

## Review

# Structure and activation of rhodopsin

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Rhodopsin is the first G-protein-coupled receptor (GPCR) with its three-dimensional structure solved by X-ray crystallography. The crystal structure of rhodopsin has revealed the molecular mechanism of photoreception and signal transduction in the visual system. Although several other GPCR crystal structures have been reported over the past few years, the rhodopsin structure remains an important model for understanding the structural and functional characteristics of other GPCRs. This review summarizes the structural features, the photoactivation, and the G protein signal transduction of rhodopsin.

**Keywords:** G-protein-coupled receptor (GPCR); rhodopsin; crystal structure; photoactivation; transduction

Acta Pharmacologica Sinica (2012) 33: 291–299; doi: 10.1038/aps.2011.171; published online 23 Jan 2012

## Introduction

Rhodopsin, a visual pigment found in the rod photoreceptor cells of the retina, is responsible for converting photons into chemical signals that stimulate biological processes in the nervous systems of humans and other vertebrate animals, allowing them to sense light<sup>[1]</sup>. Rhodopsin is a member of class A of the GPCR superfamily<sup>[2]</sup>, which is a large group of cell surface signaling receptors that transduce extracellular signals into intracellular pathways through the activation of heterotrimeric G proteins. The human GPCR superfamily, comprised of about 900 members, is involved in many aspects of human physiology and diseases, and it represents the most important protein targets for pharmaceutical drug discovery<sup>[2]</sup>.

The crystal structure of ground-state bovine rhodopsin containing the reverse agonist 11-*cis*-retinal was the first high-resolution GPCR structure solved by X-ray crystallography<sup>[3–6]</sup>. Recently, crystal structures have been published for both opsin and rhodopsin in active conformations, with or without the binding of a peptide derived from the C-terminal helix  $\alpha 5$  of the  $\alpha$  subunit of G protein transducin<sup>[7–11]</sup>. The crystal structures of rhodopsin and the results of related biochemical and biophysical studies<sup>[12]</sup> have revealed the molecular mechanisms of photoactivation and visual signal transduction, leading to a significant progress in understanding the signaling

pathways of the entire GPCR superfamily<sup>[13]</sup>.

As the first solved GPCR crystal structure, bovine rhodopsin has been used as a structural template in many efforts of molecular modeling and in designing therapeutic drugs for human diseases related to GPCR signaling pathways<sup>[14]</sup>. More recently, crystal structures have been published for  $\beta 1$ -adrenergic receptor<sup>[15]</sup>, A<sub>2A</sub> adenosine receptor<sup>[16]</sup>,  $\beta 2$ -adrenergic receptor<sup>[17–21]</sup>, and the complex of  $\beta 2$ -adrenergic receptor with a trimeric G protein<sup>[21]</sup>. These results have further enriched our understanding of ligand-induced activation and downstream signal transduction by GPCRs.

In this review we summarize the structural features, the photoactivation, and the G protein signal transduction of rhodopsin.

## Overall structure of bovine rhodopsin

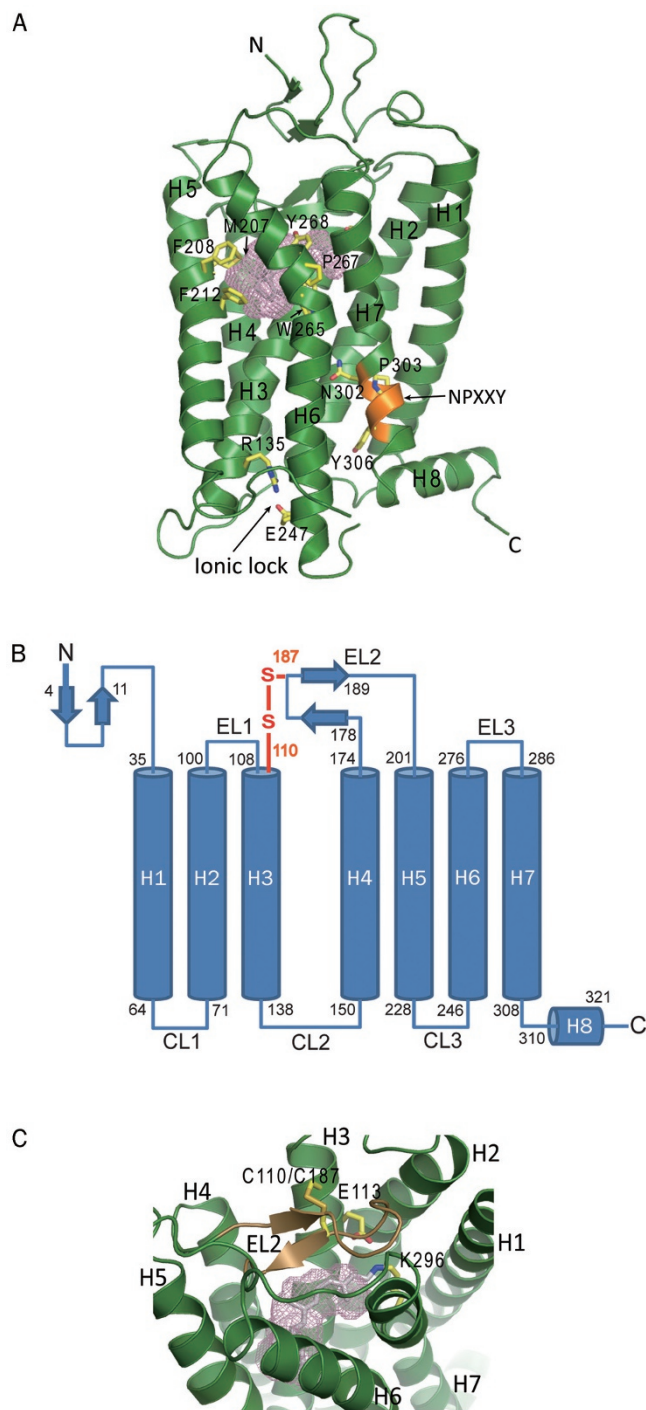
The crystal structure of inactive, 11-*cis*-retinal-bound bovine rhodopsin was first determined by Palczewski *et al* in 2000<sup>[3]</sup> and later by a few other groups<sup>[4–6]</sup>. The bovine rhodopsin structure features a seven-transmembrane (7TM) helix core architecture with three loop regions on both the extracellular and the cytoplasmic side of the membrane (Figure 1A and 1B). The N-terminus of rhodopsin, located on the extracellular side, consists of a two-stranded  $\beta$  sheet stretching from Gly4 to Pro11, followed by a loop region of about 24 amino acid residues (Figure 1A and 1B). The residues Asn2 and Asn15 are glycosylation sites for the receptor, and mutations that replace these residues with alanine lower the receptor's light-sensing activity<sup>[22]</sup>. The Thr4, Asn5, Thr17, Pro23, and Asn28 residues

