A G protein-coupled receptor at work: the rhodopsin model

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G protein-coupled receptors (GPCRs) are ubiquitous signal transducers in cell membranes, as well as important drug targets. Interaction with extracellular agonists turns the seven transmembrane helix (7TM) scaffold of a GPCR into a catalyst for GDP and GTP exchange in heterotrimeric Gαβγ proteins. Activation of the model GPCR, rhodopsin, is triggered by photoisomerization of its retinal ligand. From the augmentation of biochemical and biophysical studies by recent high-resolution 3D structures, its activation intermediates can now be interpreted as the stepwise engagement of protein domains. Rearrangement of TM5–TM6 opens a crevice at the cytoplasmic side of the receptor into which the C terminus of the Gα subunit can bind. The Gα C-terminal helix is used as a transmission rod to the nucleotide binding site. The mechanism relies on dynamic interactions between conserved residues and could therefore be common to other GPCRs.

Function of G protein-coupled receptors

To transmit extracellular signals into living cells, nature has evolved membrane-spanning receptor proteins that connect the extracellular environment to the cell interior. G protein-coupled receptors (GPCRs) are the largest family of such receptors, with approximately 800 different members in humans [1]. Environmental and physiological signals such as hormones, neurotransmitters, odorants, gustatory substances and light are received by these receptors, which are also the targets for many drugs, including β blockers and antihistamines. GPCRs are thought to respond to the binding of extracellular ligands with a conformational change in the ligand binding site [2], which extends via their seven transmembrane helix (7TM) scaffold into the intracellular domain [3,4]. The active cytoplasmic receptor surface enables binding of cognate heterotrimeric G proteins (Gαβγ) and catalysis of GDP→GTP exchange in the Gα subunit. The GTP-bound G protein then decouples from the receptor and dissociates into Gα–GTP and Gβγ subunits. Both Gα–GTP and Gβγ subunits can elicit cell-specific responses via particular effector proteins and regulation of intracellular second messenger levels. Hydrolysis of GTP to GDP within Gα and subsequent dissociation of Gα–GDP with Gβγ completes the G protein cycle. In concert with shut-off of the activated receptor by interactions with receptor kinase and arrestin, an enzymatic feedback sets the second messenger concentration back to its original level. The catalytic nature of receptor–G protein interaction results in the generation of many copies of the GTP-bound activated G protein, establishing a first step in signal amplification and regulation. The three steps of reception, amplification and feedback constitute a signaling module that might be common to signal transduction systems in general [5].

This review focuses on the first key step in G protein-mediated signal transduction, in which the signal crosses the membrane and the activated receptor couples to the G protein. New insight into this process comes from rhodopsin, the photoreceptor of the retinal rod cell and the eponym of the largest class of GPCRs, the rhodopsin-like GPCRs with ~670 members in the human genome [1]. Rhodopsin consists of the opsin apoprotein and the covalently bound messenger levels. Hydrolysis of GTP to GDP within Gα and subsequent dissociation of Gα–GDP with Gβγ completes the G protein cycle. In concert with shut-off of the activated receptor by interactions with receptor kinase and arrestin, an enzymatic feedback sets the second messenger concentration back to its original level. The catalytic nature of receptor–G protein interaction results in the generation of many copies of the GTP-bound activated G protein, establishing a first step in signal amplification and regulation. The three steps of reception, amplification and feedback constitute a signaling module that might be common to signal transduction systems in general [5].

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Box 1. GPCR toolbox

A toolbox of functional elements utilized by GPCRs can be derived from data available on their sequence, function and structure (Figure I). The two highly conserved E(D)R3.50Y and NP7.50xxY(x)5,6F motifs [28] are part of functional microdomains that constrain the 7TM helix bundle in the compact inactive conformation and undergo structural changes on receptor activation [8–32,87]. In rhodopsin, Arg1353.50 of the E(D)R3.50Y motif on TM3 plays a key role in tethering TM3 to TM6. Arg1353.50 forms a hydrogen bonding network with Glu1343.49 on TM3 and Glu2476.30 on TM6; in rhodopsin the network also includes Thr2516.34. A functional network around Arg1353.50—known as the TM3–TM6 ionic lock—was proposed for class A GPCRs (rhodopsin family) [25,26] and might also be utilized by class C GPCRs (glutamate family) [95]. The NPxxY(x)5,6F motif on TM7–H8 is functionally bipartite because it constrains TM7 with TM1 and TM2 and also constrains TM7 with H8. The latter is enabled by an electrostatic interaction between the aromatic side chains of Tyr3067.53 on TM7 and Phe3137.60 on H8, yielding the TM7–H8 microdomain. Asn3027.49 at the N-terminal part of the NPxxY(x)5,6F motif and the two residues Ala2997.46 and Ser2987.45 in the preceding helix turn are involved in a hydrogen bonding network with Asn551.50 on TM1 and Asp832.50 on TM2 (TM1–TM2–TM7 network).

TM6 is interrupted due to a proline kink induced by Pro2676.50, which is part of the conserved CWxP6.50 motif. The CWxP6.50 motif is the basis of the rotamer toggle switch hypothesis [27]. In GPCR activation, the rotamer states of Trp at position 6.48 and Phe at position 6.52 are predicted to be coupled and to change during receptor activation, thus providing a link between the CWxP6.50 motif and motion of the cytoplasmic part of TM6 [27]. In the case of rhodopsin, the phenyl side chain of Phe6.52 is missing but the β-ionone ring of retinal is in direct contact with Trp2656.48. It is assumed that the β-ionone ring, together with Ala2896.52, plays the role of Phe6.52 [76]. The Y5.58(x)5.56(R)x(R)5.66 motif on TM5 contains conserved residues (Tyr2235.58 and Lys2315.66) used as microswitches in stabilizing the active receptor state (Box 2).

