Activation of G Protein–Coupled Receptors: Beyond Two-State Models and Tertiary Conformational Changes

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signal transduction, receptor pharmacology, protein dynamics, protein conformation, allostery

Abstract

Transformation of G protein–coupled receptors (GPCRs) from a quiescent to an active state initiates signal transduction. All GPCRs share a common architecture comprising seven transmembrane-spanning α-helices, which accommodates signal propagation from a diverse repertoire of external stimuli across biological membranes to a heterotrimeric G protein. Signal propagation through the transmembrane helices likely involves mechanistic features common to all GPCRs. The structure of the light receptor rhodopsin may serve as a prototype for the transmembrane architecture of GPCRs. Early biochemical, biophysical, and pharmacological studies led to the conceptualization of receptor activation based on the context of two-state equilibrium models and conformational changes in protein structure. More recent studies indicate a need to move beyond these classical paradigms and to consider additional aspects of the molecular character of GPCRs, such as the oligomerization and dynamics of the receptor.

OVERVIEW OF G PROTEIN–COUPLED RECEPTOR STRUCTURE

GPCR: G protein–coupled receptor

7-transmembrane (7-TM) domain: the region of the receptor that forms seven α helices and spans across the membrane

Orthosteric ligand-binding site: the primary binding site in the receptor for endogenous agonists

Rhodopsin: visual pigment responsible for scotopic (dim light) vision. Rhodopsin specifically refers to the apo-protein opsin covalently linked to the chromophore 11-*cis*-retinal

G protein–coupled receptors (GPCRs) are found in most eukaryotic organisms (1), where they modulate a wide range of biological processes. Human GPCRs can be separated into four major groups based on sequence similarity: classes 1, 2, and 3 and the frizzled family (2). All GPCRs are predicted to share a common 7-transmembrane (7-TM) α-helical structure and signal via heterotrimeric G proteins coupled on their cytoplasmic surface. The greatest structural variability among GPCRs occurs in the amino terminal region, which forms the orthosteric ligand-binding domain for some receptors. Although crystal and NMR structures are available for the ligand-binding domains of several GPCRs, the only structures of the 7-TM domain are crystal structures and electron microscopy reconstructions of rhodopsin (**Figure 1**), the light receptor found in the rod outer segments of the retina (3). General features revealed in the transmembrane region of rhodopsin in crystal structures are predicted to be conserved across all GPCRs (4).

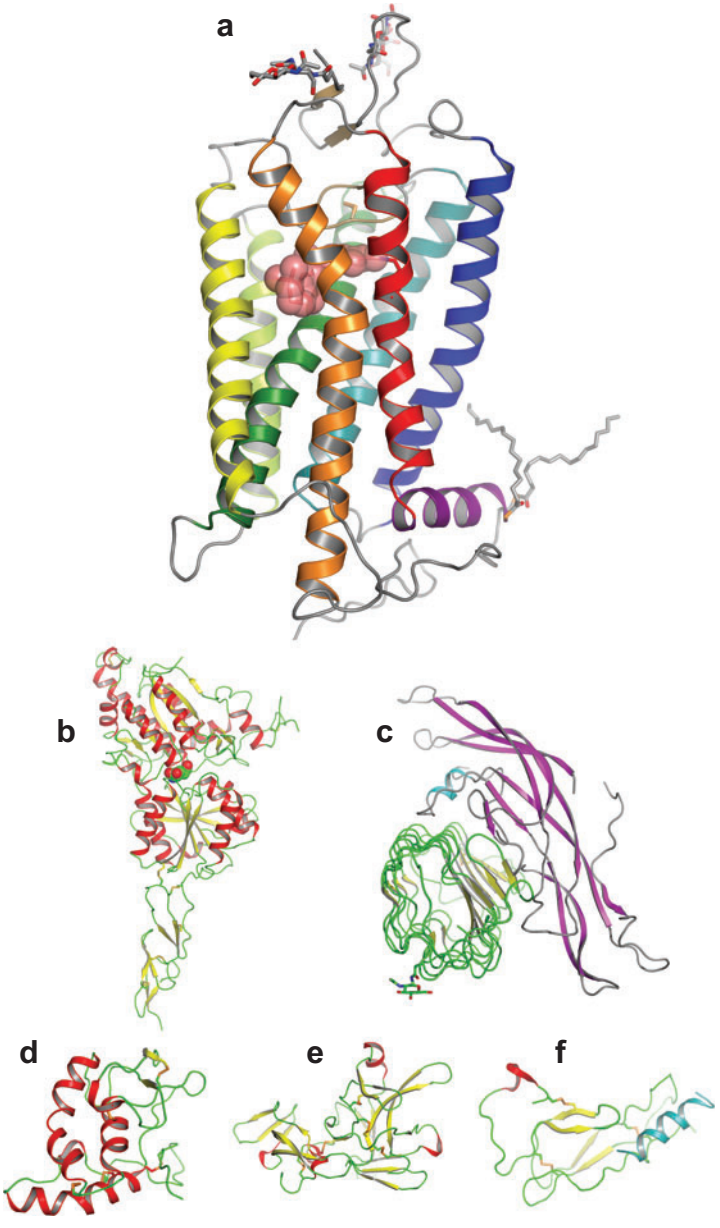
Diversity in the Ligand-Binding Pocket of GPCRs

GPCRs are activated by a broad spectrum of stimuli. Based on the location of the orthosteric ligand-binding region, GPCRs can be divided into two broad classes. One class forms its ligand-binding pocket within a cavity formed by the transmembrane helices, whereas the other class binds ligand in a large amino terminal region that forms a distinct domain. The amino terminal ligand-binding domain of some GPCRs has been shown to attain the proper fold and bind ligand independently of the transmembrane region of the receptor. It is unclear how agonist-promoted structural changes in an amino terminal ligand-binding domain are translated into activating structural changes within the 7-TM domain of the receptor. Orthosteric ligand-binding domains with a known structure are briefly discussed here.

Figure 1

Diversity of the orthosteric ligand-binding domain of various GPCRs. (*a*) Crystal structure of dark state bovine rhodopsin (PDB ID: 1U19). Rhodopsin is covalently bound to 11-*cis*-retinal (*pink spheres*). Helices are denoted by the following colors: TM-I, blue; TM-II, cyan; TM-III, dark green; TM-IV, lime green; TM-V, yellow; TM-VI, orange; TM-VII, red; and H-8, purple. (*b*) The ligand-binding domain of the group II metabotropic glutamate receptor (PDB ID: 2E4U). Glutamate (*red and green spheres*) binds in a crevice formed by the two lobes of the ligand-binding domain. (*c*) The complex between the ligand-binding domain of the follicle stimulating hormone receptor (*green* and *yellow*) and its ligand, follicle stimulating hormone (*pink* and *cyan*) (PDB ID: 1XWD). (*d*) The cysteine-rich domain of murine frizzled 8 (PDB ID: 1IJY). (*e*) Crystal structure of the ligand-binding domain of the *Drosophila methuselah* receptor (PDB ID: 1FJR). (*f*) A representative structure of the ligand-binding domain from the NMR ensemble of structures for the corticotropin releasing factor II (CRFR-II_β) receptor with bound agonist astressin (*cyan*) (PDB ID: 2JND). All amino terminal ligand-binding domains are oriented such that the putative location of the 7-TM domain would be located below the structure with the exception of the CRFR-II_β receptor ligand-binding domain for which the orientation is unknown.

The binding pocket for retinal in rhodopsin may represent a prototype for the ligand-binding pocket of GPCRs that bind small molecules within the transmembrane region (**Figure 1*a***). The 11-*cis*-retinal chromophore of rhodopsin is covalently linked to a lysine residue in transmembrane helix VII and acts as an inverse agonist, locking the receptor in the inactive state. The retinal-binding cavity is closer to the



FSH: follicle-stimulating hormone

CRF: corticotropin-releasing factor

VFT: Venus flytrap

extracellular surface than to the cytoplasmic surface. The ligand-binding cavity of GPCRs that bind amine ligands also appears to be located in a corresponding position (5). The amino-terminal region and extracellular loops of rhodopsin form a plug covering the retinal-binding cavity and also contact the chromophore (6). A similar plug-like feature has been proposed for both the D_2 dopamine and M_3 muscarinic receptors (7, 8).