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Received 2011-09-23 Accepted 2011-11-12



**Figure 1.** Overall structure of ground state bovine rhodopsin and its key features (PDB: 1F88). (A) The seven-transmembrane helix domain with the retinal in gray stick and the ligand binding pocket shown as a pink mesh. Major ligand binding residues around the ligand binding pocket are shown as yellow sticks and are labeled. Other features include the ionic lock (yellow sticks) and the NPXXY motif (orange). (B) Two-dimensional sequence of bovine rhodopsin with the starting and ending residues of secondary structural elements indicated. The disulfide bond connecting EL2 to helix 3 is shown in orange. N, amino terminus; C, carboxyl terminus; EL, extracellular loop; CL, cytoplasmic loop. (C) The ligand binding pocket (pink mesh) of rhodopsin with EL2 (the lid of the pocket) shown in dark brown. The disulfide bond between C110 and C187 is labeled.

in this region have been proved to be crucial for the correct folding of the receptor, and mutations in these residues can lead to autosomal dominant retinitis pigmentosa (ADRP)<sup>[23, 24]</sup>.

The second extracellular loop region (EL2), between helix 4 and helix 5 or from Gly175 to Asn200, is the longest among three extracellular loop regions, and it is essential for ligand binding of the receptor (Figure 1C). Biochemical data have shown that the interaction of EL2 with the helix bundle is important for the correct folding and biological function of the receptor<sup>[25]</sup>. The majority of this loop region, from Tyr178 to Ile189, forms a twisted two-stranded  $\beta$  sheet that is positioned at the opening of the ligand binding pocket, serving as a “lid” to block rapid exit of the ligand from the pocket (Figure 1C). The position of this  $\beta$  sheet is stabilized by hydrophobic interactions between residues Tyr178, Pro180, Met183 (Leu in humans), Cys185, and Cys187 of EL2, and surrounding residues of the helix bundle, especially those from helices 1, 2, 3, and 7. It is also stabilized by a disulfide bond between Cys187 of the  $\beta$  sheet and Cys110 of helix 3, and by a hydrogen bonding network among Glu181 and Tyr192 of EL2, Tyr268 of helix6 and a few water molecules. Between the two strands of this  $\beta$  sheet is a salt bridge formed by Asp190 and Arg177, which is a key interaction for maintaining the functional conformation of this lid over the retinal binding site (Figure 1C)<sup>[26]</sup>.

The architecture of the 7TM domain is a common feature across the GPCR superfamily with many conserved residues<sup>[16–20]</sup>. Among the seven helices, helix 6, from Lys246 to His276 in the ground-state model, bends about 36° at Pro267, with both ends facing away from the core of the 7TM domain (Figure 1A)<sup>[3]</sup>. Upon photoactivation, the cytoplasmic end of this helix shifts further away from the 7TM core, and this shift creates a crevice in the cytoplasmic side of the receptor for binding the  $\alpha$  subunit of G protein transducin<sup>[8, 11]</sup>.