In rhodopsin, both ends of the retinal ligand are stabilized by nearby residues. Glu1133.28 on TM3 and Glu181 in loop E2 form the complex counterion of the protonated retinal Schiff base. The β-ionone ring of retinal is surrounded by His2115.46, Trp1263.41 and Glu1223.37 (forming the TM3–TM5 hydrogen bonding network) and Trp2656.48 of the CWxP6.50 motif. On light-induced cis–trans isomerization of retinal, the TM3–TM5 network is weakened and the dominant contribution to the counterion shifts from Glu1133.28 on TM3 to Glu181.

Figure I. Conserved residues and functional microdomains in GPCRs. (a) Rhodopsin (PDB accession 1GZM) with bound inverse agonist 11-cis-retinal is shown as a representative GPCR. The basic GPCR architecture consists of 7TM helices, linked by cytoplasmic (C1–C3, top) and extracellular (E1–E3, bottom) loops. The cytoplasmic helix H8 follows directly after TM7 and is frequently terminated by one or two palmitoylated Cys residues (Cys322 and Cys323 in rhodopsin). Oligosaccharide chains are often attached at the N terminus (Asn2 and Asn15 in rhodopsin). A conserved disulfide bridge constrains the extracellular end of TM3 and the middle of loop E2 (Cys1103.25 and Cys187 in rhodopsin). The most conserved residue in each TM helix is shown in blue. According to Ballesteros-Weinstein numbering, these residues are designated x.50, where x is the TM helix number, and other residues are designated relative to the reference residue on each helix [94]. Shaded areas and color-coded residues in (a) correspond to the Logos color-coded sequence. For generation of the Logos sequence (http://weblogo.berkeley.edu), GPCR sequences covering the α-group of rhodopsin-like GPCRs [1] for which crystal structures are available [19] were used. (b) Rhodopsin-specific microdomains (rhodopsin numbering). These include the TM3–TM5 network (blue shading) with Glu1223.37, Trp1263.41 on TM3 and His2115.46 on TM5, and the Schiff base network (purple shading) with the protonated Schiff base (PSB) linkage between Lys2976.43 on TM7 and the inverse agonist 11-cis-retinal. All figures were prepared using PyMol software (www.pymol.org).
chromophore 11-cis-retinal, which acts in the dark as a strong inverse agonist and constrains rhodopsin in the inactive conformation (Box 1) [6–8]. Absorption of a photon causes cis—trans isomerization of the retinal and generates the agonist all-trans-retinal in situ, which activates the receptor. Although rhodopsin responds to light and not to diffusible ligands, it bears close similarities to ligand-activated GPCRs. The photofunctional core, which alone distinguishes rhodopsin from other GPCRs, is responsible for the fast irreversible reactions linked to the photoisomerization event. However, the conversions that render the receptor competent to interact with its cognate G protein occur subsequently on a slower time scale. These so-called Meta states are in G protein-dependent equilibrium and are analogous to the high- and low-affinity ligand binding states in other GPCRs [9,10]. Eventually, after the activation event, the conversions that render the reactivated GPCRs. The photofunctional core, which alone dis- 
solved at 2.9 and 3.2 Å, respectively (Box 2). The G protein network including Glu1223.37, Trp1263.41 and His2115.46 motif in TM5, featuring two residues (Tyr2235.58 and Lys2315.66 in rhodopsin) that in the active receptor state undergo specific interactions with residues released from the TM3–TM6 ionic lock (Box 2). Also highly conserved are proline residues Pro2155.50, Pro2676.50 and Pro3037.50, which induce kinks in TMs, as well as a disulfide bridge between Cys1104.25 on TM3 and Cys187 of the extracellular loop E2 connecting TM4 and TM5 [6,28].

**Rhodopsin-specific microdomains**

In rhodopsin, only one pair of ligands is natively present, 11-cis-retinal and all-trans-retinal, which act as inverse agonist or agonist, respectively, with light energy transforming the inverse agonist into an agonist. As a light-operated switch for a single G protein pathway, rhodopsin lacks the complexity of diffusible ligand-activated GPCRs that can couple to different pathways [29,30]. In concert with the covalent Schiff base linkage between retinal and Lys2967.43 on TM7, the inactive ground-state stability of rhodopsin is ensured by two additional constraints. One is the Schiff base network, a hydrogen bonding network around the chromophore. The network includes residues in extracellular loop E2 and water molecules, as well as the protonated Schiff base, which forms a salt bridge with Glu1133.26 of its complex counterion [7,9,31]. The second constraint tethers TM3 and TM5 by a hydrogen bonding network including Glu1223.37, Trp1263.41 and His2115.46 (Box 1) [8,32]. These residues can be expected to show sensitivity to movements of the β-ionone ring, with which Glu1223.37 is in direct contact. The β-ionone ring is further restricted by hydrophobic residues Phe2125.47, Phe2616.44 and Trp2656.48 and is thus in contact with TM6 [7].