The crystal structure of the amino terminal ligand-binding domain of human follicle-stimulating hormone (FSH) receptor bound to its glycoprotein ligand, FSH, has been determined (9). The ligand-binding domain contains multiple leucine-rich repeats flanked on each side by cysteine-rich regions. However, the crystallized ligand-binding domain is a truncated form that excludes the carboxy-terminal cysteine-rich region, suggesting that the latter is not required for binding FSH (10). The FSH receptor ligand-binding domain is tube-shaped with a slight bend that binds FSH by a hand-clasp binding mechanism in which the tube-like structure of the FSH receptor and the FSH molecule wrap around one another (**Figure 1*c***). A similar structure also has been determined for the thyroid-stimulating hormone receptor ligand-binding domain bound to an activating antibody (10a). Thus, the FSH receptor ligand-binding domain structure may be conserved among other class 1 glycoprotein-binding receptors.

The NMR structure of the amino-terminal ligand-binding domain of mouse corticotropin-releasing factor (CRF) receptor 2β has been determined both in the presence and absence of ligand (11–13) (**Figure 1*f***). This structure is stabilized by three disulfide bonds, two tryptophan residues, and one salt-bridge. The ligand-binding domain of the CRF receptor may be a prototype for other class 2 receptors that bind peptide ligands (14). Methuselah is a GPCR found in *Drosophila* that is closely related to class 2 receptors. The ligand-binding domain of Methuselah has been expressed and its crystal structure determined (15) (**Figure 1*e***). This ligand-binding domain contains three subdomains that are composed mainly of β-sheets with five disulfide bonds. A solvent-exposed tryptophan residue present in a shallow groove may represent the ligand-binding site.

The amino-terminal region of most class 3 GPCRs consists of a Venus flytrap (VFT) ligand-binding domain linked to a cysteine-rich domain (reviewed in 16). The VFT domain is linked both structurally and functionally to the 7-TM domain by the cysteine-rich domain, except in the case of $GABA_B$ receptors (17). Structures of the VFT domains of rat $mGluR_1$, $mGluR_3$, and $mGluR_7$ were determined by X-ray crystallography and serve as a prototype for the structure of VFT domains of other class 3 receptors (18–20). The crystallized amino-terminal region of mGluR3 contains both the VFT domain and cysteine-rich domain (**Figure 1*b***). All VFT structures from the different subtypes of the mGluR are structurally similar (20). The VFT consists of two lobes with the ligand-binding site located in the crevice between them. The VFT domain can exist in either an open or closed conformation depending on the occupancy of its ligand-binding site. The closed conformation results in receptor activation (21).

The frizzled family of GPCRs comprises frizzled and smoothened, which are closely related to class 2 receptors (reviewed in 22, 23). The ligand-binding domain

of frizzled contains a cysteine-rich domain that binds the endogenous ligand Wnt, a palmitoylated glycoprotein agonist. This cysteine-rich domain alone can bind Wnt (24). The crystal structure of the cysteine-rich domain of murine Frizzled 8 has been solved (25) (**Figure 1*d***). All cysteine residues in this ligand-binding domain are involved in disulfide bonds. Mapping of residues involved in Wnt binding obtained from mutational studies indicate a surface on the cysteine-rich domain that may form the binding site for Wnt (25).

A Common Activation Mechanism Within the Transmembrane Domain of GPCRs

Despite variations in the modes of ligand binding to the different classes of GPCRs, all activation processes are likely to include similar changes within the 7-TM domains. The 7-TM domain of several class 3 GPCRs folds correctly and is targeted to the cell surface even in the absence of the VFT and cysteine-rich domains (26–28). The 7-TM domains of these class 3 receptors alone can produce a cellular response and their activities can be altered by positive and negative allosteric modulators that bind within this domain (26) (for additional information, see the sidebar Modulation of GPCR Activity by Allosteric Ligands). An ionic network similar to that found in class 1 GPCRs, which stabilizes the inactive state of the receptor, is also predicted to exist within the 7-TM domain of class 3 receptors (29). These studies reveal a striking similarity between class 3 and class 1 GPCRs when only the 7-TM domains are considered. Likewise, activation of class 2 GPCRs may also require structural changes within the 7-TM region similar to those required for the activation of class 1 receptors (30). Furthermore, GPCRs from different classes can signal by coupling to the same type of heterotrimeric G proteins, which are comparatively few in variety (31). Thus, despite variations in the location of the orthosteric ligand-binding site in the receptor, all activation processes likely involve similar changes within the transmembrane helices of the receptor to propagate an external signal to the heterotrimeric G protein. The remainder of this review will focus on the activation mechanism as it relates to the structurally conserved 7-TM domain.

CURRENT IDEAS ABOUT G PROTEIN–COUPLED RECEPTOR ACTIVATION

Two-State Thermodynamic Equilibrium Models of Receptor Activation

The linkage between ligand binding and activity is at the heart of many biological processes including that of GPCR signaling. Such processes have been traditionally described by two-state models (32–34), in which the effect of ligands on the equilibrium between two distinct conformations or states of the protein underlies the function and activity of the molecule. The most commonly used two-state model to describe the action of GPCRs is the ternary complex model (**Figure 2*a***). This model was formulated based on observations made in studies using radiolabeled ligands (33).

MODULATION OF GPCR ACTIVITY BY ALLOSTERIC LIGANDS

The term allostery is derived from the Greek words allos (other) and stereos (solid) and refers to the topological linkage between two distinct sites. Allosteric ligands bind to receptor molecules at sites that are structurally distinct from the orthosteric ligand-binding site and modulate the activity of the receptor by either enhancing (allosteric activators) or decreasing (allosteric inhibitors) the action of the orthosteric agonist. Allosteric ligands are thought to exert their effects on receptor activity by changing the affinity, binding kinetics, or intrinsic activity of orthosteric agonists, or by shifting the equilibrium in favor of either the active or inactive states of the receptor. Binding of the G protein or accessory proteins to the receptor provides an additional dimension of complexity to the allosteric behavior of the receptor. Endogeneous allosteric ligands include ions, lipids, amino acids, and peptides; their physiological role may be to fine-tune the signaling response of GPCR systems. Exogenous allosteric ligands provide an additional means to modulate the activity of the receptor and provide a potential for the development of novel pharmaceutical agents.

Radioligand binding studies played an important role in providing initial molecular and mechanistic descriptions of GPCRs at a time when structural insights were limited or nonexistent (35) (for additional information, see the sidebar Molecular Discovery of GPCRs).

Radioligand binding studies have revealed that agonists for many GPCRs display shallow curves with Hill coefficients less than 1 (**Figure 2*b***), suggesting a heterogeneous population of receptors and interpreted as multiple states of the receptor. These states are interconvertible and sensitive to guanyl nucleotides, thereby implicating the involvement of heterotrimeric G proteins. The effect of guanyl nucleotides on the affinity of an agonist for its receptor was demonstrated early on for the glucagon receptor, where GTP reduces the apparent affinity of the agonist ^{125}I-glucagon (36). The heterogeneity revealed by agonists and the effect of guanyl nucleotides were described quantitatively by the ternary complex model (33) (**Figure 2*a***).

In the ternary complex model, the ternary complex consists of the receptor (R), agonist (A), and the G protein (G). The shallow curves observed in agonist binding assays are attributed to two interconvertible states of the receptor that coexist in equilibrium in the absence of guanyl nucleotides and bind agonists with different affinities. Activation of the receptor is dependent on its ability to form a complex with the G protein and therefore the active state is a property of the receptor–G protein complex. Coupling of the receptor to the G protein (RG) results in an active state of the receptor that binds agonist with high affinity, whereas the uncoupled receptor (R) is inactive and exhibits low affinity for agonist. Guanyl nucleotides are thought to shift the equilibrium in favor of the low-affinity or uncoupled state (**Figure 2*b***).

MOLECULAR DISCOVERY OF GPCRs

Studies on hormone-binding GPCR systems have played a large role in the development of the receptor concept. The action of hormones on their receptors was first quantified by Clark in 1933 by analyzing their dose-response relationships. Clark's theory was later modified by Ariens (1954), Stephenson (1956), and Furchgott (1966) to include properties of the receptor such as intrinsic activity and efficacy. The availability of radiolabeled ligands for GPCRs allowed direct measurements of hormone-receptor interactions and labeling of the receptor molecule itself. The first report of radiolabeled ligand usage was by Paton & Rang who, in 1965, reported the saturable binding of radiolabeled ligands to the muscarinic receptor. Hormone-binding GPCRs were initially conceptualized by Earl Sutherland to be a part of the same molecule as the effector adenylate cyclase. In 1977, the groups of Gilman, Lefkowitz, and Rodbell demonstrated that the receptor for radiolabeled hormones was molecularly distinct from adenylate cyclase. Lefkowitz's group went on to establish an affinity purification scheme for the β_2 adrenergic receptor in 1979, which eventually led to the cloning of the gene encoding this hormone-binding GPCR in 1986. The predicted amino acid sequence of the β_2 adrenergic receptor and other related gene products revealed similarity to the well-studied visual pigment rhodopsin, suggesting a structural link between these receptors. Completion of the full genomic sequences for many different organisms shows that a large fraction of the genome encodes for structurally related GPCRs.

