Structure of rhodopsin and the superfamily of seven-helical receptors: the same and not the same

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The crystal structure of rhodopsin provides significant insights concerning structure/activity relationships in visual pigments and related G-protein-coupled receptors. The specific arrangement of seven-transmembrane helices is stabilized by a series of intermolecular interactions that appear to be conserved among Family A receptors. However, the potential for structural and functional diversity among members of the superfamily of seven-helical receptors presents a significant future challenge.

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Abbreviation
GPCR G-protein-coupled receptor

Introduction

The photoreceptors of the vertebrate visual system are rods and cones, associated respectively with nocturnal and diurnal vision. Rhodopsin, the visual pigment of the vertebrate rod cell, has been studied intensively for at least two decades because it is both fascinating and accessible [1•]. Rhodopsin comprises an opsin apoprotein and a retinylidene chromophore moiety linked via a protonated Schiff base to a conserved lysine residue within its membrane-embedded core (Figure 1). The chromophore is tuned to absorb a green photon, and, with exceptional speed and quantum efficiency, its C_{11}–C_{12} *cis* double bond photo-isomerizes to the *trans* conformation. Photolyzed rhodopsin switches to its active state, which catalyzes the uptake of GTP by the heterotrimeric G protein transducin. Rhodopsin can be defined functionally as a G-protein-coupled receptor (GPCR) because it couples to transducin during signal transduction.

Transducin and other G proteins have been crystallized and high-resolution structures are available for a number of their forms, both inactive and active [2,3], most recently in complex with downstream effectors and regulators [4,5]. However, the recent crystal structure of bovine rhodopsin is the first report of a high-resolution structure of a GPCR [6••].

What a relief that the first high-resolution structure of a seven-helical receptor confirmed the existence of seven transmembrane helices [6••]. Earlier projection density maps derived from cryoelectron microscopy of rhodopsin reconstituted into bilayers had provided a two-dimensional glimpse [7], which was incredibly useful in the design and interpretation of literally hundreds of experiments. The assignment of each of the projection densities to specific transmembrane segments, and thus their connectivities, had been based upon inference [8]. The exhaustive analysis of Baldwin *et al.* turned out to be correct, and the molecular graphics models of the transmembrane core of rhodopsin and other related GPCRs were likewise satisfactory. We did not know for sure until recently, however, that seven-helical receptors really do have seven transmembrane helical segments.

What else have we learned from the crystal structure of bovine rhodopsin and its subsequent refinements reported at about 0.28 nm resolution [6••,9•]? How much of what we learn about rhodopsin can be applied to other seven-helical receptors, which may make up the largest gene family in the human genome? These questions are addressed within this short review. The subtitle of this article — *The Same and Not the Same* — is taken from a book of the same title by Roald Hoffmann, who presents the concept of molecular mimicry in chemical structures, a concept that has been revisited in thinking about how seven-helical receptors perform a wide range of biological functions [10].