**Active conformation of the cytoplasmic domain in the opsin apoprotein**

A year ago, the structures of ligand-free opsin [33], alone and in complex with a short G protein fragment [34], were solved at 2.9 and 3.2 Å, respectively (Box 2). The G protein fragment was prepared as an 11-mer peptide derived from the key receptor binding site on the C terminus of the Gz subunit, which is known to be mandatory for signal transfer from the receptor to the nucleotide binding site of Gz [35–38]. The overall structures of opsin and opsin in complex with the Gz-derived peptide are similar to bovine...
rhodopsin in its 11-cis-retinal-bound ground state, the first GPCR structure solved in 2000 [6]. The smallest differences are observed in the extracellular domain. There the extracellular loops and the N terminus form the compact retinal plug structure [39,40], which remains intact although no ligand is present in the retinal binding pocket of opsin. A core structure composed of TM1–TM6 is also virtually unchanged. Relative to rhodopsin, prominent structural changes are observed for TM5 and TM6. The cytoplasmic part of TM6 is tilted outwards from the helix bundle by 6–7 Å. TM5 is extended by 1.5–2.5 helix turns (depending on the reference structure and corresponding crystal form) and moves 2–3 Å towards TM6. Due to this helix rearrangement a deep crevice is formed at the cytoplasmic side of the receptor, which contains conserved Arg1353.50 of the E(D)R3.49Y motif at its floor. This opening, or metaphorically speaking blossoming [41], of the receptor enables binding of the Ga C terminus, as observed in co-crystals of opsin and the corresponding Go fragment. This provides one of several lines of evidence for identification of the two available opsin structures as an active opsin state (denoted as Ops*), in which the cytoplasmic domain is in the active G protein-binding conformation (Box 2) [34]. In Ops*, the residues of the TM3–TM6 ionic lock and NPxxY(x)5,6F microdomains form new interactions. Interactions occur with residues of the Y(x)5.58(K(R))5.66 motif in TM5, where Tyr2235.58 and Lys2315.66 stabilize the Ops* conformation by tethering TM5-TM3 (linkage between

**Box 2. Crystal structures of inactive and active GPCR conformations**

The identical interaction of the Go-derived peptide with opsin and light-activated rhodopsin in native disk membranes [99,100]. In addition, X-ray and NMR analysis yielded the same angle of the Go-derived peptide helix relative to the membrane normal [100]. EPR experiments also indicated that the Go-derived peptide binds to the open receptor [59]. The interaction between activated GPCR and G protein [87,96], Glu1343.49 and Arg1353.50 of the E(D)R3.49Y motif also play a pivotal role in this regard [97,98]. The bound Go-derived peptide forms a near ideal α-helix with a C-terminal open reverse turn (C cap) [34]. Although the peptide mainly forms hydrophobic contacts to the inner surface of TM5 and TM6, its recognition by the receptor seems to be more driven by geometry and less by specific side chain contacts. Two backbone carbonyl oxygens of the C cap of the Go-derived peptide form a hydrogen bonding network to Arg1353.50 and Gln3127.59, respectively (Figure Ic). Importantly, the structure of the peptide in the crystal is virtually identical to the structures of two homologous peptides as determined by NMR spectroscopy in their conformation bound to light-activated rhodopsin in native disk membranes [99,100]. In addition, X-ray and NMR analysis yielded the same angle of the Go-derived peptide helix relative to the membrane normal [100]. EPR experiments also indicated that the Go-derived peptide binds to the open receptor [59]. The identical interaction of the Go-derived peptide with opsin and light-activated rhodopsin led us to identify both opsin structures as active opsin (Ops*) conformations (Figure II).

**Figure I. Crystal structures of inactive rhodopsin and active opsin (Ops*) conformations.** Cytoplasmic and lateral views of (a) inactive rhodopsin ground state (shown in green, PDB accession 1U19) [7] and (b) ligand-free opsin (shown in orange, PDB accession 3CAP) [33]. Residues of the TM3–TM6 ionic lock (Glu1343.49, Arg1353.50, Glu2476.30), the Y(x)5.58(K(R))5.66 motif (Tyr2235.58, Lys2315.66) and the TM7–H8 microdomain (Tyr3067.53, Phe3137.60) are shown as stick models. Side chain movements on receptor activation are indicated by black arrows, whereas helix movement is indicated by a yellow arrow. (c) Lateral view and close-up of the cytoplasmic domain of opsin in complex with a C-terminal peptide derived from the last 11 residues of the transducin Go subunit (shown in blue and magenta, respectively; Go peptide residues are labeled in italics; PDB accession 3DQ8) [34]. The peptide binds into the cytoplasmic crevice of opsin opened by movement of TM5 and TM6. The C cap of the peptide helical structure is involved in a hydrogen bonding network with Arg1353.50 in the E(D)R3.49Y and Gin3127.59 in the NPxxY(x)5,6F motifs, respectively.
Tyr223\textsuperscript{5.58} and Arg135\textsuperscript{3.50}) and TM5–TM6 (linkage between Lys231\textsuperscript{5.66} and Glu247\textsuperscript{4.30}), respectively. Also in Ops*, Tyr306\textsuperscript{7.53} shows a rotamer change and release from Phe313\textsuperscript{7.60} on H8. Thus, Arg135\textsuperscript{3.50} and Tyr306\textsuperscript{7.53} are microswitches that have different roles and interactions in the inactive and active state, respectively (Box 2) [33,34].

Conformation and gating of the retinal binding domain in opsin

Profound structural rearrangements in Ops* compared to 11-cis-retinal-bound rhodopsin are also observed in the retinal binding domain [33] (Box 2). Changes along the retinal binding pocket occur in regions adjacent to the retinal attachment site Lys296\textsuperscript{7.43}, the C19 methyl group in the middle and the \(\beta\)-ionone ring at the end of retinal. The side chain of Lys296\textsuperscript{7.43} seems to be flexible, as suggested by the lack of a defined electron density. A salt bridge between Lys296\textsuperscript{7.43} and Glu113\textsuperscript{3.28} as in rhodopsin is not evident. Instead, Glu181 in loop E2 is located closer to Lys296\textsuperscript{7.43}, indicating a shift of the interaction from Glu113\textsuperscript{3.28} to Glu181. This is reminiscent of the light-induced counterion shift in the metarhodopsin states [31,42,43].