Recognition that receptors exhibit constitutive activity (37, 38) has led to the idea that the active state of the receptor is an intrinsic property of the receptor itself rather than that of the receptor–G protein complex. This view is also supported by alkylation studies of purified M_2 muscarinic receptor devoid of G proteins (39). Mutations in the receptor can increase the level of constitutive activity and agonists bind these mutants with higher affinity than wild-type receptor (37, 38). However, the high-affinity binding of agonists to constitutively active mutants was independent of the G protein (38), which could not be accommodated by the classical ternary complex model and therefore required the formulation of the extended ternary complex model (38). In this model, the receptor exists in two interconvertible states that are an intrinsic property of the receptor itself: an inactive state, R, that binds agonist with low affinity and an active state, R^*, that binds agonist with high affinity. The G protein binds exclusively to the R^* state, leading to the cellular response.

Although the quantitative accuracy and application of the ternary complex model in describing experimental data are uncertain (40, 41), they have been instrumental in shaping our view of the activation process. While these thermodynamic equilibrium schemes do not provide direct information about protein structure, they have led to the view that receptors exist in two discrete conformations, an active and inactive state.

Constitutive activity: the production of a cellular response in the absence of an agonist

R: inactive receptor state

R^*: active receptor state

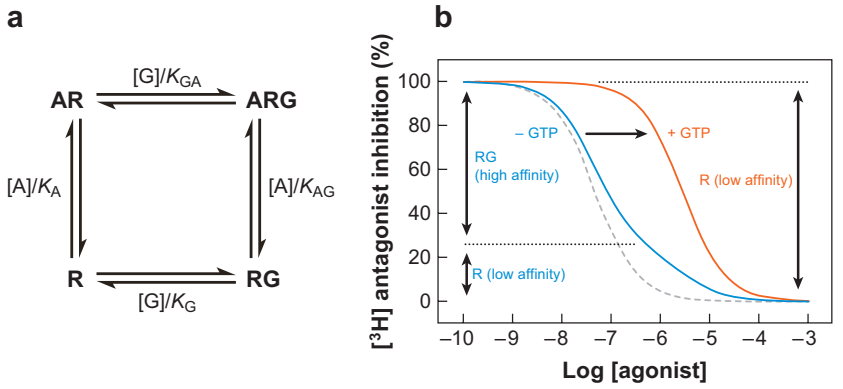


Figure 2

Ternary complex model of receptor activity. (*a*) The classical ternary complex model is shown. Agonists (A) bind free receptor (R) or receptor coupled to the G protein (RG) with the dissociation constants K_A and K_{AG}, respectively. The G protein (G) binds free receptor or receptor occupied by agonist (AR) with the dissociation constants K_G and K_{GA}, respectively. (*b*) Curves represent typical patterns revealed in competition studies between a fixed concentration of a radiolabeled antagonist and graded concentrations of an unlabeled agonist. The curves were simulated using parameters obtained from Reference 42 by a two-state multisite model in which a radiolabeled antagonist and an unlabeled agonist compete for sites that are mutually independent and noninterconverting. In the absence of GTP (*blue curve*), the agonist reveals both a high-affinity state attributed to the receptor coupled to the G protein (RG) and a low-affinity state attributed to free receptor (R). In the presence of GTP (*orange curve*), the equilibrium appears to shift toward the free receptor state, thereby revealing a single low-affinity state for the agonist. The grey dashed line represents the simulated curve for a receptor exhibiting a single high-affinity state. The ternary complex model is often used to conceptualize the effects shown here; however, this model may not be adequate when applied quantitatively.

Within the context of the ternary complex model, a ligand shifts the equilibrium in favor of the state for which it has higher affinity. Thus, agonists promote activation of the receptor because they bind the active state with higher affinity compared with the inactive state. Conversely, inverse agonists exhibit the opposite effect of agonists by binding the inactive state of the receptor with higher affinity compared to the active state, thereby shifting the equilibrium in favor of the inactive state. Antagonists show no preference in affinity between the two states of the receptor, thus neither state is favored and the equilibrium is unaffected (for additional information, see the sidebar Classification of Ligands).

Can Two-State Thermodynamic Equilibrium Models Adequately Describe Receptor Function?

On a superficial level, the ternary complex model appears to be consistent with the pattern of curves obtained from radioligand-binding assays and can explain a variety of effects. For instance, the intrinsic activity or efficacy of agonists for the adrenergic receptor and muscarinic receptor correlate with the fraction of high-affinity binding

CLASSIFICATION OF LIGANDS

Ligands that bind to the receptor can be broadly categorized as either agonists or antagonists. The words agonist and antagonist come from the Greek agōnistēs (combatant) and antagōnistēs (rival), respectively. Agonists bind the receptor to promote a cellular response and are classified as either full or partial depending on their ability to promote maximal activity or the manifestation of efficacy in the system. Antagonists oppose the action of agonists by blocking the binding of an agonist to the receptor thereby preventing a cellular response. In contrast to full and partial agonists, inverse agonists produce a negative cellular response, and in contrast to antagonists, inverse agonists can decrease the basal level of activity. Until studies were performed on constitutively active receptors, antagonists and inverse agonists could not be readily distinguished. The mechanism by which each of these types of ligands achieves its action and the molecular description of efficacy continue to be areas of discussion and debate.

sites revealed by radioligand-binding curves (42, 43). This correlation appears to be consistent with the idea that full agonists shift the equilibrium in favor of the active state to a greater extent than do partial agonists. Constitutive activity can be accommodated within the context of the extended ternary complex model by changes in the equilibrium existing between R and R* states. Mutations that increase the level of constitutive activity alter this equilibrium in favor of the R* state and result in higher binding affinities for agonists and lower binding affinities for inverse agonists (38, 44, 45). This supports the idea that agonists and inverse agonists have reciprocal effects in shifting the equilibrium between two states of the receptor.

Results from several biophysical studies also appear to be in qualitative agreement with an activation process involving two states of the receptor (e.g., 46–49). For instance, effects of agonists and inverse agonists on the intramolecular fluorescence resonance energy transfer (FRET) signal in the α_{2A} adrenergic receptor tagged with both cyan fluorescent protein (CFP) and yellow fluorescent protein (YFP) as well as circular dichroism difference spectra of purified 5-HT_{4A} receptor were in the opposite direction (47–49). Moreover, the magnitude of the signal change correlated with the efficacy of the agonists tested. A similar effect of agonists was observed in the quenching of a fluorescent probe covalently linked to the cytoplasmic end of transmembrane helix VI in the β_2 adrenergic receptor (46).

Upon closer examination, however, the data point to a more complex situation wherein the action of ligands occurs through multiple states of the receptor. The kinetics of fluorescence quenching changes in the β_2 adrenergic receptor and intramolecular FRET changes in α_{2A} adrenergic receptor are inconsistent with two distinct states, but rather, indicate multiple states of the receptor (47, 48, 50, 51). Studies on the β_2 adrenergic receptor using a bimane-tryptophan quenching system also support the notion that different types of agonists promote different receptor conformations rather than simply modulating the equilibrium between an active and

Fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET): nonradiative transfer of energy from a fluorescent or bioluminescent donor molecule to a fluorescent acceptor molecule

CFP: cyan fluorescent protein

YFP: yellow fluorescent protein

inactive state (52). The effect of different agonists on a specific conformational switch in the β_2 adrenergic receptor was monitored by bimane-tryptophan quenching in a receptor construct engineered with an exogenous cysteine residue and tryptophan residue. Results from this study suggest that the activity of the receptor is determined by multiple conformational switches in the receptor and that different agonists have different abilities to modulate these switches.

Rigid-body movement: movement of a region within a protein with minimal disruption of secondary structure

Predicted Conformational Changes Upon Receptor Activation

Conformational changes within the transmembrane helices of the receptor thought to accompany activation of GPCRs have been probed by several indirect methods, including site-directed spin labeling, site-directed fluorescence quenching, sulfhydryl accessibility, and disulfide cross-linking (reviewed in 53–56). Spin labeling studies of rhodopsin in solution suggest that rigid-body movements of helices underlie the activation of this receptor (57).