The ligand binding site of rhodopsin is a pocket on the extracellular side of the transmembrane bundle. It is surrounded by hydrophobic residues that stabilize the polyene backbone of the retinal, among which are Met207, Phe208, and Phe212 from helix 5, and Trp265 and Tyr268 from helix 6 (Figure 1A and 1C)<sup>[3, 4, 6]</sup>. The positions and the conformations of those hydrophobic residues undergo dynamic changes during the photoactivation of the retinal and the conformational changes in the receptor<sup>[7, 8, 11]</sup>.

Another important feature of this receptor is the “ionic lock”, a salt bridge between Arg135 of helix 3 and Glu247 of helix 6 (Figure 1A)<sup>[3, 4, 6]</sup>. This salt bridge blocks the G protein binding site of the receptor in its inactive conformation. Upon photoactivation, the transmembrane bundle undergoes a conformational change: the cytoplasmic side of helix 6 bends further away from the 7TM core and the ionic lock breaks, resulting in an opening on the cytoplasmic side of the receptor for G protein interaction<sup>[7, 8, 11]</sup>.

A conserved NPXXY motif on helix 7 is also common to all the GPCR family members based on sequence alignment and crystal structures<sup>[3, 16–18]</sup>. It has an important role in receptor activation: this motif shifts toward helix 6 and the key residue Tyr306 on this motif flips toward helix 6, helping breaking the

ionic lock and pushing helix 6 away from the transmembrane bundle (Figure 1A).

The C-terminus of rhodopsin is on the cytoplasmic side of the membrane, extending from residue Met309 at the C-terminal end of helix 7 to the last residue of the receptor, and featuring a short amphipathic helix (helix 8) perpendicular to helix 7 (Figure 1A and 1B)<sup>[3, 4, 6]</sup>. It forms hydrophobic interactions by its residues Phe313, Cys316, and Met317 with residues Leu57, Val61 of helix 1 and His65 in the loop following helix 1, and is covalently anchored to the membrane by palmitoylation of residues Cys322 and Cys323 in the loop following helix 8. The C-terminal loop following residue Asn326 is disordered in all the crystal structures. In this region there are serine residues Ser334, Ser338, and Ser343, whose phosphorylation is important for arrestin binding to terminate the cycle of G protein activation<sup>[27]</sup>.

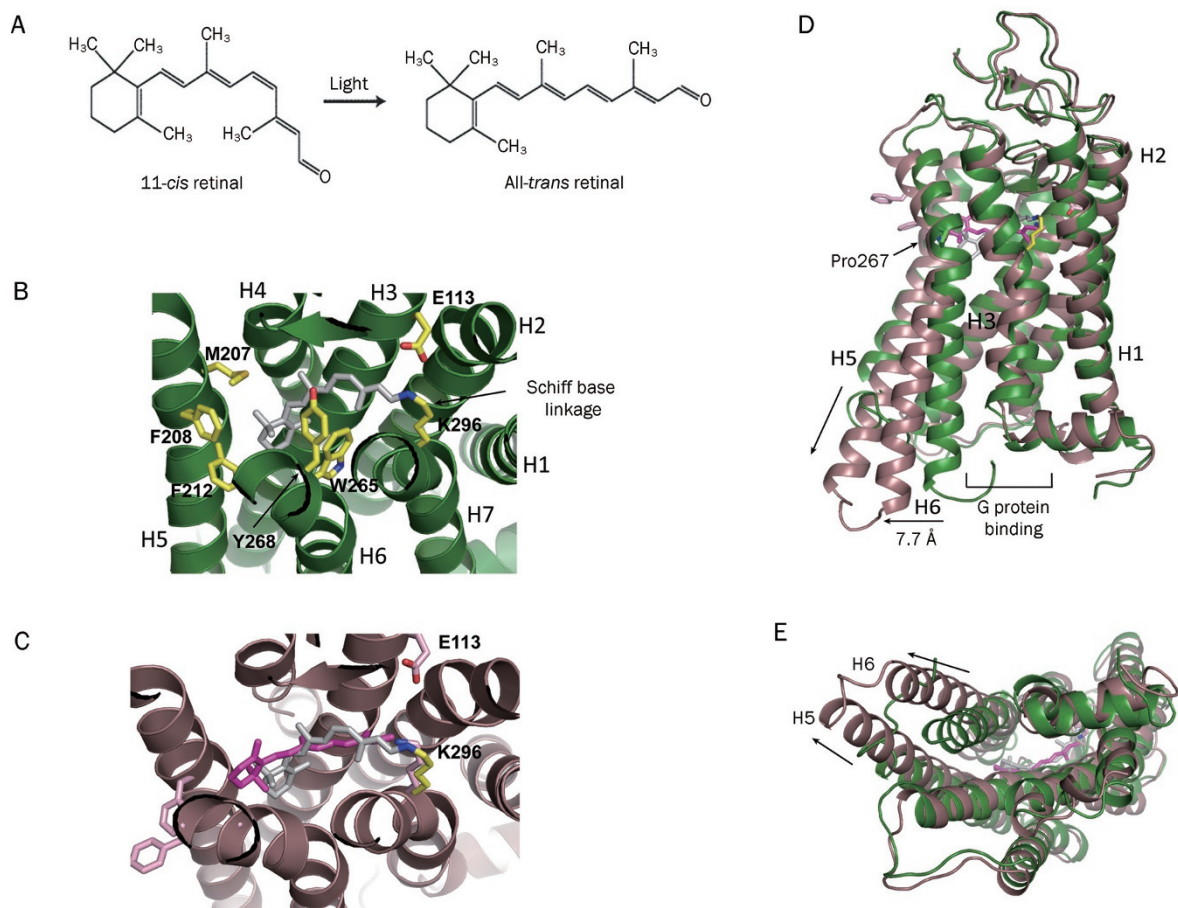
The loop region CL3 between helices 5 and 6 on the cytoplasmic side is flexible and largely disordered in ground-