The two bulky hydrophobic residues Phe261\textsuperscript{6.44} and Trp265\textsuperscript{6.48} on TM6 are moved due to the outward tilt of TM6. The Trp265\textsuperscript{6.48} side chain is shifted towards the position that is occupied by the retinal \(\beta\)-ionone ring in rhodopsin. Tentatively, it is possible that part of the space occupied by retinal in rhodopsin might be used by water in Ops*, as indicated by weak but at present uninterpretable electron density in the retinal binding pocket. Interestingly, in the Ops* structure with bound \(\text{Go}\)-derived peptide [34], the presence of the peptide seems to have a distant effect on the retinal attachment site. In the complex between Ops* and \(\text{Go}\)-derived peptide, electron density is observed for the Lys296\textsuperscript{7.43} side chain, indicating a potential stabilizing network between Lys296\textsuperscript{7.43} and the residues Ser186 and Glu181 from loop E2. Such long-range effects are consistent with allosteric changes in the ligand and G protein binding domains, and thus with the postulate in classical receptor theory that binding of G protein and ligand are coupled in the active receptor conformation [44].

The position and flexibility of Lys296\textsuperscript{7.43} are also relevant to its capacity to form the retinal Schiff base during rhodopsin regeneration and for the entrance of 11-cis-retinal to and exit of all-trans-retinal from their common central binding pocket. The retinal plug tightly shields the retinal binding pocket from the extracellular environment in both rhodopsin and Ops*, so the ligand binding site can only be accessed from the membrane phase, in agreement with the regeneration observed for the N2C/D282C rhodopsin mutant in which the retinal plug is tightly fixed by an engineered disulfide bond [45,46]. The Ops* structures reveal two openings of the retinal binding pocket located in the extracellular half of the membrane spanning domain between TM1 and TM7 and TM5 and TM6, respectively (Box 2) [33,34].

Figure II. Structure of Ops* and model of channel for retinal through Ops*. (a) Lateral view of a surface model of Ops* (PDB accession 3CAP) showing two openings of the retinal binding pocket (A,B) and a computationally docked retinal in its binding site (red). (b) A defined electron density for Lys296\textsuperscript{7.43} is missing in the crystal structure. Depending on the rotamer state of Lys296\textsuperscript{7.43} (shown as orange or black stick model) and its interaction with Ser186 and Glu181 or Tyr268\textsuperscript{6.51}, respectively, a channel for retinal through the protein is open or closed [47]. (c,d) Coplanar cut through opsin revealing the channel with openings A and B. Electrostatic surface potentials are contoured at \(\pm 20\) kT/e with negatively and positively charged surface areas as red and blue, respectively. Depending on the rotamer state of Lys296\textsuperscript{7.43} (indicated schematically as an orange or black bar), the channel is closed (c) or open (d). In the model of the open channel, the amino group of Lys296\textsuperscript{7.43} is above the cutting plane, explaining the apparent lack of positive surface charge (blue).
7TM bundle and links the two openings [47]. In the situation discussed above, where Lys2967.43 is part of a network with Ser186 and Glu181, a channel large enough for retinal would not continuously traverse the protein. This is only the case when the rotamer state of Lys2967.43 is such that it can be hydrogen bonded to Tyr2686.51 [47] (Box 2, Figure II). Lys2967.43 might thus function as a gate allowing retinal to pass the channel [47].

Because the retinal channel is only found in Ops* and not in all other known rhodopsin crystal structures, the question arises as to whether the Ops* conformation is mandatory for uptake or release of the retinal ligand. For the release reaction, this indeed seems to be the case, because the release rate of all-trans-retinal is maximum when the receptor is stabilized by the Gα-derived peptide and the amount of retinal released has the pH dependence of the active conformation [48]. No clear answer yet exists for retinal uptake. On the one hand, opsin recombines readily with 11-cis-retinal at intracellular pH values at which virtually no Ops* is present [11–13,49]. On the other hand, intact rod cells show a short threshold elevation within the time frame of 11-cis-retinal uptake, which could reflect transient formation of Ops* [50]. The information revealed by modeling work is that any function of the channel in retinal passage through the receptor requires conformational adjustments. A deeper understanding of the mechanism of 11-cis-retinal uptake will require a high-resolution structure of the inactive opsin conformation.

**Box 3. Photoactivation of rhodopsin and global changes for G protein coupling**

Light-induced activation of rhodopsin starts with cis→trans isomerization of the retinal and energy storage in a twisted all-trans-retinylidene-Lys2967.43 conformation (Figure I). Concomitant with the release of strain in retinal, fast transformations in the protein occur. The succession of corresponding spectrally distinguishable photo-intermediates – i.e. Batho, Lumi and Meta I, absorbance maxima at 540, 497 and 478 nm, respectively – constitutes a fast photofunctional core process. Up to Meta I, a state that is reached within a few microseconds, the activation remains near the retinal binding pocket and only minor structural changes occur in the more distant parts of the protein [51,52,101]. With the formation of Meta I, the activation path reaches the level of the Meta intermediates. The spectroscopically identical Meta II substates Meta Ila, Meta Iib and Meta IibH+ sequentially develop from one another until an equilibrium between all the states is reached after a few milliseconds. At physiological pH and temperature, Meta IibH+ accumulates in the reaction sequence [69]. In the course of the Meta conversions, deprotonation of the retinal Schiff base and protonation of Glu113 3.22 of its complex counterion (step 1), motion of TM6 (step 2) and proton uptake to the TM3–TM6 ionic lock microdomain (step 3) occur sequentially. Thus, two of the main constraints of the inactive ground state, the retinal Schiff base network and the TM3–TM6 ionic lock, are broken one after another [59].