The most pronounced movements upon activation of rhodopsin were predicted to involve the outward movement of the cytoplasmic ends of transmembrane helices III, VI, and VII, and possibly a rotation of transmembrane helix VI about its helical axis (53). Measurements of the movement of spin labels attached to side chains within these helices indicate that displacements of the spin labels can exceed 10 Å upon activation of rhodopsin in detergent solution. These inferred movements have been proposed to open up the cytoplasmic region of the receptor permitting interaction with and activation of the G protein. Similar conformational changes have been suggested to occur with other GPCRs (58–60). Movements of helices have been observed in spin-labeling studies of rhodopsin reconstituted into lipid bilayers; however, the mobility of the spin labels was restricted compared with detergent-solubilized receptor (61). Similarly, disulfide cross-linking studies with the M_3 muscarinic receptor showed that agonist-promoted structural changes were much more restricted when studies were carried out in the membrane rather than in solution (62). Thus, the movement of helices that accompany receptor activation in a lipid bilayer environment may be more restricted compared with those observed in solution.

Linkage Between Receptor Activation and G Protein Activation

The action of GPCRs may be analogous in some respects to regulatory enzymes where the ligand-binding site is discrete from the active site (63). The active site in the case of enzymes is the catalytic domain and in the case of GPCRs is the nucleotide-binding site within the G protein Gα subunit. The ligand-binding site in the receptor and the nucleotide-binding site in the G protein are tightly coupled despite their distance from each other. Binding of an agonist at the ligand-binding site in the receptor leads to the activation of the G protein by promoting the exchange of bound GDP for GTP in the nucleotide-binding pocket. Transmission of this signal from the ligand-binding site in the receptor to the nucleotide-binding site in the G protein is commonly thought to occur through the transient association between the two molecules.

The precise determinants and timing for the functional and structural interactions between the receptor and G protein are unknown. The classical view of signal transduction depicts the association between receptor and G protein to occur only after receptor activation. In the visual system, the photoreceptor G protein transducin is present at a concentration of approximately 500 μM (64). Prior to illumination, 30% of transducin is bound to rod outer segment disc membranes with a dissociation constant of 10 μM (65). The binding site for transducin prior to illumination is presumed to be dark-state rhodopsin (66), suggesting that activation of the receptor may not be a prerequisite for binding G protein and that some G protein may be bound to inactive receptor in the basal state. This idea of precoupling is supported by bioluminescence resonance energy transfer (BRET) and FRET studies and computational considerations (67–70). Moreover, GDP-bound transducin has been shown to bind to metarhodopsin I (MI), an intermediate state of rhodopsin that precedes the metarhodopsin II (MII) active state, albeit with a weaker affinity than to MII (71). Binding of transducin to the preactivated form of the receptor does not initiate GDP-GTP exchange.

Transducin: a heterotrimeric G protein localized to the outer segment of photoreceptor cells that couples specifically to the visual pigments rhodopsin or cone opsins

MI: metarhodopsin I

MII: metarhodopsin II

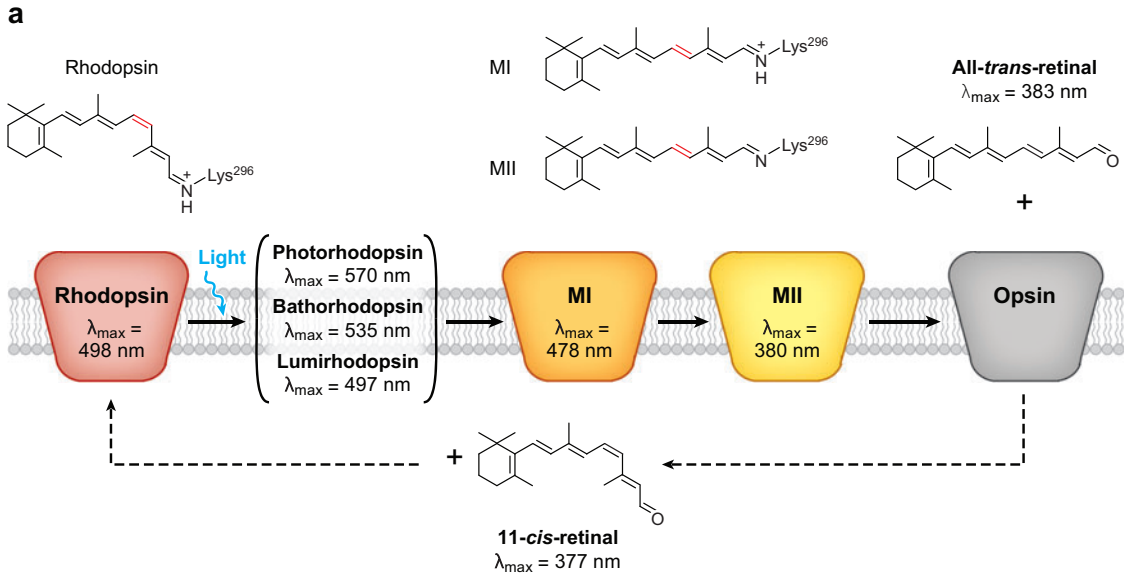
Activation of rhodopsin in rod outer segment membranes decreases the dissociation constant for binding transducin to 200 nM (72). The heterotrimeric G protein Gα subunit is normally bound to GDP prior to binding the receptor, and in the absence of bound guanyl nucleotide, transducin binds even more tightly to activated rhodopsin with a dissociation constant of 1 nM (72). Similar affinities of transducin for rhodopsin were observed in an artificial lipid bilayer using surface plasmon-waveguide resonance spectroscopy; transducin bound inactive rhodopsin with a dissociation constant of 64 nM, and activation of the receptor resulted in a decrease in the dissociation constant to 0.7 nM (73).

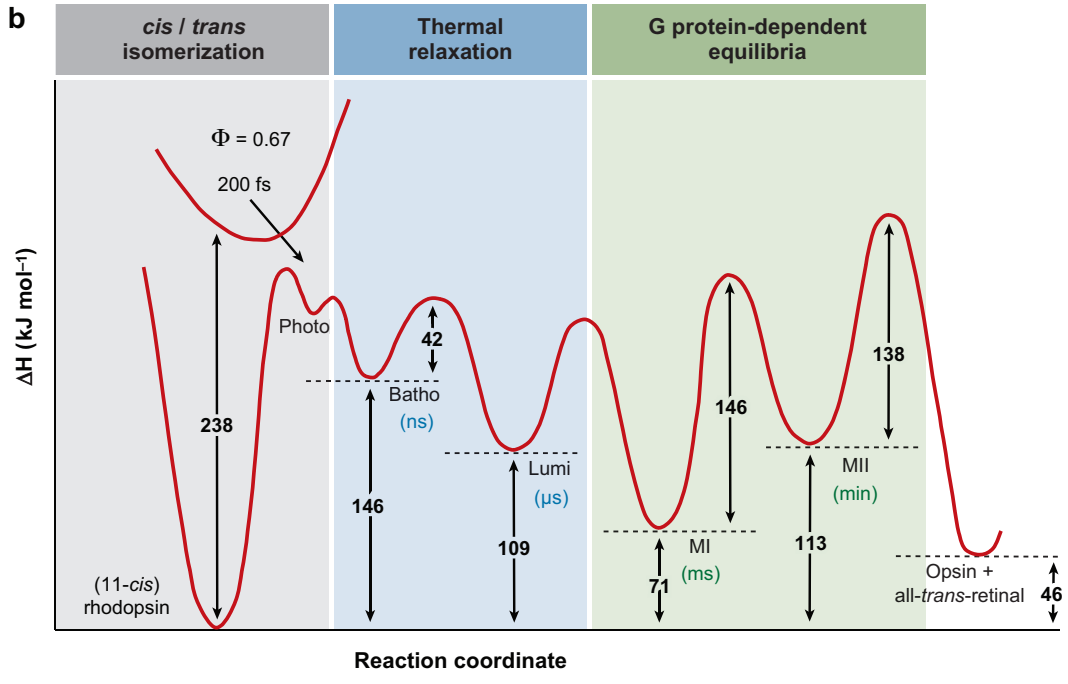
Once the receptor and G protein form a complex, the ligand-binding site of the GPCR and nucleotide-binding site of the G protein are tightly coupled. The effect of guanyl nucleotides on the binding of agonists to the receptor is often the focus of studies aimed at understanding the functional coupling between the receptor and G protein. Interestingly, agonists affect the binding of guanyl nucleotides to the G protein in a manner that mirrors the effect of guanyl nucleotides on the binding of agonists to the receptor (74, 75). Activated rhodopsin can form a stable complex with transducin when the guanyl nucleotide binding site in the Gα subunit is unoccupied (76). The empty guanyl nucleotide-binding pocket in the G protein prevents the classical MII decay of activated rhodopsin, which leads to the release of the chromophore from the ligand-binding site. Communication between the two sites is bidirectional.

