophore is a derivative of vitamin A<sub>1</sub>, with a total of 20 carbon atoms (Fig. 2). The binding site of the chromophore lies within the membrane-embedded



**Figure 2** Photoisomerization of the 11-*cis*-retinylidene chromophore (RET) to its 11-*trans* form is the only light-dependent event in vertebrate vision. The RET chromophore is a derivative of vitamin A<sub>1</sub> with a total of 20 carbon atoms. The structure of the chromophore in rhodopsin appears to be 6*s-cis* 11-*cis* 12*s-trans* 15-*anti*-retinylidene protonated Schiff base. The planar surfaces are meant to depict the twists about the C-6–C-7 and C-12–C-13 bonds. Photoisomerization in Rho occurs on an ultrafast time scale, with photorhodopsin as the photoproduct formed on a femtosecond time scale [23]. The photolyzed pigment then proceeds through a number of well-characterized spectral intermediates. As the protein gradually relaxes around 11-*trans* RET, protein–chromophore interactions change and distinct  $\lambda_{\text{max}}$  values are observed. Important photochemical properties of Rho in the rod cell disc membrane include a very high quantum efficiency ( $\approx 0.67$  for Rho versus  $\approx 0.20$  for RET in solution) and an extremely low rate of thermal isomerization.

domain of the receptor (Fig. 3). All seven transmembrane segments and part of the extracellular domain contribute interactions with the bound chromophore. The chromophore is located closer to the extracellular side of the transmembrane domain of the receptor than to the cytoplasmic side. Glu-113 serves as the counterion for the Schiff base attraction of the chromophore to Lys-296. In all, at least 16 amino acid residues are within 4.5 Å of the chromophore: Glu-113, Ala-117, Thr-118, Gly-121, Glu-122, Glu-181, Ser-186, Tyr-191, Met-207, His-211, Phe-212, Phe-261, Trp-265, Tyr-268, Ala-269, and Ala-292. The most striking feature of the binding pocket is the presence of many polar or polarizable groups to coordinate an essentially hydrophobic ligand.

The cytoplasmic domain of Rho is comprised of three cytoplasmic loops and the carboxyl-terminal tail: C1, C2, C3, and CT. Loops C1 and C2 are resolved in the crystal structure, but only residues 226 to 235 and 240 to 246 are resolved in C3. CT is divided into two structural domains. C4 extends from the cytoplasmic end of H7 at Ile-307 to Gly-324, just beyond two vicinal Cys residues (Cys-322 and Cys-323), which are posttranslationally palmitoylated. The remainder of CT extends from Lys-325 to the carboxyl terminus of Rho at Ala-348. The crystal structure does not resolve residues 328 to 333 in CT.



**Figure 3** The RET chromophore-binding pocket of bovine Rho. The RET chromophore-binding pocket is shown from slightly above the plane of the membrane bilayer looking between transmembrane segments H1 and H7. Several amino acid residues are labeled, including the Schiff base counterion Glu-113. At least three residues appear to interact with the C-19 methyl group of the chromophore: Ser-118, Ile-189, and Tyr-268. The C-19 methyl group might provide a key ligand anchor that couples chromophore isomerization to protein conformational changes. Some additional key amino acid residues are labeled, including the Cys-187, which forms a highly conserved disulfide bond with Cys-110.

A number of cytoplasmic proteins are known to interact exclusively with the active state of the receptor (R<sup>\*</sup>). Because the crystal structure depicts the inactive Rho structure that does not interact significantly with cytoplasmic proteins, the structure can provide only indirect information about the relevant R<sup>\*</sup> state. Perhaps the most extensively studied receptor–G-protein interaction is that of bovine Rho with G<sub>t</sub>. Detailed biochemical and biophysical analysis of the R<sup>\*</sup>–G<sub>t</sub> interaction has been aided by mutagenesis of the cytoplasmic domain of bovine Rho. Numerous Rho mutants defective in the ability to activate G<sub>t</sub> have been identified. Several of these mutant receptors were studied by flash photolysis [9], light-scattering [10], or proton-uptake assays [11]. The key overall result of these studies is that C2, C3, and H8 are involved in the R<sup>\*</sup>–G<sub>t</sub> interaction.