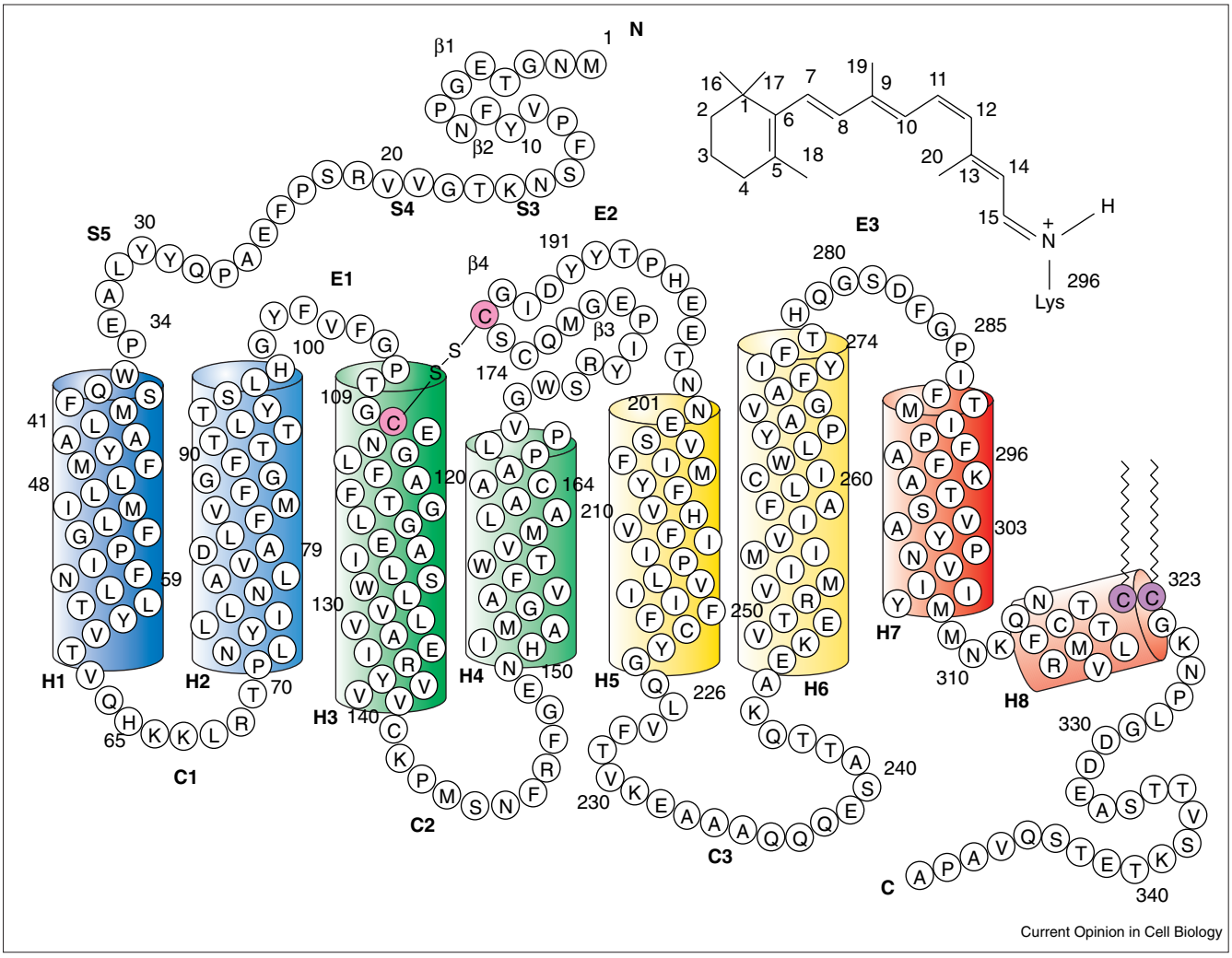
Rhodopsin structure

A ribbon diagram of rhodopsin is illustrated in Figure 2. Seven transmembrane segments (H1–H7), predominantly helical, are linked together sequentially by extracellular (E1, E2 and E3) and cytoplasmic loops (C1, C2 and C3). The amino-terminal tail is extracellular and the carboxy-terminal tail cytoplasmic. The retinylidene chromophore (magenta, Figure 2) is situated more toward the extracellular boundary of the plane of the putative membrane bilayer. The transmembrane helices are tilted to varying degrees with respect to the putative plane of the membrane bilayer.

One of the most striking features of the structure of rhodopsin is the presence and positioning of the β4 strand (Ser186–Cys187–Gly188–Ile189) within E2 (Figure 3). The β4 strand runs almost parallel along the length of the polyene chain of the chromophore, which seems to be held very firmly in place by multiple contacts. The β4 strand is stabilized by a highly conserved disulfide bond between Cys110 and Cys187. The E2 loop also provides potential contacts with the chromophore through Glu181 and Tyr191.