STRUCTURES OF INACTIVE AND PHOTOACTIVATED RHODOPSIN: IMPLICATIONS FOR GPCR ACTIVATION

Intermediates in Vertebrate Rhodopsin Activation

The activated state of rhodopsin, MII, is thought to be attained through the formation of multiple intermediate states (77, 78) (**Figure 3**). The dark-state of rhodopsin is maintained by the inverse agonist 11-*cis*-retinal, which is covalently linked to the





receptor via a protonated Schiff base. In the absence of the chromophore, opsin exhibits constitutive activity that equals 10^{-6} of the activity observed in the active MII state (79). The dependency of the spectral properties of the chromophore on the protein environment in rhodopsin allows the precise monitoring of the multiple intermediates owing to the distinct absorbance spectrum exhibited by each intermediate (**Figure 3*a***). The series of events leading to rhodopsin activation is initiated by the absorption of a single photon by 11-*cis*-retinal, which results in the isomerization of 11-*cis*-retinal to all-*trans*-retinal occurring on a femtosecond time scale with high quantum yield (0.67) (78). Isomerization of the chromophore leads to the formation of multiple intermediate states prior to the formation of the active MII state. These intermediate states of rhodopsin can be trapped at low temperatures and can be detected at physiological temperatures in two-dimensional crystals or at room temperature in polyacrylamide gels (80, 81). The Schiff base linkage becomes deprotonated upon attainment of MII and decay of this active state involves the hydrolysis and release of all-*trans*-retinal (82). All-*trans*-retinal is enzymatically converted back to 11-*cis*-retinal in the retinal pigment epithelium cells so that rhodopsin can be regenerated (83).

Structures of Inactive States of Rhodopsin

Several crystal structures are now available for the dark-state form of rhodopsin (84–89). Each structure reveals the same overall arrangement of helices and, while not identical, these structures are similar to each other especially with respect to the transmembrane helices (**Figure 4*a*,*c***). The most significant differences are found within the cytoplasmic loops, which are flexible. Several water molecules have been resolved in dark-state crystals of rhodopsin that point to a functional role for water in the spectral tuning of visual pigments and in the regulation of receptor activity (86). Some water molecules are bound to residues that are highly conserved among GPCRs, suggesting that functional roles for water-mediated contacts may be conserved across members of this family of receptors (90).

Structures for three of the inactive intermediates of rhodopsin are also available (91–93). Crystal structures for the bathorhodopsin and lumirhodopsin intermediates

Figure 3

Intermediates of rhodopsin. (*a*) Rhodopsin is covalently linked to 11-cis-retinal via a protonated Schiff base at Lys^{296} in transmembrane helix VII. Upon absorption of a photon of light, the chromophore is isomerized to all-*trans*-retinal. This isomerization leads to the formation of a series of intermediates each exhibiting a distinct absorbance spectrum. The MII state is the active form of the receptor that binds to and activates the G protein. The λ_{max} shown for each intermediate are those from bovine samples (77, 78). The λ_{max} for free 11-*cis*-retinal and all-*trans*-retinal are those obtained in ethanol (156). This figure is adapted from References 157, 158. (*b*) Schematic of the reaction coordinate during rhodopsin activation. Isomerization of 11-*cis*-retinal to all-*trans*-retinal occurs within femtoseconds with a high quantum yield (Φ). Enthalpies and activation enthalpies for rhodopsin and its intermediates are indicated. Lifetimes of intermediates are shown in parentheses. This figure was adapted from References 77, 82.

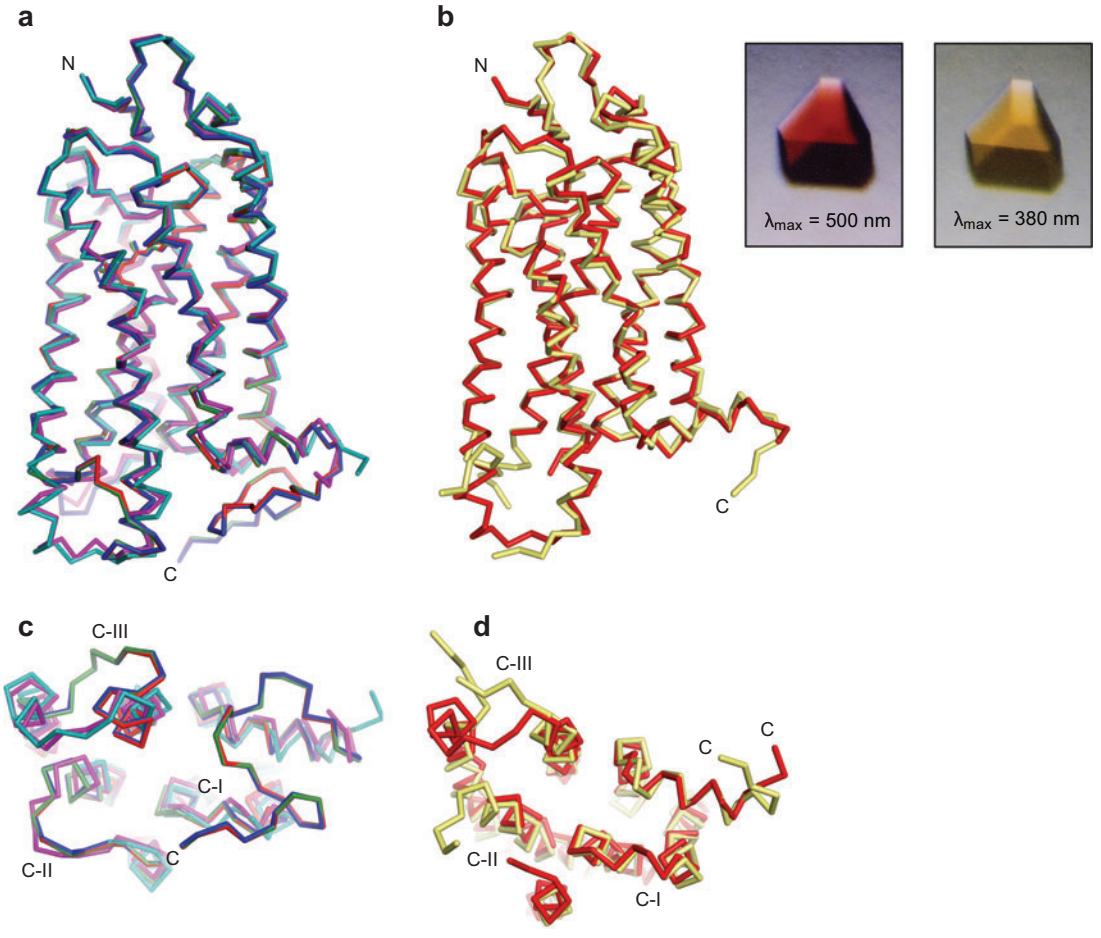


Figure 4

Structural differences between the dark-state and photoactivated states of rhodopsin. (*a*, *c*) Superposition of the three dark-state (PDB ID: 1U19, *red*; 1GZM, *purple*; 2I36, *cyan*) and the early intermediates, bathorhodopsin (PDB ID: 2G87, *green*) and lumirhodopsin (PDB ID: 2HPY, *dark blue*). No significant changes are observed except in the region comprising residues 230–243. *Panel c* is rotated 90° about the x axis. (*b*, *d*) Structural superposition of the dark-state (PDB ID:2I36) and the photoactivated state (PDB ID:2I37). Images of crystals for the dark-state and the photoactivated state and their λ_{max} values are shown in the insets. Structural changes observed are limited to cytoplasmic loops II (C-II) and III (C-III) and the cytoplasmic tail. *Panel d* is rotated 90° about the x axis. The amino terminus and carboxyl terminus are labeled as N and C, respectively.

are almost completely isomorphous to the dark state receptor structure (91, 92) (**Figures 4*a*,*c***). The chromophore appears nearly to adopt its full all-*trans*-retinal form by the lumirhodopsin stage. Achievement of the all-*trans*-retinal form results in minor local changes in the structure of the receptor, but no large global changes were detected at this stage. FTIR spectroscopy at low temperatures also indicates

that there is little global change in the structure of lumirhodopsin as compared to dark-state rhodopsin (94). Large conformational changes also seem to be absent in the late MI inactive state of rhodopsin (95). The low-resolution structure of the MI state of rhodopsin in 2-D crystals showed no significant differences when compared with the dark-state receptor (93).