H8 is a cationic amphipathic helix that may bind a phospholipid molecule, especially a negatively charged phospholipid such as phosphatidylserine. In fact, spectroscopic evidence has been reported to show an interaction between Rho and a lipid molecule that is altered in the transition of Rho to metarhodopsin II, the spectrally defined form of R<sup>\*</sup> [12]. H8 points away from the center of Rho, and the area of the membrane surface covered by the entire cytoplasmic surface domain appears to be roughly large enough to accommodate G<sub>t</sub> in a one-to-one complex.

## Molecular Mechanism of Receptor Activation

Although the crystal structure of Rho does not provide direct information about the structure of R\* or about the dynamics of the Rho to R\* transition, it does provide a wealth of information that should help to design experiments using existing methods to address specific questions regarding the molecular mechanism of Rho activation. An inactive receptor conformation must be capable of changing to an active conformation which catalyzes nucleotide exchange by a G protein. In Rho, the chromophore is in its "off" state, but switches to the "on" state 11-*trans* geometry by photoisomerization, which leads to the R\* conformation of the receptor. Recent studies have suggested that steric and/or electrostatic changes in the ligand-binding pocket of Rho may cause changes in the relative disposition of transmembrane (TM) helices within the core of the receptor. These changes may be responsible for transmitting a "signal" from the membrane-embedded binding site to the cytoplasmic surface of the receptor. Trp mutagenesis [13], mutagenesis of conserved amino acid residues on H3 and H6 [14,15], and the introduction of pairs of His residues at the cytoplasmic borders of TM helices to create sites for metal chelation [16] have recently provided insights regarding the functional role of specific helix-helix interactions in Rho. These results indicated a direct coupling of receptor activation to a change in the spatial disposition of H3 and H6. This could occur if movements of H3 and H6 were coupled to changes in the conformation of the connected intracellular loops, which are known to contribute to binding surfaces and tertiary contacts of Rho with G<sub>t</sub>.

More direct evidence for changes in interhelical interactions upon receptor activation were provided by extensive site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy studies of the transition of Rho to R\* in modified, or expressed, mutant pigments. The results suggested a requirement for rigid body motion of transmembrane helices, especially H3 and H6, in the activation of Rho [17]. A slight reorientation of helical segments upon receptor activation is also supported by experiments using polarized attenuated total reflectance infrared difference spectroscopy [18]. Finally, movement of H6 was also detected by site-specific chemical labeling and fluorescence spectroscopy [19]. The structural rearrangement of helices upon activation might not result in an R\* structure that is drastically different from that of Rho as an engineered receptor with four disulfide bonds (between the cytoplasmic ends of H1 and H7, and H3 and H5, and the extracellular ends of H3 and H4, and H5 and H6) was still able to activate G<sub>t</sub> [20].

Because the arrangement of the seven transmembrane segments is likely to be evolutionarily conserved among the family of GPCRs, the proposed motions of H3 and H6 may be a part of a conserved activation mechanism shared among all receptor subtypes [21,22]. In other class A GPCRs, agonist ligand binding would be coupled to a change in the orientations of H3 and H6.