Opsin is reached with hydrolysis (in minutes) of the retinylidene Schiff base and release of all-trans-retinal. In the living eye, fresh 11-cis-retinal from a complex metabolism regenerates the light-sensitive rhodopsin ground state [11]. Purified opsin, devoid of any retinoids but in its native membrane host and at neutral pH, is several orders of magnitude less active towards the G protein than Meta II [12,13], but lower pH enhances its activity [62,102].

The residual activity of opsin can be explained by an active (Ops*) conformation in pH-dependent equilibrium with inactive opsin [49,62,103]. The free energy gap between Meta II and Ops* can be estimated from the apparent pKₐ at which the respective species forms. Meta II appears at an apparent pKₐ of >7.5, whereas the pKₐ for Ops* formation is 4.1 [49,69]. The difference in pKₐ equates to a difference in free energy of approximately 5 kcal/mol, which can be assigned to the stabilizing effect of the all-trans-retinal agonist present in Meta II but not in Ops*. Consistently, Meta II and Ops* exhibit a similar difference in their pH-rate profiles for G protein activation [62,102–104].

**Figure I. Rhodopsin and light-activated photoproducts.** The early photoproducts of rhodopsin related to the reactions of its photosensory core are underlined in red and the late Meta intermediates in blue.
The receptor activation path: unifying two paradigms
As outlined above, the main difference between rhodopsin as a photoreceptor and other GPCRs is the photofunctional core with its fast light-induced transformations that precede G protein-dependent conformational conversions in equilibrium. The ease of triggering the activation process by light facilitates the monitoring of fast transformations in the protein, which follow the cis—trans isomerization of 11-cis-retinal (Box 3) [51–53]. Throughout all the conversions from bathorhodopsin (Batho) via lumirhodopsin (Lumi) to the late metarhodopsin (Meta) photo-intermediates, retinal remains in the all-trans configuration and its Schiff base bond remains in the anti configuration. The photointermediates reflect stages of interaction between the chromophoric ligand and its immediate protein environment, the ligand pocket. A specific structural change that occurs with the formation of the Meta I intermediate is a change in the complex counterion of the protonated Schiff base, which shifts its center of interaction from Glu1133.28 on TM3 to Glu181 in loop E2 [31,42]. At this point in the photoexcitation pathway, we can assume that the interaction between the newly formed agonist all-trans-retinal and the opsin apoprotein is firmly established. Meta I has limited signaling capacity. An interaction with rhodopsin kinase has been observed [54] and there is also a non-productive interaction with the G protein in which GDP is not released [55].

With the Meta I state, the activation path enters a system of coupled equilibria between intermediates that all bear all-trans-retinal bound by an intact but deprotonated retinal Schiff base. This is the definition of the bleached intermediate Meta II [9]. Meta II formation is enhanced at the expense of Meta I by interaction with the G protein (and also arrestin), which established Meta II as the active G protein-binding species. Analogously, ligand-activated GPCRs show active (G protein binding) and inactive states characterized by high and low affinity for agonists, respectively [9,10,44]. Enhancement of Meta II at the expense of Meta I is also observed when rhodopsin is solubilized in detergents such as dodecyl maltoside and octyl glucoside [56,57]. The steps up to lumirhodopsin formation are detergent-insensitive [57]. Early on it was noted, paradoxically, that Meta II formation involves proton uptake from solution, although the Schiff base becomes deprotonated [9,56,58]. This is now explained by proton transfer in two different but thermodynamically coupled functional domains. These are the retinal Schiff base network and the TM3–TM6 ionic lock. In the course of rhodopsin activation, proton transfer from the retinal Schiff base to Glu1133.28 of its complex counterion and proton uptake to the TM3–TM6 ionic lock with Glu1344.49 as proton acceptor occur sequentially [9,59,60]. Proton transfer reactions per se are very fast, depending on the relative orientation and distance of proton donor and acceptor groups and on the electric field in their vicinity [61]. The observed reprotonation step is delayed because the system waits for the opportunity for proton transfer. Protonation changes are thus both monitors and co-determinants of conformational changes, in which donor and/or acceptor groups are brought into a new environment, thereby generating the opportunity for proton transfer.

It can be considered a paradigm of rhodopsin activation that protonation changes and release of inactivating constraints, occurring in the retinal Schiff base and in the TM3–TM6 ionic lock microdomains, are coupled [6,62,63]. Protonation switches that govern the transition between inactive and active states of a GPCR have also been postulated for the β2-adrenoceptor, in which a conformational change is thought to influence the hydrophobic or hydrophilic character of Asp1423.45 and thus the protonation state of this residue [64]. A second paradigm of rhodopsin activation was noted from observations of light-induced changes in the electron paramagnetic resonance (EPR) spectrum of site-specific spin-labeled rhodopsins, which occurred on the time scale of Meta II formation and reversed with its decay [65]. Later work revealed that the motion of TM6 was the dominant event and that both helix motion and activation of Gt could be prevented by TM crosslinking using engineered disulfide bridges or metal ion binding sites [66,67]. The effect was interpreted as a solid-state motion and outward tilt of TM6. Recent work has confirmed the TM6 helix motion paradigm by distance measurements using double electron–electron resonance (DEER) spectroscopy on pairs of spin labels attached to rhodopsin in a site-directed manner. The DEER measurements quantified a 5 Å outward movement of TM6 and identified smaller relative motions of TM1, TM7 and the C-terminal domain [68].