Although the role of the E2 loop in rhodopsin function is not yet known, it is tempting to speculate that the β4 strand provides a cap or lid which traps the 11-*cis*-retinylidene

Figure 1



The secondary structure of bovine rhodopsin. Amino acid residues are depicted in single letter code. The amino-terminal tail (N) and extracellular domain is toward the top and the carboxy-terminal tail (C) and cytoplasmic domain is shown toward the bottom. Transmembrane α-helical segments (H1–H7) and the cationic amphipathic helix H8 are shown as cylinders. An essential disulfide bond links Cys110 and Cys187 (pink). Cys322 and Cys323 (purple) are palmitoylated. Extracellular domains E1–E3, cytoplasmic loops C1–C3 and the amphipathic helix H8 are shown. Inset: structure of the 11-*cis*-retinylidene chromophore. Carbon atoms are numbered 1–20.

chromophore in its binding pocket. However, preliminary experiments in our laboratory suggest that its main role might be to regulate the stability of the active state of the receptor in which the all-*trans* chromophore acts, akin to an agonist ligand in other GPCRs. If this is the case, the E2 loop in other GPCRs also might be expected to provide contacts with agonist ligands.

The involvement of the extracellular region of rhodopsin in direct interactions with the chromophore was somewhat unexpected. Mutations of extracellular loop residues generally did not have effects on spectral properties of recombinant mutant pigments. However, the involvement of putative extracellular loop regions in rhodopsin was anticipated, in early studies by Khorana and co-workers [11,12], who proposed that the extracellular domain of rhodopsin might fold into a defined tertiary structure. In addition, several neuropeptide and peptide hormone receptors employ extracellular loops to bind their ligands. Both mutational mapping and photo-affinity ligand analogue cross-linking experiments suggested that the ligand-binding domains of these receptors might extend beyond a binding crevice comprising residues from the transmembrane helices alone. Perhaps the most detailed work has been carried out on the mammalian tachykinin receptors [13] and the thyrotropin-releasing hormone receptor [14]. Moreover, major interactions between ligand and extracellular loops have been reported in receptors not normally grouped with rhodopsin, including the vasopressin receptor [15], gonadotropin-releasing hormone receptor [16], formyl–Leu–Met–Phe receptor, and the complement factor C5A receptor [17].

What about the cytoplasmic surface of rhodopsin? In general, the cytoplasmic surface loops C2 and C3 and the carboxy-terminal tail are not as well resolved as other parts of the structure. Many residues in the C3 loop and carboxy-terminal tail, including backbone atoms, are not seen at all. Despite this limitation, one of the most striking features of the structure is the presence of an apparent cationic amphipathic helix (H8), which begins just beyond H7 and appears to lie roughly parallel to the plane of the membrane. H8 extends from Asn310 to Cys323 and is probably situated in the interfacial region of the bilayer [18] where it might remain anchored by palmitoyl ester cysteine residues, which can be seen well in the refined crystal structure [9•]. H8 might facilitate interaction of rhodopsin with a specific phospholipid molecule in the disc membrane of the rod outer segment [19]. Interestingly, a specific lipid–protein interaction changes upon rhodopsin activation [20], which also induces a change in the distribution of phosphatidylserine between the two leaflets of the disc membrane [21].

This region of rhodopsin also interacts with transducin directly [22,23]. The other interesting point that stems from the disposition of H8 is that it expands the apparent functional surface area of the receptor. Transducin might fit onto rhodopsin in a bimolecular complex [24], which must account for the extensive contacts known to occur between loops C2, C3 and H8 of rhodopsin and the carboxy-terminal tail of the α subunit of transducin; and possibly between the α5 helix of the α subunit of transducin [25] and the carboxy-terminal tail of the γ subunit of transducin (Figure 2).