Structure of a Light-Activated Deprotonated Intermediate of Rhodopsin

A purification and crystallization scheme that allowed for photoactivation of rhodopsin in crystal form was established (96), which permitted the first crystal structure determination of an activated form of a GPCR (89). Such photoactivated crystals of rhodopsin displayed the characteristic absorbance spectrum of the active MII state, contained only all-*trans*-retinal and, upon solubilization from the crystal form, were capable of activating transducin. Fine details of changes that accompany activation of the receptor were not available owing to the resolution of the crystal structures. However, the resolution was sufficient to detect any large rigid-body movements of transmembrane helices if they occurred. Surprisingly, such movements were absent in photoactivated rhodopsin crystals. Differences were observed in the cytoplasmic loops and carboxyl terminal region of the receptor, where some regions became more flexible or disordered, whereas others assumed different orientations when compared with the dark-state structure of the same crystal form (**Figures 4*b*,*d***). Although the characteristic absorbance spectrum for the active MII state can be attained without significant conformational changes, it is unclear whether large-scale rearrangements of the 7-TM region are inhibited by the crystallization conditions employed or by crystal packing constraints. It is interesting to note that large differences in absorbance spectra between rhodopsin and its intermediates arise from minimal structural changes in the protein molecule.

Thermodynamic Considerations in Rhodopsin Activation

Thermodynamic studies suggest that intermediates leading up to the inactive MI state result in a buildup of molecular interactions that are lost upon formation of MII. The transition from lumirhodopsin to MI results in a decrease in both enthalpy and entropy. The reverse trend is observed for these thermodynamic parameters in the transition from MI to MII (97). This trend led to the speculation that molecular interactions are built up during the transition from lumirhodopsin to MI, whereas the transition from MI to MII results in a loss of molecular interactions and an increase in flexibility of the protein. But the low-resolution 2-D structure of the MI state of rhodopsin did not reveal any conformational changes even at this late inactive stage (93).

The difference in enthalpy between lumirhodopsin or MII and MI is minimal (**Figure 3**). It would seem intuitive that if a significant conformational change were to occur upon transition from MI to MII, a more significant structural change would be observed in the transition from lumirhodopsin to MI. This speculation assumes

that the molecular interactions built up in MI are merely released in MII. Large entropic changes were observed upon transition to MII from MI (97), which may suggest that entropic effects play a significant role in the activation of the receptor.

Does GPCR Function Require Large Tertiary Structural Changes?

Bacteriorhodopsin is an archaeal proton pump that has a 7-TM structure with a retinal chromophore and photocycle intermediary states analogous to those of rhodopsin. Structural lessons learned from bacteriorhodopsin may provide some insight into both the interpretation of results from the recent crystal structure of photoactivated rhodopsin and understanding the structural requirements for GPCR activation. The M intermediate of bacteriorhodopsin is analogous to the MII intermediate of rhodopsin. Similar to the MII state of rhodopsin, the M intermediate of bacteriorhodopsin was predicted to undergo large-scale conformational changes within the transmembrane regions as indicated by low-resolution neutron and X-ray diffraction studies (98, 99). Although several crystal structures have been reported for the M state of bacteriorhodopsin, these structures are variable and exhibit only a fraction of the predicted large-scale conformational changes (98, 100). Studies of bacteriorhodopsin have shown that factors such as dehydration and crystal packing constraints can restrict the movement of helices while still exhibiting the characteristic absorbance spectrum of the activated M state (98, 99).

The requirement for large conformational changes to activate bacteriorhodopsin was questioned by studies on a triple mutant (D96G/F171C/F219L) of this proton pump. This mutant exhibited only minor structural changes while retaining 66% of the wild-type proton pumping activity (101). The ground state structure of the triple mutant did, however, exhibit large conformational changes typically seen in the M state of wild-type bacteriorhodopsin (101, 102). Studies of this triple mutant suggest that, although large conformational changes can occur in bacteriorhodopsin, they are not an absolute requirement for activation. Similarly, localized changes in a hydrogen-bonded network linking archaeal sensory rhodopsin II and its transducer protein HtrII are sufficient to produce a phototaxic response (103). Thus, minor changes in structure can lead to activity, albeit at lower levels.

Structural studies of bacteriorhodopsin demonstrate that a single photoactivated structure of rhodopsin may be inadequate to explain the full structural story of receptor activation. Structures of the inactive and photoactive states of rhodopsin suggest that if activation of the receptor is solely due to tertiary changes, then minor changes in conformation can result in signal propagation. Indeed, a "straitjacketed" rhodopsin created by disulfide bonding of mutant cysteine residues introduced into the rhodopsin sequence could activate transducin despite the restricted movement of its helices (104).

Small tertiary conformational changes on the order of 1 Å can lead to profound effects on the activity of proteins such as enzymes and receptors (105). Such changes result in dramatic effects that often occur at locations distal to the site of ligand binding. In the case of the bacterial aspartate receptor, little change in tertiary structure was observed in the presence and absence of ligand (106, 107). However, differences

were observed in the quaternary structure of the dimeric receptor between the ligand bound and unbound states. So a combination of small tertiary changes and larger quaternary changes likely defines the signal transduction properties of this system. Similarly, GPCR activity may also involve quaternary changes that occur within an oligomeric arrangement of receptor molecules.

Allosterism in proteins can be achieved in the absence of conformational changes through changes in thermal fluctuations of the protein (108, 109). Such thermal fluctuations usually are considered to be random and are rarely considered to have any effect on the activity of GPCRs. The large entropic contribution to the formation of the MII state of rhodopsin may indicate a need to reconsider thermal fluctuations of the receptor as an integral part of the activation mechanism (97).

Atomic force microscopy (AFM): a high-resolution method that permits imaging, manipulation, and detection of chemical and physical properties of biological material

OLIGOMERIC CONTEXT OF G PROTEIN–COUPLED RECEPTOR ACTIVATION

Oligomerization of GPCRs

Most membrane proteins, including GPCRs, can form dimers or larger oligomers (35, 110–113). However, the existence of monomers and their possible physiological function cannot be ruled out for GPCRs (114–116). Organization of GPCRs into oligomeric clusters has been inferred from studies using several different methods, including radioligand binding, coimmunoprecipitation, BRET, FRET, and atomic force microscopy (AFM). Cryo-electron tomography images of minimally perturbed murine rod outer segments display a highly concentrated heterogeneous distribution of rhodopsin in disc membranes (117). AFM images of rhodopsin in native disc membranes provide the clearest structural picture of the oligomeric arrangement of a GPCR to date (118, 119). Rows of rhodopsin dimers have been observed directly by AFM and the spatial constraints from these studies have been used to construct a molecular model of a rhodopsin oligomer (120) (**Figure 5**). This model indicates that the rhodopsin dimer offers a complementary platform for the binding of a single transducin or arrestin molecule, which each exhibit a "footprint" larger than that of a rhodopsin monomer (121, 122).

The dimer interface of GPCR oligomers likely involves the transmembrane regions of the receptor. The large amino-terminal regions that form distinct ligand-binding domains in some GPCRs may also form a dimer interface because those regions have a propensity to form oligomers, even in the absence of the transmembrane region of the receptor. The amino-terminal region of the mGluR, FSH receptor, and frizzled all form dimers in crystal structures in a manner compatible with dimerization of the 7-TM region (9, 18, 19, 25). In the case of the mGluR family of receptors, the VFT domain dimer is maintained via disulfide bond linkage (123, 124) and the activation mechanism involves a change in quaternary structure of the two monomers with respect to each other (11, 18, 19).

Perhaps the clearest demonstration that GPCRs can exist and function physiologically as oligomers comes from examples of receptors that form hetero-oligomers, which permit a more diverse signaling repertoire from a limited pool of gene

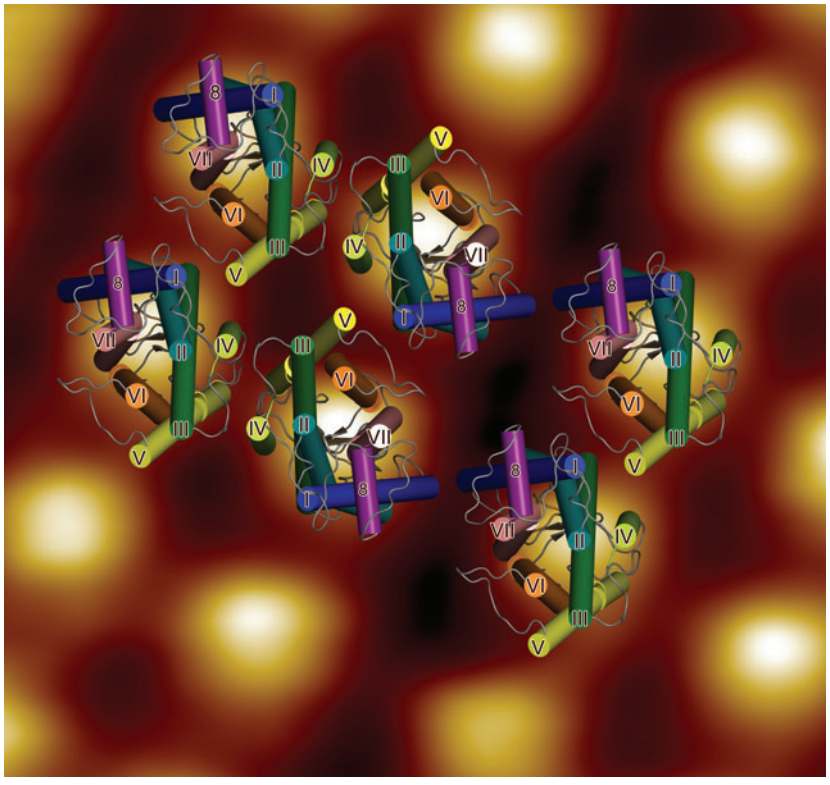


Figure 5

Model of a rhodopsin oligomer. A model of a rhodopsin oligomer based on distance constraints measured in AFM images of native murine disc membranes is displayed (119) (PDB ID: 1N3M). A top view is shown with helices represented as cylinders and numbered. Two rows of rhodopsin dimers are shown. A pair of rhodopsin dimers packed in the same row is shown on the left. Only one receptor molecule from rhodopsin dimers in the adjacent row on the right is shown. The model is positioned on top of an averaged AFM image of rhodopsin molecules created from raw data presented in Reference 118.