To unify the two paradigms, the temporal sequence of events was determined by measuring light-induced TM6 motion and protonation changes of spin-labeled rhodopsin in parallel as a function of pH and temperature. Proton uptake in the TM3–TM6 ionic lock microdomain occurs as a consequence of TM6 motion [59]. Both these events occur ten times more slowly than retinal Schiff base deprotonation at 30 °C, but at the same rate at T < 10 °C. The data suggest a temporal sequence of events (Box 3). Importantly, TM6 motion is a thermally activated process in Meta II and occurs as a delayed consequence of Schiff base deprotonation. The motion of TM6 most probably results in a change in the pK_a of Glu1344.49 and subsequent proton uptake from solution [9]. The scheme in Box 3, which was obtained from data on solubilized rhodopsin, has now been confirmed by two independent studies on native membranes [69,70]. Although the techniques applied cannot specifically identify helix motion, a general activation scheme was deduced with the order (i) Schiff base deprotonation, (ii) structural changes and (iii) proton uptake.

Structural correlation of activation steps
We can now attempt to combine the above data on receptor activation steps and receptor structure to derive a possible rhodopsin activation scenario (Figure 1 and Table 1). This will provide the basis for a description of the intermediates as the stepwise engagement of structural changes in conserved regions (Box 1). Formation of the active state is initiated by release of strain in the protein-bound retinal caused by cis—trans isomerization-induced retinal elongation [53]. Both ends of retinal are fixed in the ligand binding pocket, so the Schiff base and TM3–TM5 networks respond first to retinal isomerization. The structural elements that undergo changes in the transition to the
active receptor state are sequentially engaged, which corresponds to development of the Meta states (Box 3). The altered retinal–protein interactions affect the Schiff base environment and lead to a shift of the Schiff base complex counterion from Glu1133.28 on TM3 to Glu181 in loop E2 in Meta I [31,42]. Recent solid-state NMR experiments revealed that during rhodopsin activation a ~2 Å translation of retinal towards TM5 occurs and loop E2 moves away from the retinal binding site [71,72]. Although this determination yields a retinal translation that is smaller than anticipated in earlier work (see the references cited in Ref. [72]), it would allow the β-ionone ring to alter the TM3–TM5 hydrogen bonding network that includes Glu122 3.37, Trp1263.41 and His2115.46 and to induce a rearrangement of TM5 (Box 1) [8,32]. This rearrangement in turn enables the cytoplasmic part of TM5 above the Pro2155.50-induced kink to approach the TM3–TM6 ionic lock. The Tyr2235.58 side chain of the Y5.58(x)7K(R)5.66 motif can thereby unlock the ionic lock by affecting the salt bridge between Glu1343.49 and Arg1353.50. The movement of TM5 could arise in Meta I – perhaps during the counterion shift – or on conversion to Meta IIa. Indications are

Figure 1. Model of structural correlates of the rhodopsin activation scheme. The inactive rhodopsin ground-state conformation (PDB accession 1U19) is shown in black, and the active rhodopsin conformation is taken from the Ops* structure (PDB accession 3CAP) and shown in orange. (a) In the rhodopsin ground state (i), the retinal Schiff base network is highlighted. Side chains of residues of the TM7–H8 microdomain, the TM3–TM6 ionic lock, and the Y5.58(x)7K(R)5.66 motif in their inactive state are shown as green sticks. In the rhodopsin–Batho transition, retinal isomerization leads to a distorted polyene chain in which the positions of the β-ionone ring and the Schiff base do not change significantly [52,53]. The Batho–Lumi transition (ii) features retinal relaxation and a small dislocation of the β-ionone ring, as well as a small local outward movement of the polypeptide backbone in the middle of TM3 [51,53]. The concomitantly weakened electrostatic interactions in the TM1–TM2–TM7 network and between Asn782.45 and Trp1614.50, however, are not sufficient to allow protein structural changes to reach the cytoplasmic domain.}

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Table 1. GPCR signaling: what can be learned from rhodopsin?

<table>
<thead>
<tr>
<th>Light-activated rhodopsin</th>
<th>Ligand-activated GPCRs</th>
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<tbody>
<tr>
<td>Agonist formation by light in the ligand binding site (&lt;1 ps)</td>
<td>Uptake of diffusible agonist from solution or membrane</td>
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<tr>
<td>Opening of the cytoplasmic GPCR domain and presentation of the Gα binding site</td>
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<tr>
<td>(i) TM5–TM6 motion, breakage of the ionic lock</td>
<td></td>
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<tr>
<td>(ii) Rearrangement of microswitches Arg1353.50, Glu2475.30, Tyr2235.58 and Lys2345.66</td>
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<tr>
<td>Formation of new TM3–TM5 and TM5–TM6 interactions by:</td>
<td></td>
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<tr>
<td>Release of Arg3.50 and Glu3.50 by Tyr7.38</td>
<td></td>
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<tr>
<td>Release of Glu3.50 from Arg3.50 and stabilization of Glu6.50 by Lys/Arg6.66</td>
<td></td>
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<tr>
<td>(iii) Rearrangement of the NPxxY(x)5,6F microdomain (Tyr3065.73 microswitch)</td>
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</table>

| Multi-step interaction between G protein and active GPCR |
| (i) Encounter between G protein and GPCR |
| (ii) Docking of the Gα C terminus into the open cytoplasmic crevice |
| (iii) Helix switch in the Gα C terminus |
| (iv) Interaction of the Gα C terminus with Arg1363.50 of the E(D)R7.50Y motif |