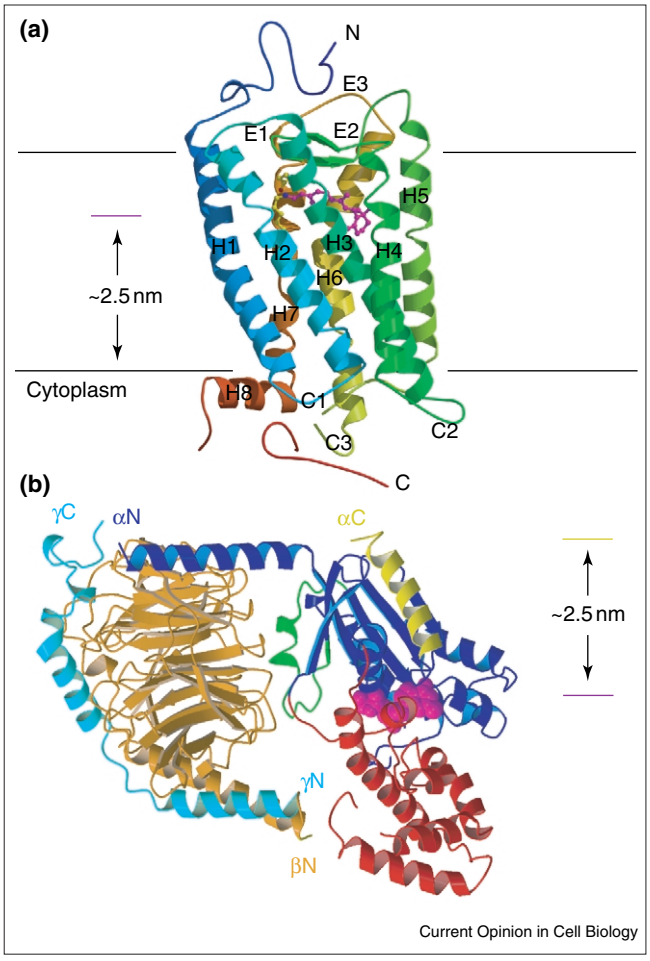
So, although it is somewhat ironic that one of the most striking findings in the first crystal structure of a seven-helical receptor is the presence of an eighth helix, H8 actually appears to cap or terminate the basic seven-helical receptor bundle. H8 may modulate rhodopsin–transducin interactions and essential rhodopsin–phospholipid interactions.

Activation of rhodopsin

Here it should be emphasized that the structure discussed above is that of the *inactive* state of rhodopsin, when it is bound to 11-*cis*-retinylidene chromophore. In the dark, rhodopsin does not bind appreciably to transducin; and further detailed analysis will require direct structural information about the active state of rhodopsin (R*), in addition to a structure of the R*–transducin complex. In the meantime, attempts to assemble a model of R* from structures of peptides using computation methods may be warranted [26]. One of the basic inherent questions is how the isomerization of the chromophore is transmitted to the guanine nucleotide-binding pocket of transducin over a distance of some 5.0 nm.

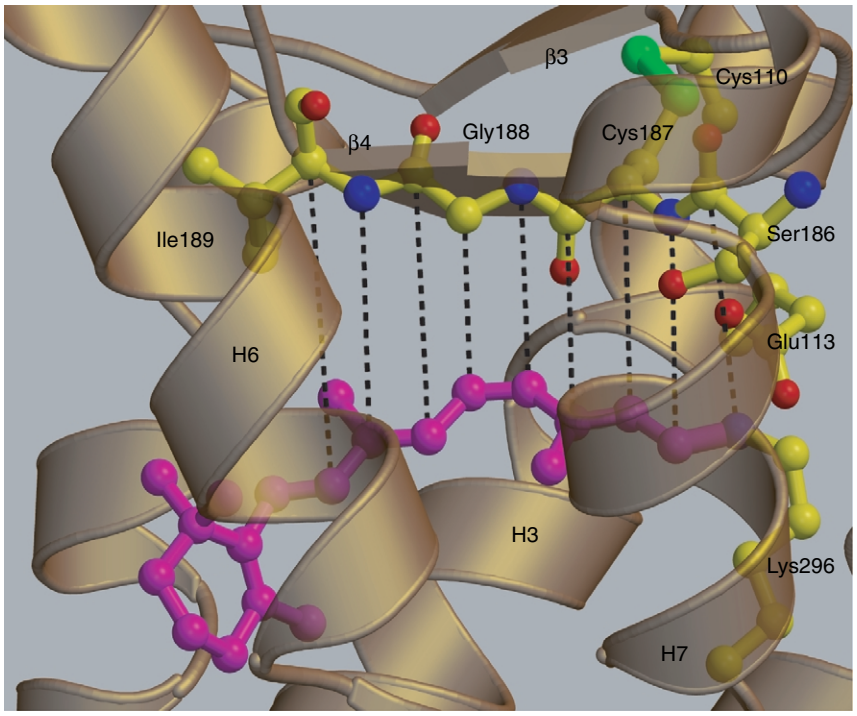
To function as a photoreceptor molecule, rhodopsin is highly specialized; but does it also share structural features with other receptors that bind diffusible ligands? Of course, GPCRs share a seven-helical pattern, but what