state rhodopsin crystal structures, but is structured in most activated rhodopsin models<sup>[8-11]</sup> (Figure 1A and 1B). Upon activation, the majority of this loop region becomes part of the elongated helix 5 that provides more interface for G protein interaction.

### Ligand-induced conformational change and activation of rhodopsin

Retinal is one of the vitamin A compounds derived from carotenoids (Figure 2A). It is the photoactive moiety of rhodopsin that captures light and converts photons into chemical signals. Vision starts with the absorption of photons, and the photon-triggered isomerization of the retinal from the 11-*cis* to the all-*trans* state, followed by conformational changes in the 7TM domain of rhodopsin to accommodate the binding of G proteins, leading to the downstream signal transduction<sup>[28]</sup>.

The ligand binding pocket of rhodopsin, with a volume of about 352 Å<sup>3</sup>, is located on the luminal side of the receptor



**Figure 2.** Ligand binding and conformational changes in rhodopsin. (A) Chemical structures of 11-*cis*- and all-*trans*-retinal. (B) 11-*cis*-retinal (in gray) in the ligand binding pocket is associated with the surrounding residues of the protein moiety (green, PDB: 1F88). (C) Conformational changes in retinal and the protein moiety of rhodopsin upon photoactivation. The photoactivated all-*trans*-retinal (PDB: 3PQR) is magenta and the ground-state 11-*cis*-retinal (PDB: 1F88, gray) is superposed on the activated all-*trans*-retinal for comparison. The protein moiety of activated rhodopsin (PDB: 3PQR) is dark brown. (D) The key conformational changes in rhodopsin upon photoactivation are the outward tilting of the cytoplasmic end of helix 6 (indicated by the horizontal arrow), creating a crevice for G-protein binding, and the elongation of the cytoplasmic end of helix 5 (indicated by the vertical arrow) that provides more interface for G-protein interaction. Green shows the ground-state conformation (PDB: 1F88), and brown shows the activated conformation (PDB: 3PQR). (E) Bottom view of panel D.

(Figure 1A, 1C, and 2B). Prior to photoactivation, 11-*cis*-retinal in the ligand binding pocket is covalently bound to Lys296 of helix 7 through a Schiff base linkage. The Schiff base is protonated and the linkage is stabilized by charge delocalization over the  $\pi$ -electron system of the polyene backbone of the retinal chromophore, and by an electrostatic interaction with the carboxyl group of Glu113 of helix 3 (Figure 2B)<sup>[3, 4, 6]</sup>. The retinal in the ligand binding pocket is also stabilized by the surrounding hydrophobic residues including Met207, Phe208 and Phe212 from helix 5 and Trp265 and Tyr268 from helix 6 (Figure 2B). The Schiff base linkage and its electrostatic interaction are essential for maintaining the ground-state conformation of rhodopsin, and a mutation that alters either Lys296 or Glu113 results in constitutive activation of the receptor<sup>[29]</sup>.

Upon light absorption, the  $\pi$ -electron system of the retinal chromophore undergoes a  $\pi \rightarrow \pi^*$  transition that lowers the potential energy barrier for *cis-trans* isomerization. The isomerization changes the retinal from a bending *cis* configuration to a straight *trans* configuration. It flips the aldehyde group and the C<sub>20</sub> methyl group about 180° around the C<sub>11</sub>=C<sub>12</sub> double bond, and tilts the  $\beta$ -ionone group toward the space between helices 5 and 6 (Figure 2C). The photon-induced *cis-trans* transition in the retinal leads to a sterically strained excited-state rhodopsin that thermally decays through a series of intermediates, photorhodopsin, bathorhodopsin, lumirhodopsin, and metarhodopsin I, and forms the active-state metarhodopsin II<sup>[7, 8, 11, 30-32]</sup>. During the thermal relaxation, the protein moiety of rhodopsin undergoes a series of conformational changes that lead to the formation of a G protein binding site on the cytoplasmic side of the 7TM bundle<sup>[7-11]</sup> (Figure 2D and 2E). The major conformational changes are the bending of helix 6 at Pro267, and the eight-residue elongation of the C-terminus of helix 5, the addition coming from the CL3 loop (Figure 2D and 2E). The bending of helix 6 results in the cytoplasmic end of this helix tilting away from the transmembrane core, and creates an opening with a diameter of about 14 Å<sup>[8, 10, 11]</sup>. The cytoplasmic end of helix 6 in all-*trans*-retinal bound rhodopsin is about 7.7 Å away from that of the *cis*-retinal bound conformation. The Pro267 residue of helix 6 is highly conserved among GPCR family members, and it is believed that it serves as a hinge for the bending because it has no hydrogen bond between its backbone nitrogen atom and the carbonyl group of the residue one helical turn upstream. The elongation of the cytoplasmic side of helix 5 expands the interface for G protein interaction, and thus contributes the binding affinity of rhodopsin for the G protein transducin (Figure 2D and 2E).