given by the crystal structures of the β-adrenoceptors and of the A2A adenosine receptor [15–18], which in their partial inverse agonist or antagonist bound state—relative to inactive rhodopsin—exhibit a clear movement of TM5 and a flip of Tyr5.50 (or Tyr227.58 Ala [15]) into the direction of the TM3–TM6 ionic lock, whereas a large TM6 movement is lacking. It is interesting to note that the structures of both the β-adrenoceptors and of the A2A adenosine receptor show stabilization of the cytoplasmic loop C2 by interaction with Arg1363.50. The crystal structure of a photoactivated intermediate of rhodopsin, which, according to its 380-nm absorbance maximum, can be either Meta IIa or Meta IIB, lacks TM5 movement and shows little if any movement of TM6 [73]. Together with the information from the EPR work, we can assign this photoactivated form to the Meta IIa state.

In the subsequent Meta IIa—Meta IIB transition of rhodopsin, TM6 moves outward in concert with disruption of the TM3–TM6 ionic lock network [59,60,74] and supported by the β-ionone ring interaction with Trp2656.48 of the CWxP motif. According to the activating Trp6.48–Phe6.52 rotamer toggle switch hypothesis [27], the retinal–β-ionone ring acts in place of the phenyl ring, which is not present in rhodopsin due to substitution of Phe with Ala2696.52 [75]. In Meta IIB, TM6 is fixed in the outward position due to rearrangement of the NPxxY(x)5,6F microdomain and the TM3–TM6 ionic lock involving a rotamer change of the Tyr3065.73 side chain and an interaction of Glu2475.30 with Lys2345.66 of the Y5.58(x)7K(R)5.66 motif. Thus, in rhodopsin the NPxxY(x)5,6F microdomain and the TM3–TM6 ionic lock exist in two well-defined on and off states. In the GPCR structures of the β1, β2 and A2A receptors, partial steps of the rearrangement of these two microdomains are observed, which might be explained by the lack of full inverse agonism of the bound ligands. However, all-trans-retinal is, as a full agonist, expected to drive the complete inactive—active conversion of the TM3–TM6 ionic lock and NPxxY(x)5,6F microdomain. Complete structural conversion to the active GPCR state is manifest in the new stabilization of Arg1363.50 by Tyr227.58 and protonation of Glu1343.49 (which is released from Arg1353.50), forming Meta IIBH7. In the active opsin structure in which an agonist is lacking, pH and crystallization conditions act as stabilizing forces. Detergents shift the metarhodopsin equilibrium to the active conformation, so it is conceivable that octyl glucoside used in crystallization favors the Op5* conformation.

In this regard the largely different conformations of rhodopsin and Op5* can be viewed as the extremes necessary for the switch-like operation of rhodopsin. However, partial agonism does exist in rhodopsin. Pigments with artificial retinals lacking either the ring or the C19 methyl group [74,76–78] are impaired in their capacity to activate the G protein, but also show an equilibrium of Meta states. One known case of a native partial agonist is all-trans-retinal bound by a reprotonated and distorted Schiff base bond (syn instead of anti configuration [79]). This so-called Meta III intermediate [48] is in equilibrium with an active form [80], similar to the equilibrium between Meta I and Meta II or Op5 and Op5*.

Signal transfer to the G protein: role of the C-terminal α5 helix in Gα

Information on the active state of rhodopsin can now be used to obtain insights into the mechanism of signal transfer to the G protein (Box 4). Available evidence suggests that GPCRs can exist as monomers and dimers and even higher oligomers [81]. Recent work has shown that active rhodopsin forms a 1:1 complex with transducin [82] and that rhodopsin and the β2-adrenoceptor as monomers can activate G proteins efficiently [82–85]. This has provided the basis for the following discussion of signal transfer in a 1:1 rhodopsin–transducin complex.

It has been firmly established that the C terminus of the Gα subunit is a key receptor interaction site [35] that undergoes a conformational change on Gβγ and receptor binding [86] and several studies have identified the C-terminal α5 helix of Gα as an indispensable part of the signal transmission machinery [36–38]. We can now also understand why cytoplasmic loops C2, C3 and C4 are all implicated in signal transmission and why impairments in the TM7–H8 microdomain lead to severe defects in Gα activation [34,87,88].
Box 4. A hypothesis of receptor–G protein coupling

Signal transfer over a cell membrane by a GPCR is based on the presentation of an active receptor. Driven by the binding of an agonist, the active conformation is selected from an ensemble of receptor conformations. A crevice within the cytoplasmic receptor surface is the main binding domain for the G protein. The activating latch of the G protein is the C terminus of the α subunit. This element is flexible before it binds, but adopts a helical conformation with a C cap structure when the G protein recognizes the active receptor. Signal transmission over a distance of >40 Å, from the agonist binding site to the nucleotide binding site of the G protein, proceeds through a well-defined solid-state rearrangement of α helices. Helix motion is supported by proton transfer reactions and fast backbone and side chain fluctuations. First, TM5 and TM6 engage in new interactions to form a crevice into which the G protein α5 helix can bind. Second, the bound α5 helix switches into a new position, thereby acting as a transmission rod to the nucleotide binding site (Figure I).