products (125). The functional unit of the $GABA_B$ receptor is an obligate hetero-oligomer composed of $GABA_{B1}$ and $GABA_{B2}$ subunits (126, 127). Taste receptors for sweet and umami responses also exist as obligate hetero-oligomers (128). Differentiation between the two taste responses results from the pairing of T1R3 with either T1R1 for umami or T1R2 for sweet. Hetero-oligomers of opioid receptors display novel pharmacological properties and have been detected in the spinal cords of mice (129, 130). Thus, oligomerization of GPCRs can occur physiologically.

Quaternary Changes Within Oligomers of GPCRs Upon Activation

Structural changes that accompany GPCR activation likely derive from a combination of both tertiary and quaternary changes. The small tertiary changes observed in the photoactivated structure of rhodopsin may point to an activation mechanism whereby small tertiary changes are amplified into larger quaternary changes. Much of the past emphasis in understanding the mechanism of GPCR activation has been at the level of tertiary structural changes. Arrangement of GPCRs into oligomeric arrays adds an additional level of complexity to this signaling system that requires further investigation. Sites within each monomer of a GPCR oligomeric complex appear to be coupled both functionally and structurally.

Communication between subunits of an oligomeric complex has been demonstrated in class 3 GPCRs. Each subunit of the $GABA_B$ receptor has a distinct role and communication between each is essential for receptor function (126). The $GABA_{B1}$ subunit binds agonists, whereas the $GABA_{B2}$ subunit is required for G protein coupling. Thus, ligand binding occurs at one subunit and that information is transmitted to the G protein via the other subunit. The requirement for only a single subunit or single 7-TM domain in an oligomeric complex to be activated for function has also been shown with the heteromeric T1R taste receptor and homomeric mGlu1 receptor (131, 132).

Single subunit activation was also observed with GPCRs from other classes (133, 134) and likely occurs in rhodopsin where a single photon can activate the system (135). Indeed, monomeric rhodopsin and monomeric β_2 adrenergic receptor reconstituted into an artificial lipid bilayer system both have been shown to bind G protein and catalyze GDP-GTP nucleotide exchange (136, 136a). Interestingly, in studies with the leukotriene B_4 receptor, binding of the G protein results in a structural asymmetry between the subunits of the oligomer, pointing to a role for the G protein in modulating communication between monomers of an oligomeric complex (134).

Changes in quaternary arrangements have been detected in the mGlu1α receptor by a FRET approach (137) and in the dopamine D_2 receptor by disulfide cross-linking (138). Changes in the intermonomeric FRET signal monitored in mGluR1α tagged with either CFP or YFP at cytoplasmic loop I or II indicate that agonists promote movements of cytoplasmic loops I away from each other and cytoplasmic loops II closer to one another. Cross-linking of substituted cysteine residues in transmembrane helix IV of the dopamine D_2 receptor implies that this helix is involved in the dimer interface. This observation is consistent with models of rhodopsin dimer derived from geometrical constraints measured by AFM studies on native disc membranes (120). Inverse agonist treatment and agonist treatment produced different sets of cross-linked substituted cysteine residues. Moreover, some cross-linked cysteine residues resulted in a constitutively active receptor, suggesting that cross-linking at the dimer interface can lock the receptor in an active state. These data point to a dynamic dimer interface and a role for quaternary changes in the activation of GPCRs.

A DYNAMIC CONTEXT OF G PROTEIN–COUPLED RECEPTOR ACTIVATION

A Funnel-Shaped Energy Landscape Description of Receptor Activation

Growing evidence that different types of agonists signal via distinct states of a receptor indicates that two-state models provide an inadequate description of receptor function. Even sequential binding schemes (e.g., 50), which are still based on the premise that each ligand follows a linear sequence of events through distinct conformations of the receptor, may be too simplistic. An emerging view of protein function is based on the idea that proteins exist as an ensemble of dynamic states and that the energy landscape describing their action is funnel-shaped (32, 139–142). This funnel-shaped

energy landscape concept initially was used to describe protein folding, but it can be extended to describe other processes involving proteins, such as ligand binding, protein-protein interactions, conformational diversity, and allosterism (143). Within the context of a funnel-shaped energy landscape, the receptor exists as an ensemble of dynamic states, and receptor activation can arise via multiple pathways that lead toward the bottom of the funnel rather than by a single linear pathway. This framework may depict the activation of GPCRs more accurately.

Dynamic Versus Conformational Changes

Dynamic and conformational types of structural changes can occur within proteins (108, 144). Dynamic changes arise from thermal fluctuations or motions within a protein that alter the position of atoms about their mean position. Conformational changes arise from distinct movements within the protein that result in a mean overall structure that is distinct from other mean conformations. Dynamic changes are mainly driven by entropy, whereas conformational changes are mainly driven by enthalpy.

Signaling via GPCRs proceeds through allosteric interactions between the ligand-binding site in the receptor and the nucleotide-binding site in the G protein. GPCR activation is typically conceptualized within the framework of conformational change. Conformational changes likely do play a role in GPCR activation and in the allosteric interchange between the receptor and G protein. However, dynamic changes must also be considered because they often can contribute significantly to allosteric processes.

NMR relaxation studies have provided insights into the effect of ligands on the dynamics of several proteins (145). In many cases, binding of a ligand reduces the level of dynamics, especially within the ligand-binding pocket. In such cases, the entropic penalty incurred upon ligand binding must be offset by an enthalpic contribution. In some systems, binding of a ligand increases the dynamics of the protein and the process therefore is largely entropically driven. Increased dynamic motions of the protein are not only observed within the immediate vicinity of the ligand-binding site but also in distal regions of the protein that may be involved in allosteric processes. Increased dynamic motion in distal regions also suggest that entropic effects can contribute to allostery.

Allostery can occur solely from dynamic structural changes in the protein in the absence of any conformational change. Theoretical considerations have demonstrated that significant changes in free energy can occur through changes in the frequency and amplitude of thermal fluctuations of a protein that can drive allosteric processes (108). Experimentally, negative cooperativity in the binding of cAMP to the dimeric catabolite activator protein has been shown to occur entirely by changes in dynamics rather than conformation (109).

Dynamic Nature of GPCRs and G Proteins

Proteins are not the static structures displayed in images and models derived from X-ray crystal structure data but rather are dynamic structures that sample many states

over time. Thermal fluctuations of a protein are not apparent in static images of crystal structures and the effect of these fluctuations are averaged out in thermodynamic equilibrium schemes. Although often ignored in explanations of protein function, apparently random fluctuations do play a role in protein function and can be channeled into productive events (146). Within the context of funnel-shaped energy landscapes, receptor activation occurs via an ensemble of different dynamic states of the protein rather than a single state. Ligands modulate the activity of receptors by altering the distribution of dynamic states. This distribution can also be modulated by other extrinsic factors that affect receptor function, such as G protein binding, mutations, lipid bilayer composition and solvent composition.

The dynamic nature of GPCRs is evident in molecular dynamics simulations (e.g., 147, 148). Thermal fluctuations observed during such simulations reflect the dynamic nature of the receptor. Molecular dynamics simulations of the β_2 adrenergic receptor either free of ligand, bound to an agonist, or bound to an antagonist suggest that ligands can exert effects on the extent of fluctuations in the protein (147). The free or agonist-bound receptor is more flexible in its transmembrane regions than the antagonist-bound form.