### Rhodopsin-G protein interaction

The binding of the C-terminus of the  $\alpha$  subunit of transducin to rhodopsin was defined by the crystal structure of rhodopsin in complex with a synthetic peptide, GaCT (Ga C-terminus), derived from the C-terminus of helix  $\alpha 5$  of the  $\alpha$  subunit<sup>[8, 10, 11]</sup> (Figure 3A). The outward movement of helix 6 of rhodopsin breaks the ionic lock between Arg135 on helix 3 and Glu247 on helix 6, thus creating an opening that

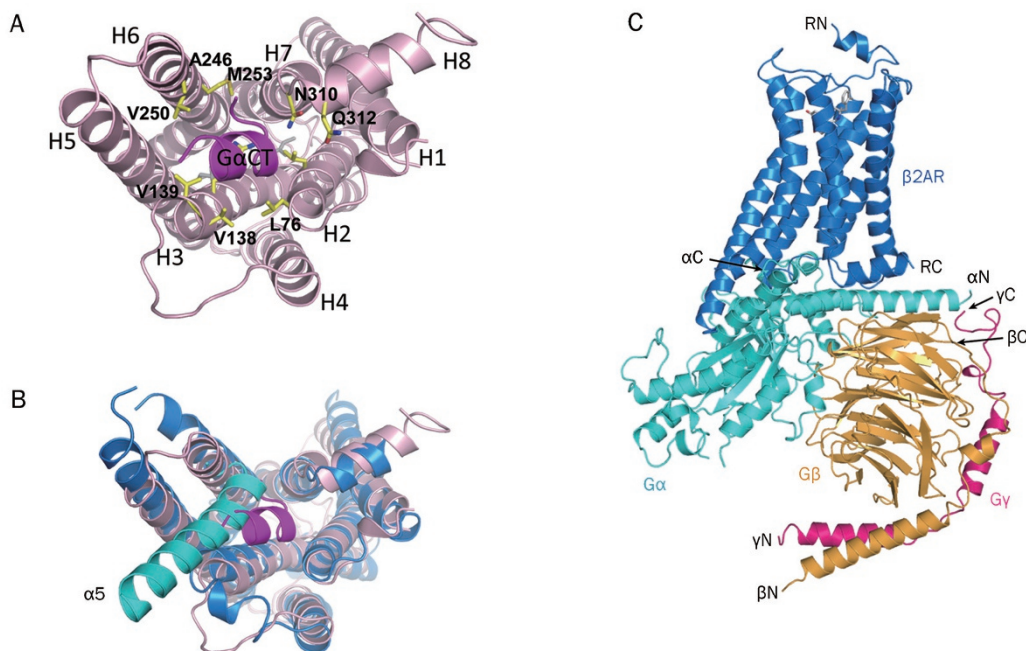
accommodates the C-terminal helix of the  $\alpha$  subunit of transducin. The binding of the  $\alpha$  peptide on the cytoplasmic side of rhodopsin is largely facilitated by two sets of interactions: a) hydrophobic interactions between the peptide residues Leu341, Leu344, Val347, Leu349, and Phe350, and the receptor residues Ala246, Val250, and Met253 on helix 6, Val138 and Val139, and the backbone of Arg135 on helix 3, and Leu72 and Leu76 on helix 2, and 6) polar interactions between the carbonyl groups of residues Gly348 and Lys345 of the peptide, and the residues Asn310 and Q312 of helix 8 (Figure 3A). The binding of the  $\alpha$  subunit of transducin to rhodopsin induces the exchange of GDP for GTP on the  $\alpha$  subunit, and this exchange triggers the dissociation of the GTP-bound  $\alpha$  subunit from the  $G\beta\gamma$  dimer and the receptor. Both the dissociated GTP-bound  $\alpha$  subunit and the  $\beta\gamma$  dimer of transducin then activate downstream signaling pathways<sup>[33]</sup>.