### Box 5. Outstanding questions

- In rhodopsin, presentation of a crevice-like binding site for the G protein requires motion and rearrangement of transmembrane helices. Is this a general principle in GPCRs?
- What is the role of the cytoplasmic loops of GPCRs in the rearrangement of transmembrane helices and how do they contribute to G protein specificity?
- Do the two elements TM5 and TM6 move simultaneously or sequentially? Can the two motions be assigned to specific intermediates, as outlined in Box 3 and Figure 1?
- The Ops* conformation has a ligand channel with two openings into the hydrophobic lipid environment. Is this the channel for retinal uptake and release and is the channeling unidirectional? Is such a channel common to all GPCRs that bind hydrophobic ligands?
- GPCRs bind the α5 helix of the Gα subunit to trigger GDP release from the nucleotide binding pocket. How are other structural elements in the G protein involved?
- The C terminus of the α5 helix is flexible before it binds into the receptor crevice, but is bound as an α helix with a C cap structure. What is the nature of this conversion (induced fit or conformational selection) and is it a general principle of signal transfer between receptors and cognate proteins?

Switches and lubricants behind signal transmission

As soon as the agonist all-trans-retinal is fitted in its binding pocket (Box 3 and Figure 1), the rhodopsin activation process is initiated and proceeds through changes in microdomains using highly conserved microswitches. How does the signaling free energy flow from the ligand binding pocket to the binding domain for the G protein? Nygaard and co-workers [3] have discussed alternative mechanisms for the propagation of structural changes, namely: (i) a domino effect from residue to residue and (ii) an allosteric effect that mobilizes larger domains in a concerted action of the Monod–Wyman–Changeux type, termed a global toggle switch. A continuous domino effect through the whole protein is unlikely because of the variable thermodynamic coupling between the two protonation switches in the retinal Schiff base network and at the TM3–TM6 ionic lock (transitions between metarhodopsin states; Box 3).

The data available for rhodopsin and opsin consistently support a mechanism of the solid state and thus of the global toggle switch type. This does, however, not exclude – but rather requires – microscopic rearrangement of amino acid side chains, which has a role in both switching and sliding of helices or domains. The large conformational switches observed in helix motion alternate with local changes in protonation in a cause–consequence relationship (Box 3). The motion of TM6 at 30 °C occurs ten times more slowly than the preceding reaction, deprotonation of the retinal Schiff base. During this time TM6 remains locked in its inward position (corresponding to Meta IIa), presumably stabilized by hydrophobic and/or van

techniques to investigate the dynamic motion of the α5 helix within the G protein, which occur on interaction with light-activated rhodopsin. They found a distance change between the α5 helix and the β2 strand of Gα consistent with a rotation and translation of the α5 helix [37]. In recent work, a disulfide bond was engineered to constrain the α5 helix in its receptor-associated conformation [89]. The level of basal nucleotide exchange was strongly enhanced, supporting the role of the α5 helix as a transmission rod from the receptor–G protein interface to the nucleotide binding site.

A complementary molecular modeling approach revealed two modes of interaction of the α5 helix with Ops* [88]. One of them closely matches the position of the Gα-derived peptide in the crystal structure and reproduces the hydrogen bonding network at the C cap of the peptide (Box 2). In the alternative fit, the α5 helix binds to the inner surface of the TM5–TM6 helix pair and runs parallel to the membrane. This was interpreted as a switch from an initial transient interaction to the final stable interaction within the open crevice of the active receptor (helix switch [88]). Additional conformational changes in the G protein accompany the motion of α5 and additional interactions between the receptor and G protein are required to provide a firm basis for the α5 switch [38,88].

![Figure I. The rhodopsin model: Signal transmission from the retinal binding site to the nucleotide binding site in the G protein.](image-url)
nder Waals interactions (Box 3). Interactions relevant to switching occur not only between residues of the protein, but also between water molecules in strongly hydrophobic-bonded water networks or in protonated water clusters [90]. Any such process is based on the precise and evolutionarily conserved [23,24] arrangement of water molecules in the protein matrix.

Water is likely to have a role not only in the protonation switches between the global solid-state motions, but also in supporting the sliding motion of helices by solvating helix–helix contacts [91]. Generally, hydrogen bonding networks might open or close in a coordinated manner and define the solid-state motion or sliding of helices [91]. Within the hydrophobic milieu of the membrane, the strength of hydrogen bonds will be diminished by the high dielectric effect of buried water molecules and the competition from water for hydrogen bonds. Moreover, surfaces of solvated contacts are more easily separated [92]. This could play a role in the interaction of the Gα C-terminal α5 helix with opsin, in which hydrogen bonding networks between the C cap and the opsin binding pocket likely include interfacial water molecules to guide the α5 helix through the helix switch [88].

These considerations teach us that understanding signal transduction in GPCRs will not only require knowledge of the frozen conformations in active and inactive states, the function of the intermediates, and the thermodynamic and kinetic properties of their interconversion. It will also be necessary to understand the underlying protein dynamics and the specific conformational space that the signaling proteins can explore [93]. Questions that need to be addressed are listed in Box 5.

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References


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55 Morizumi, T.
66 Farrens, D.L.
54 Pulvermüller, A.
et al.
58 Arnis, S.
72 Ahuja, S. et al. (2009) Location of the retinal chromophore in the activated state of rhodopsin*. J. Biol. Chem. 284, 10190–10201
75 Crocker, E. et al. (2006) Location of Trp265 in metarhodopsin II: implications for the activation mechanism of the visual receptor rhodopsin. J. Mol. Biol. 367, 163–172
79 Vogel, R. et al. (2003) Deactivation of rhodopsin in the transition from the signaling state meta II to meta III involves a thermal isomerization of the retinal chromophore C/D. Biochemistry 42, 9863–9874
97 Franke, R.R. et al. (1990) Rhodopsin mutants that bind but fail to activate transducin. Science 250, 123–125