Figure 2



Interactions of rhodopsin with transducin at the cytoplasmic surface of the rod outer segment disc membrane. **(a)** Ribbon diagram of rhodopsin prepared from the crystal structure coordinates of the A chain of the published crystal structure coordinates (PDB 1f88). The amino terminus (N) and extracellular surface are shown toward the top of the figure and the carboxyl terminus (C) and intracellular surface toward the bottom. Seven transmembrane segments (H1–H7), characteristic of GPCRs, are shown. The retinylidene chromophore (magenta) is shown in a ball-and-stick format. The crystal structure does not resolve a small segment of the C3 loop linking H5 and H6 or a longer segment of the carboxy-terminal tail distal to H8. Asparagine-linked carbohydrates and palmitoyl groups revealed in the structure are not shown for clarity. The Schiff base linkage of the chromophore lies approximately 2.5 nm from the cytoplasmic surface of the membrane bilayer. **(b)** The GDP-bound form of transducin (PDB 1got), with the surface that presumably interacts with rhodopsin facing upward. The Ras-like domain of the α subunit (blue) is seen above the GDP-binding pocket and the helical domain below (red). The α5 helix is shown in yellow and the Switch II region green. The β subunit (gold) and γ subunit (turquoise) are to the left. The bound GDP (magenta) may be up to 2.5 nm from the surface of transducin. The amino and carboxyl termini of each subunit are labeled. Structures thought to interact with rhodopsin or the membrane, or both – including the amino and carboxyl termini of the α subunit and the carboxyl terminus of the γ subunit – cluster on a common surface of transducin. The relative orientation of the cytoplasmic surface of rhodopsin and the rhodopsin-binding surface of transducin is arbitrary. Upon formation of the rhodopsin–transducin complex, the chromophore-binding pocket of rhodopsin becomes allosterically coupled to the nucleotide-binding pocket of transducin, which is approximately 5.0 nm away. The structure of the active state of rhodopsin (R*), or of the R*–transducin complex, has not yet been determined.

Figure 3



Interactions between the retinylidene chromophore and the β4 strand of the second extracellular loop of rhodopsin. The chromophore-binding pocket is shown from within the plane of the membrane bilayer. The extracellular surface is toward the top and intracellular surface is toward the bottom. Transmembrane segments are shown in ribbon format and the chromophore and selected amino acid residues are shown in ball-and-stick format and are labeled. The chromophore is situated such that its proximal end (approximately C_9 to C_{15}) lies along the β4 strand, and its distal end (approximately C_9 to the cyclohexenyl ring) lies along H3.

about the precise orientation and disposition of the helices? And what about other high-resolution structural features observed for the first time in the rhodopsin crystal structure? The easy answer to both questions is that rhodopsin should be expected to provide useful information about large numbers of GPCRs. However, members of the superfamily of seven-helical receptors perform diverse functions. They bind diverse ligands, couple to many G protein classes, and also signal through G-protein-independent pathways [27].