Comparison of the rhodopsin-G $\alpha$  peptide structure with the recently published crystal structure of the  $\beta 2$ -adrenergic receptor in complex with its trimeric G protein<sup>[21]</sup> revealed noteworthy differences. Helix  $\alpha 5$  of the intact  $\alpha$  subunit binds to the receptor in a position tilted about 38° toward helix 6 and 2 Å away from the bottom of the crevice relative to the position of the  $\alpha$  peptide in the active rhodopsin-peptide complex (Figure 3B and 3C). The intact G protein  $\alpha$  subunit does not fit deeply in the crevice of the receptor as the peptide does, probably because the whole  $\alpha$  subunit is much larger, and therefore is not able to fit in the deep crevice in the transmembrane bundle of the receptor. Also in the  $\beta 2$ -AR-G protein complex, helix 6 is bent outward about 26° more than that in the activated rhodopsin structure, and the opening of the crevice is larger than that of rhodopsin, to accommodate the intact C-terminal helix of the  $\alpha$  subunit in the  $\beta 2$ -AR-G protein complex (Figure 3B and 3C). Because the crystal structure of a rhodopsin-full length G protein complex is not available, we still do not have the whole picture of the interface of rhodopsin with the  $\alpha$  subunit as a full length protein in the context of intact trimeric transducin.

### Structural understanding of disease-related rhodopsin mutations

Rhodopsin is central to the process of vision. Mutations in rhodopsin are major causes of vision diseases or disorders. More than 120 point mutations have been identified in human gene of rhodopsin, many of which lead to vision diseases such as ADRP and congenital stationary night blindness (see <http://www.retina-international.org/sci-news/rhomut.htm> for most disease-linked mutations). Crystal structures have provided us molecular basis for understanding how mutations in rhodopsin influence the protein folding, stability and/or biological functions of the receptor.

Retinal is the photoactive chromophore in rhodopsin, and is the key to the entire light signal transduction. Crystal structures have revealed that 11-*cis*-retinal is covalently bound by Lys296 of helix 7 in ground state rhodopsin (Figure 2A and 2B). Mutations of this retinal binding residue, K296M and K296E, cause severe ADRP<sup>[34]</sup>. These mutants have been



**Figure 3.** Rhodopsin-G protein interface. (A) Rhodopsin in complex with a synthetic peptide derived from helix  $\alpha 5$  of the  $G\alpha$  subunit of transducin (PDB: 3PQR). The rhodopsin residues interacting with the  $G\alpha$  peptide are labeled. (B) Comparison of the binding mode of rhodopsin with the synthetic peptide and that of the  $\beta 2$ -adrenergic receptor with intact G protein (PDB: 3SN6). The  $\beta 2$ -adrenergic receptor is blue and the  $G\alpha$  subunit is cyan. For clarity, only helix  $\alpha 5$  of the  $G\alpha$  subunit is shown. (C) The whole-complex model of the  $\beta 2$ -adrenergic receptor with intact G protein (PDB: 3SN6). The  $\beta 2$ -adrenergic receptor is blue, the  $G\alpha$  subunit is cyan, the  $G\beta$  subunit is brown, and the  $G\gamma$  subunit is pink. RN, N-terminus of the receptor; RC, C-terminus of the receptor;  $\alpha N$ , N-terminus of the  $G\alpha$  subunit;  $\alpha C$ , C-terminus of the  $G\alpha$  subunit;  $\beta N$ , N-terminus of the  $G\beta$  subunit;  $\beta C$ , C-terminus of the  $G\beta$  subunit;  $\gamma N$ , N-terminus of the  $G\gamma$  subunit;  $\gamma C$ , C-terminus of the  $G\gamma$  subunit.

found to be folded properly, but constitutively activated<sup>[35]</sup>. Substitutions of other residues interacting with or at proximity of retinal can change retinal binding affinity of the receptor. It has been identified that mutations of those residues M44T<sup>[36]</sup>, G114D<sup>[37]</sup>, G114V<sup>[38]</sup>, L125R<sup>[39]</sup>, C167R<sup>[40]</sup>, Y178N<sup>[41]</sup>, Y178C<sup>[42]</sup>, E181K<sup>[40]</sup>, S186P<sup>[40]</sup>, S186W<sup>[43]</sup>, G188R<sup>[44]</sup>, G188E<sup>[45]</sup>, M207R<sup>[46]</sup>, M216R<sup>[47]</sup>, and M216K<sup>[48]</sup> (Lue216 in bovine), cause different levels of ADRP disease. Mutations of G90D<sup>[49]</sup> and A292E<sup>[50]</sup>, two other retinal binding residues, have been found in patients with congenital stationary night blindness.

The conserved residue Pro267 is at the kink in helix 6 and serves as a hinge for the bending of helix 6 to create the G protein binding site at cytoplasmic side of rhodopsin. Replacement of this residue with other non-proline residues affects the conformation of the G protein binding site of rhodopsin and the kinetics of transducin activation<sup>[51, 52]</sup>. Mutations of P267L and P267R have been reported to cause ADRP<sup>[41, 52, 53]</sup>.