The dynamic nature of the β_2 adrenergic receptor has been demonstrated experimentally by single-molecule fluorescence spectroscopy (149). Monitoring the fluorescence signal of single receptor molecules covalently tagged with a fluorescent probe revealed that the protein is dynamic and that multiple states exist in the unliganded state. Inclusion of agonist shifted the distribution of fluorescent signals of individual receptor molecules and also displayed multiple, well-populated states that differed from those observed in the unliganded state.

The dynamic nature of GPCRs also has been demonstrated experimentally in solution NMR of α-^{15}N-labeled rhodopsin in the dark state (150, 151). Fluctuations of rhodopsin in the dark state are not only observed in the flexible loops of this receptor but also within the transmembrane region. Flexibility of the protein in the dark state is not evenly distributed throughout the molecule. Moreover, the dynamics of the backbone and side chain of a single tryptophan residue can be different as well. With the exception of a few specific regions of the protein that are proposed to play a role in locking the receptor in an inactive state, the receptor molecule is flexible and exhibits fluctuations. Activation of the receptor releases these localized constraints and that information is propagated via dynamic fluctuations throughout the protein.

More recent studies utilizing electron paramagnetic resonance spectroscopy and solution NMR methods have revealed changes in dynamics in addition to conformational changes accompanying G protein activation (152–155). Activity of the G protein, similar to the receptor, probably does not proceed simply through an equilibrium between two conformationally distinct states. Site-directed spin labeling of $G\alpha_{i1}$ revealed that activation is accompanied by distinct conformational changes in switch regions that are consistent with those predicted by crystal structures (152, 153). The same studies also revealed dynamic fluctuations promoted by activation, suggestive of an ensemble of states rather than a single active or inactive state. Likewise, increased levels of dynamic motion in the $G\alpha$ subunit of transducin were promoted by interactions with activated receptors in solution NMR studies performed on a

^{15}N-labeled chimera of the Gα_t subunit stably coupled to light-activated rhodopsin in both nucleotide-free and GTPγS-bound forms (154, 155). Taken together, these data suggest an activation process that involves both distinct conformational changes and dynamic changes.

CONCLUDING REMARKS

Accumulation of crystal structures of rhodopsin and of data from more recent approaches to the study of GPCRs has not ended the debate about the mechanisms

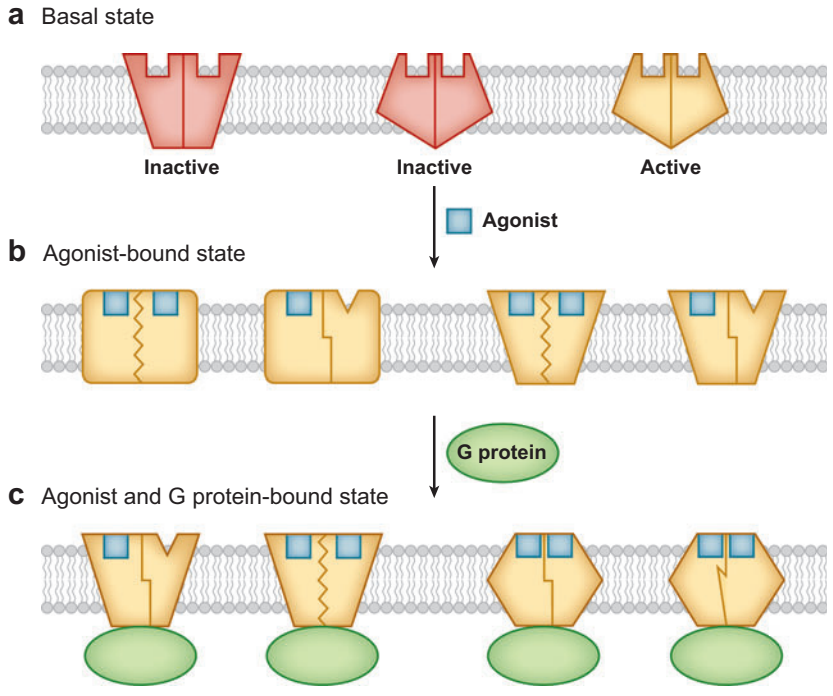


Figure 6

Oligomeric and dynamic context of receptor activation. (*a*) The basal state of a dimeric receptor. Thermal fluctuations within the receptor can result in multiple dynamic states of the receptor. Most of the states will be inactive (*red*) but a minor population may be constitutively active (*yellow*). Different receptor shapes shown represent both dynamic and conformational structural differences. (*b*) Agonist-bound state of a dimeric receptor. Binding of an agonist (*blue square*) leads to the activation of the receptor (*yellow*). An agonist potentially can cause several types of changes in the receptor that result in activity. Binding of an agonist to the receptor can change the distribution or population of receptor states, alter the dimer interface through quaternary changes, and cause cooperativity that affects binding of the next equivalent of an agonist. (*c*) Binding of both an agonist and a G protein (*green*) to the receptor can cause further changes in the structure of the receptor compared with those changes caused by agonist alone. Effects of the agonist and G protein on the dynamics and conformation of the receptor at the tertiary and quaternary levels will define the activation of the receptor.

underlying the regulation and activation of GPCRs, but rather, has opened up new areas for consideration and discussion. These new data do not necessarily negate old concepts but they do point in a direction that must extend beyond classical paradigms of receptor activation. Approaches that permit single-molecule measurements will be needed to tap into details of the signaling process that have been masked in bulk equilibrium studies. Structural approaches that provide dynamic information about the receptor are required to complement the static models of receptor structure derived from approaches such as X-ray crystallography to provide a better understanding of the dynamic contributions to receptor activation.

The activation mechanism of GPCRs is not merely an on-off switch dictated solely by conformational structural changes within the protein. The oligomeric and dynamic properties of the receptor must also be considered to understand the activation process (**Figure 6**). Thermal fluctuations exhibited by all proteins likely play some role in receptor activation. These dynamic contributions may result in an ensemble of states with many routes to receptor activation down a funnel-shaped energy landscape. GPCRs can exist as dimers or larger oligomers; the physiological roles of these structures need better understanding. Activation of some GPCRs appears to result in quaternary structural changes. But how these changes are coupled to tertiary changes in each subunit and what role they play in the activation process also require further investigation. Activity of the receptor is functionally and structurally linked to the G protein and vice versa. The G protein will exhibit dynamic and conformational changes as well as quaternary changes between its three subunits. Only with a detailed understanding of all these interconnected events and changes in dynamics and conformation of both the oligomeric GPCR and that of the heterotrimeric G protein will the mechanism of signal propagation through the 7-TM region of GPCRs be understood.

SUMMARY POINTS

1. The orthosteric ligand-binding pocket of GPCRs exists in many different types of structural forms to accommodate a broad range of external stimuli. Despite such variation, all GPCRs share a common 7-TM architecture and likely a common mechanism through this region to propagate external signals across the membrane.
2. The activity of GPCRs is often conceptualized within the context of two-state thermodynamic equilibrium models, such as the ternary complex model or extended ternary complex model where the receptor exists in equilibrium between an inactive state (R) and an active state (RG or R*), which can be modulated by ligands, G protein, or mutations. Receptor activity is presumed to be governed by an on-off molecular switch involving rigid-body conformational movements.
3. Minimal changes are noted between dark state and photoactivated state crystal structures of rhodopsin. This observation suggests that significant

conformational changes may not be the sole determinant of receptor activation. Activity, likely partial, may still be present in the absence of large tertiary structural changes.

4. GPCRs can exist in oligomeric clusters and the quaternary changes between each monomeric unit are likely to be important determinants in the mechanism of activation.
5. GPCRs are dynamic molecules. Their activity is better represented within the context of a funnel-shaped energy landscape in which the receptor exists in an ensemble of states and activation proceeds via multiple pathways.

FUTURE ISSUES

1. High-resolution structures of GPCRs other than rhodopsin with bound agonists and antagonists are needed. Structures of the receptor–G protein complex must also be determined.
2. The molecular/structural mechanisms of G protein coupling to GPCRs, including mechanisms of guanyl nucleotide exchange in the G protein induced by activated GPCRs and the reciprocal effects of the two components of the signaling complex, need to be better understood.
3. A greater understanding of GPCR action and properties in native tissues and in physiologically relevant cellular conditions is required.
4. The physiological role for GPCR oligomerization and the quaternary structural changes that accompany receptor activation need a greater molecular understanding.
5. The dynamic and conformational changes in the receptor that are central to the activation mechanism must be determined.
6. The energy landscape of GPCRs and the changes that occur in the energy landscape upon binding ligand and the G protein must be understood.

DISCLOSURE STATEMENT

Dr. Palczewski cofounded a company called Polgenix Inc., which is involved in developing general methods to study G protein–coupled receptors on the structural level.

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