The seven-helical pattern is also found in evolutionary extremes from archaebacteria to humans. Although the archaebacterial light-driven proton pump bacteriorhodopsin and the visual pigment rhodopsin employ different isomeric forms of the same retinylidene chromophore and share the seven-helical pattern, there is no evidence that these two proteins are orthologs. So, what does the structure of rhodopsin teach us about bacteriorhodopsin and related halorhodopsins? This question remains to be answered fully [9•,28]. However, a more incisive question might be to ask what the earlier high-resolution structures of bacteriorhodopsin [29,30] taught us about rhodopsin. High-resolution structures of bacteriorhodopsin have provided significant insights toward understanding retinylidene photochemistry, coupled intramolecular protein transfer reactions, and membrane protein electrostatics. But they probably do not teach us how a hormone binds to its receptor.

The molecular mechanism of receptor activation may be a different story. Studies of light-dependent conformational changes in bacteriorhodopsin preceded those in rhodopsin. The approach of site-directed spin labeling was used, by Hubbell, Khorana and others, to study bacteriorhodopsin and subsequently rhodopsin. The development of the concept of the helix movement model of receptor activation, as applied to rhodopsin and other Family A GPCRs [31–34], was inspired by the work of Subramanium, Henderson, Osterhelt and co-workers, who obtained the first structures of photocycle intermediates of bacteriorhodopsin [35–37]. Here again, even bacteriorhodopsin and rhodopsin are the same and not the same — helices appear to move but the movements are probably different.

The helix movement model of GPCR activation was proposed on the basis of the study of rhodopsin [31–33]. The crystal structure of rhodopsin seems to provide a structural basis for the coupling of chromophore isomerization to movement of helices H3, H6 and H7. It also provides insights for understanding constitutive receptor activity. It is likely that the helix movement mechanism of receptor activation will apply to most, if not all, GPCRs. The implications of the rhodopsin structure for understanding receptor activation were reviewed recently [1•,38].

Classification of seven-transmembrane GPCRs

Among all putative seven-helical receptors, there is no conserved structural feature beyond their shared

membrane topology. At least two recent developments suggest that functional classifications of apparently related receptors may not prove reliable. First, GPCRs do not segregate into groups based on the G proteins to which they couple and many receptors can signal via G-protein-independent mechanisms [27]. Second, genomic analysis shows that seven-helical receptors are widely divergent in sequence even within the same organism. Genomic analysis and bioinformatic approaches should allow a more objective and comprehensive strategy to classify seven-helical receptors [39,40].

Classification of seven-transmembrane receptors is complicated because the seven-helical motif is found widely among eukaryotes and even in archaebacteria. The database is huge, and growing. From mammalian genomes alone, about 4300 putative seven-transmembrane GPCRs have been posted on various electronic databases [41]. Early attempts at classification generally relied on alignments of subsets of sequences derived from the putative transmembrane domains. This strategy was required because the extracellular amino-terminal tails, cytoplasmic loops and carboxy-terminal tails of the receptors vary dramatically in length. For example, the length of the amino-terminal tail in different receptors ranges from seven to around 2500 amino acid residues.

Mammalian seven-transmembrane GPCRs tend to fall into one of three families, termed A, B, and C. (See Gether [42] for a more detailed discussion of GPCR classification.) Family A is the rhodopsin-like or adrenergic-receptor-like family; Family B is the glucagon-receptor-like or secretin-receptor-like receptor family; and Family C is the metabotropic glutamate neurotransmitter receptor family. Upon cursory inspection, generally the classifications are reassuring, because structural features other than the alignment scores of the transmembrane segments tend to cluster. For example, Family A receptors display relatively short amino-terminal tails and have at least one highly conserved amino acid residue in each transmembrane helix. Family B receptors display longer amino-terminal tails with a set of six conserved cysteine residues. Although a few transmembrane helical residues are conserved, they are not the same as those conserved among the Family A receptors. Family B receptors employ multiple extracellular contact sites to bind specific peptide ligands [43–45]. Family C receptors generally display much longer amino-terminal tails (500–600 residues) than both Family A and B receptors, but very few conserved transmembrane helix residues, although several residues are conserved on their cytoplasmic surface loops. The domain comprising the amino-terminal tail binds the ligand in Family C receptors.