Based on the crystal structure of rhodopsin in complex with peptide  $G\alpha CT$ <sup>[8, 10, 11]</sup>, the binding of the  $\alpha$  subunit of transducin to rhodopsin is facilitated by hydrophobic residues at the interface of the two proteins. Cys140 in CL2 is an important residue at rhodopsin side for G protein binding<sup>[54]</sup>. Mutation C140S that lowers the hydrophobicity of the residue has been found associated with ADRP<sup>[45]</sup>.

Mutations of residue Cys110 to Arg, Phe, or Tyr<sup>[37, 55]</sup>, or Cys187 to Tyr<sup>[56]</sup>, have been identified in ADRP patients. The

conserved residues Cys110 and Cys187 forms a disulfide bond that constrains EL2, the lid of the ligand binding pocket, and is required for the biological function of the receptor (Figure 1B)<sup>[57]</sup>. Mutations of those residues break the disulfide bond, thus affect the folding and biological function of the receptor.

GPCRs are membrane proteins and their interactions with the phospholipid bilayer membrane have great influence on their physiological functions<sup>[58]</sup>. Mutations at the membrane interaction interface of rhodopsin can change the affinity of rhodopsin to the phospholipid bilayers and influence the functions of the receptor. Lue46 is a hydrophobic residue at the membrane interaction interface of rhodopsin, and mutation of L46R largely affects the membrane-receptor interaction and causes severe ADRP<sup>[59]</sup>. Other disease-linked mutations at the receptor-membrane interface are F45L<sup>[60]</sup>, P53R<sup>[61]</sup>, and V209M<sup>[45]</sup>.

Some disease-causing mutations in rhodopsin occur at post-translational modification residues including glycosylation or phosphorylation sites. Glycosylation is important for correct folding and stability of most eukaryotic proteins. N-terminal glycosylation sites of rhodopsin are located at Asn2, Thr4, Asn15 and Thr17, and mutations of those residues, T4K<sup>[62]</sup>, N15S<sup>[63]</sup>, and T17M<sup>[60]</sup>, are responsible for ADRP. Rhodopsin phosphorylation is a key step of the regulatory mechanism of light signal transduction. Activated rhodopsin is phosphorylated by rhodopsin kinase at multiple sites in its C-terminal

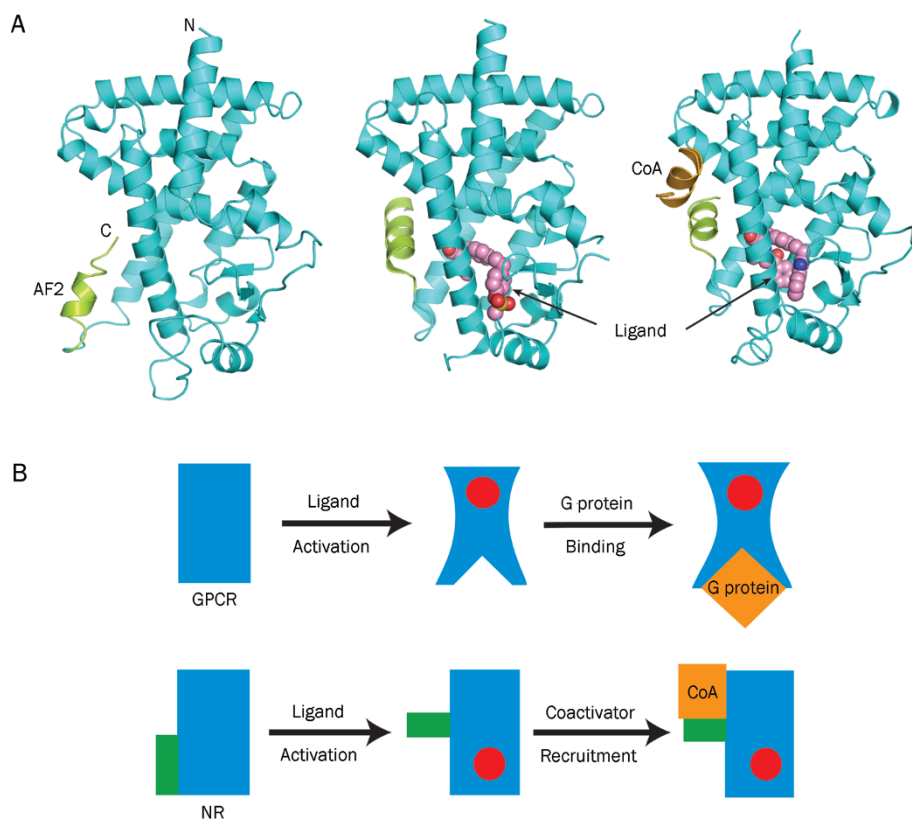
region, and the phosphorylation facilitates the association of arrestin that completely terminates light signal transduction. Mutations of a rhodopsin kinase binding residue Arg135 to Gly<sup>[45]</sup>, Trp<sup>[60]</sup>, Pro<sup>[64]</sup>, or Leu<sup>[60]</sup>, and the mutation of a phosphorylation site Thr342 to Met, which affect phosphorylation and rhodopsin-arrestin interaction, lead to ADRP<sup>[65]</sup>.