## References

1. Menon, S. T., Han, M., and Sakmar, T. P. (2001). Rhodopsin: structural basis of molecular physiology. *Physiol. Revs.* **81**, 1659–1688.
2. Sakmar, T. P., Menon, S. T., Marin, E. P., and Awad, E. S. (2002). Rhodopsin: insights from recent structural studies. *Annu. Rev. Biophys. Biomol. Struct.* **31**, 443–484.
3. Gether, U. (2000). Uncovering molecular mechanisms involved in activation of G protein-coupled receptors. *Endocr. Rev.* **21**, 90–113.
4. Franke, R. R., Konig, B., Sakmar, T. P., Khorana, H. G., and Hofmann, K. P. (1990). Rhodopsin mutants that bind but fail to activate transducin. *Science* **250**, 123–125.
5. Franke, R. R., Sakmar, T. P., Graham, R. M., and Khorana, H. G. (1992). Structure and function in rhodopsin. Studies of the interaction between the rhodopsin cytoplasmic domain and transducin. *J. Biol. Chem.* **267**, 14767–14774.
6. Palczewski, K., Kumasaka, T., Hori, T., Behnke, C. A., Motoshima, H., Fox, B. A., Le Trong, I., Teller, D. C., Okada, T., Stenkamp, R. E., Yamamoto, M., and Miyano, M. (2000). Crystal structure of rhodopsin: a G-protein-coupled receptor. *Science* **289**, 739–745.
7. Unger, V. M., Hargrave, P. A., Baldwin, J. M., and Schertler, G. F. (1997). Arrangement of rhodopsin transmembrane alpha-helices. *Nature* **389**, 203–206.
8. Le Gouill, C., Parnet, J. L., Rola-Pleszczynski, M., and Stankova, J. (1997). Structural and functional requirements for agonist-induced internalization of the human platelet-activating factor receptor. *J. Biol. Chem.* **272**, 21289–21295.
9. Ernst, O. P., Meyer, C. K., Marin, E. P., Henklein, P., Fu, W. Y., Sakmar, T. P., and Hofmann, K. P. (2000). Mutation of the fourth cytoplasmic loop of rhodopsin affects binding of transducin and peptides derived from the carboxyl-terminal sequences of transducin alpha and gamma subunits. *J. Biol. Chem.* **275**, 1937–1943.
10. Ernst, O. P., Hofmann, K. P., and Sakmar, T. P. (1995). Characterization of rhodopsin mutants that bind transducin but fail to induce GTP nucleotide uptake. Classification of mutant pigments by fluorescence, nucleotide release, and flash-induced light-scattering assays. *J. Biol. Chem.* **270**, 10580–10586.
11. Arnis, S., Fahmy, K., Hofmann, K. P., and Sakmar, T. P. (1994). A conserved carboxylic acid group mediates light-dependent proton uptake and signaling by rhodopsin. *J. Biol. Chem.* **269**, 23879–23881.
12. Isele, J., Sakmar, T. P., and Siebert, F. (2000). Rhodopsin activation affects the environment of specific neighboring phospholipids: an FTIR study. *Biophys. J.* **79**, 3063–3071.
13. Lin, S. W. and Sakmar, T. P. (1996). Specific tryptophan UV-absorbance changes are probes of the transition of rhodopsin to its active state. *Biochemistry* **35**, 11149–11159.
14. Han, M., Lin, S. W., Minkova, M., Smith, S. O., and Sakmar, T. P. (1996). Functional interaction of transmembrane helices 3 and 6 in rhodopsin. Replacement of phenylalanine 261 by alanine causes reversal of phenotype of a glycine 121 replacement mutant. *J. Biol. Chem.* **271**, 32337–32342.
15. Han, M., Lin, S. W., Smith, S. O., and Sakmar, T. P. (1996). The effects of amino acid replacements of glycine 121 on transmembrane helix 3 of rhodopsin. *J. Biol. Chem.* **271**, 32330–32336.
16. Sheikh, S. P., Zvyaga, T. A., Lichtarge, O., Sakmar, T. P., and Bourne, H. R. (1996). Rhodopsin activation blocked by metal-ion-binding sites linking transmembrane helices C and F. *Nature* **383**, 347–350.
17. Farrens, D. L., Altenbach, C., Yang, K., Hubbell, W. L., and Khorana, H. G. (1996). Requirement of rigid-body motion of transmembrane helices for light activation of rhodopsin. *Science* **274**, 768–770.
18. DeLange, F., Bovee-Geurts, P. H., Pistorius, A. M., Rothschild, K. J., and DeGrip, W. J. (1999). Probing intramolecular orientations in rhodopsin and metarhodopsin II by polarized infrared difference spectroscopy. *Biochemistry* **38**, 13200–13209.

19. Dunham, T. D. and Farrens, D. L. (1999). Conformational changes in rhodopsin. Movement of helix f detected by site-specific chemical labeling and fluorescence spectroscopy. *J. Biol. Chem.* **274**, 1683–1690.
20. Struthers, M., Yu, H., and Oprian, D. D. (2000). G protein-coupled receptor activation: analysis of a highly constrained, “straitjacketed” rhodopsin. *Biochemistry* **39**, 7938–7942.
21. Gether, U. and Kobilka, B. K. (1998). G protein-coupled receptors. II. Mechanism of agonist activation. *J. Biol. Chem.* **273**, 17979–17982.
22. Ji, T. H., Grossmann, M., and Ji, I. (1998). G protein-coupled receptors. I. Diversity of receptor–ligand interactions. *J. Biol. Chem.* **273**, 17299–17302.
23. Wang, Q., Schoenlein, R. W., Peteanu, L. A., Mathies, R. A., and Shank C. V. (1994). Vibrationally coherent photochemistry in the femtosecond primary event of vision. *Science* **266**, 422–424.