The only possible structural element that might be conserved among all seven-transmembrane receptors is a disulfide bond, which links H3 with loop E2 and corresponds to the Cys110–Cys187 disulfide bond in rhodopsin.

Family A receptors can be divided further into six groups. Vertebrate opsins are grouped with receptors for cholecystokinin, endothelin, tachykinin, neuropeptide Y, thyrotropin-releasing hormone, neurotensin, bombesin, and growth hormone secretogogues. It is likely that the structure of rhodopsin will provide the most detailed insights regarding structure/activity relationships in these receptors. Although the amine receptors fall into a different group within Family A, they have been studied intensively, using a number of approaches. Ballesteros *et al.* [46•] recently carried out the first detailed critical analysis of the rhodopsin structure as it applies to amine receptors, in particular the dopamine D_2 receptor. They concluded that the structures of rhodopsin and amine receptors might be very similar, although some structural divergence is likely, as exemplified by the presence of a proline residue in H2 in all Family A receptors, but at different positions. Structural divergence in the ligand-binding pocket may be introduced by helix kinks at proline residues or helix bending or twisting at cysteine, serine or threonine residues. These minor structural changes in helix orientation, as well as variation in amino acid sidechains that do not contribute to intramolecular interactions, might account for the structural differences, however subtle, required for Family A receptors to bind a structurally diverse collection of ligands. Additional detailed analyses will be required to exploit the information provided by the crystal structure of rhodopsin.

Structural information has also been reported recently on the extracellular amino-terminal domains of other seven-helical receptors. The recently described crystal structure of the extracellular amino-terminal domain of a metabotropic glutamate receptor indicates that ligand-mediated receptor dimerization is most likely to play a role in its activation mechanism [47•]. Other Family C GPCRs include the γ-aminobutyric acid (GABA) receptor, the calcium-sensing receptor and some pheromone receptors. The crystal structure of the amino-terminal cysteine-rich domain of the mouse Frizzled 8 protein, which binds Wnt, was also reported recently [48]. Frizzled receptors probably constitute a distinct group of receptors that fall broadly within Family B [49].

Conclusions

Rhodopsin displays several unique and fascinating properties that allow it to function as a visual photoreceptor. It also serves as a prototype of the largest family of membrane receptors in the human genome. The recent report of the crystal structure of bovine rhodopsin [6••] provides a unique opportunity to address questions related to the structural basis of rhodopsin function in the vertebrate visual transduction cascade. The structure reveals the precise orientation of seven-transmembrane helices, a unique extracellular loop structure, and an amphipathic cationic helix within the carboxy-terminal tail. The rhodopsin structure seems to provide a satisfactory template for other Family A GPCRs, as demonstrated in a

critical evaluation of dopamine D_2 receptor structure/activity and modeling studies. However, structural information from rhodopsin may provide less information about other divergent seven-helical receptors, although several fundamental properties — such as the molecular mechanism of receptor activation — may be conserved.

Several important questions will require additional structural information at higher resolution: what is the precise structural basis of ligand specificity in a particular receptor, and how can the basic seven-helical structure be tuned to bind such a large and chemically diverse spectrum of ligands? What is the precise molecular mechanism of ligand-dependent activation and of constitutive activation? What is the role of protein–phospholipid interactions in receptor activation? What are the G protein contact sites on the receptor, and what is the atomic pathway of signal transduction from the ligand-binding pocket on the receptor to the nucleotide-binding pocket of the G protein? What is the structural basis of G-protein-independent signaling displayed by many seven-helical receptors? And finally, what is the role of receptor dimerization in GPCR function? The structure described herein provides a significant step toward answering these questions.

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