### Comparison of the activation of GPCRs and nuclear hormone receptors

Nuclear hormone receptors (NRs) and GPCRs are two different groups of protein receptors that sense extracellular signals and transduce those signals to the inside of cells. GPCRs transmit signals by coupling ligand-receptor interactions to G protein activation. Activated G proteins then trigger the downstream signaling cascades in the cytoplasm to regulate various biological events in the cells. In contrast, ligand binding converts NRs into transcriptional activators that directly bind target genes and stimulate their expression. A typical nuclear receptor ligand binding domain forms a conserved helical sandwich fold that harbors a ligand binding pocket adjacent to its C-terminal activation function helix AF2.

Ligand binding does not change the helical sandwich scaffold but induces the rearrangement of the receptor's AF2 helix and the formation of a coactivator binding cleft for the recruitment of transcriptional coactivators, which further facilitate the formation of transcription complexes and subsequent gene expression<sup>[66]</sup> (Figure 4A). In the cascade of NR activation, the conformation of the receptor core structure remains unchanged. That is a major difference from the activation of a GPCR, in which the core 7TM domain is rearranged upon ligand binding and receptor activation and the core domain rearrangement creates a crevice at the cytoplasmic side of the receptor for G protein interaction and signal transduction (Figure 4B)<sup>[10, 11, 21]</sup>.

While GPCRs and NRs are functionally comparable, the GPCR group has many more members than the NR family, and senses a wide variety of environmental signals. It is interesting that the GPCR group members, in spite of their highly diverse ligands, share a highly conserved 7TM core architecture for ligand binding and G protein interaction, whereas the NRs share a conserved three-layered helix bundle for ligand binding and coactivator recruitment. In summary, ligand



**Figure 4.** A comparison of the ligand-induced activation modes of GPCRs and nuclear receptors. (A) Ligand-induced rearrangement of the C-terminal AF2 helix and coactivator recruitment of a peroxisome proliferator-activated receptor ligand binding domain (PPAR LBD). At left is an apo PPAR LBD (PDB: 1PRG)<sup>[69]</sup>; middle, the LBD upon the ligand binding-induced conformational change in the AF2 helix and the formation of the coactivator binding site (PDB: 1I7G)<sup>[70]</sup>; at right, the LBD upon the subsequent coactivator recruitment (PDB: 1K7L)<sup>[71]</sup>. The LBD core structure is cyan; the AF2 helix, green; and the coactivator motif, brown. (B) Cartoon presentation showing that ligand activation induces conformational changes in the core domain of GPCRs but not in that of NRs. Blue are the core domains of both receptors; Red are the ligands; Orange are the G protein binding to the GPCR and the coactivator binding to the NR.

binding and activation in GPCRs involve a much more extensive rearrangement of the 7TM helical core than the helical sandwich of NRs.

### Rhodopsin as a molecular model for GPCR studies

During the long period before the second GPCR crystal structure was published in 2007<sup>[17, 18]</sup>, rhodopsin was the only GPCR crystal structure available, and it has been used extensively as a model for understanding the structural and functional characteristics of other GPCRs<sup>[67, 68]</sup>. Whereas more GPCR crystal structures have been published and the importance of the rhodopsin structure as a molecular template for modeling other GPCRs has been correspondingly diminished, rhodopsin still remains a prototype of the GPCR superfamily and a model system for all 7TM domain proteins.

All GPCR structures have a conserved transmembrane core domain followed by helix 8 on the cytoplasmic side. The root mean square deviation (RMSD) of the residues of the 7TM core structures between rhodopsin and other GPCRs whose structures have been solved are among 1.2 to 1.4 Å, indicating a close similarity and high level of conservation. The most significant structural differences between rhodopsin and other GPCRs are in the ligand binding pocket and the lid covering the pocket, the EL2 loop region. The EL2 of rhodopsin adopts a  $\beta$ -sheet fold, which tightly plugs into the entrance of the pocket, while the EL2 regions of other solved GPCR structures are loosely positioned above the ligand binding pocket and can be more easily opened for ligands to move in or out. This is consistent with the observation that rhodopsin is activated by the photon-triggered isomerization of retinal in the ligand binding pocket, which requires the ligand not only being bound but also being tightly hold in the pocket, while most other GPCRs are activated by simply binding to the ligands. Although the mechanism of activation differs, rhodopsin and other solved GPCR structures share conserved core residues that define the conformation of the seven transmembrane domain and the molecular basis of the conformational change upon ligand activation, G protein interaction, and downstream signal transduction.

### Acknowledgements

We thank D NADZIEJKA for editing the manuscript. This work was supported in part by the Jay and Betty Van Andel Foundation, and National Institute of Health grant GM087413 (H Eric XU).

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