

G Protein-coupled Receptors

II. MECHANISM OF AGONIST ACTIVATION*

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The majority of transmembrane signal transduction in response to hormones and neurotransmitters is mediated by G protein-coupled receptors (GPCRs).¹ Moreover, GPCRs are the principal signal transducers for the senses of sight and smell. GPCRs are characterized by seven membrane-spanning domains with an extracellular N terminus and a cytoplasmic C terminus (Fig. 1) (GPCR structure reviewed in Refs. 1–3). Based on certain key sequences, GPCRs can be divided into three major subfamilies, receptors related to rhodopsin (type A), receptors related to the calcitonin receptor (type B), and receptors related to the metabotropic receptors (type C). Of these, the rhodopsin subfamily is by far the largest and most extensively investigated and will be the focus of the present review.

GPCR domains involved in ligand binding are nearly as diverse as the chemical structures of the known agonists (1, 3, 5). Small molecular weight ligands bind to sites within the hydrophobic core formed by the transmembrane (TM) α -helices, whereas binding sites for peptides and protein agonists include the N terminus and extracellular hydrophilic loops joining the transmembrane domains (2, 5). Domains critical for interaction with the G protein have been localized to the second and third cytoplasmic loops and the C terminus (2).

High resolution structural analysis of GPCRs has been hindered by their low natural abundance and the difficulty in producing and purifying significant quantities of recombinant protein. A low resolution structure of rhodopsin obtained from electron diffraction of two-dimensional crystals has been useful in predicting the arrangement and relative orientation of the transmembrane domains (6, 7) (Fig. 2A). Mutagenesis studies aimed at identifying intramolecular interactions have provided evidence for a similar arrangement in other GPCRs (8–12), and several molecular models of different GPCRs have been developed (13–15).

This review will examine current progress in addressing a fundamental question in the field of GPCR-mediated signal transduction: the nature of the physical changes in receptors that link agonist binding to G protein activation. It will explore the differences and similarities in the activation of rhodopsin and other GPCRs by focusing on two questions. What conformational changes take place during receptor activation? How do agonists induce these conformational changes in the receptor?

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¹ The abbreviations used are: GPCR, G protein-coupled receptor; TM, transmembrane; NBD, *N,N'*-dimethyl-*N*-(iodoacetyl)-*N'*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-ethylenediamine.

Extended Ternary Complex Model

Perhaps the most widely accepted model used to describe agonist activation of GPCRs is the ternary complex model, which accounts for the cooperative interactions among receptor, G protein, and agonist (16). This model has recently been extended to accommodate the observation that many receptors can activate G proteins in the absence of agonist and that mutations in different structural domains of the receptor can enhance this agonist-independent activity (17, 18). The extended ternary complex model also accounts for the effects of different classes of drugs (full agonists, partial agonists, neutral antagonists, and inverse agonists) on receptor signaling. The model proposes that the receptor exists in an equilibrium of two functionally distinct states: the inactive (**R**) and the active (**R***) state. In the absence of agonists, the level of basal receptor activity is determined by the equilibrium between **R** and **R***. The efficacy of ligands is thought to be a reflection of their ability to alter the equilibrium between these two states. Whereas most properties of GPCRs can be explained by this model, several studies suggest that a more complex model may be necessary (reviewed by Kenakin (19, 20)). Nevertheless, for the purpose of our discussion we will use the terms **R** to refer to the inactive conformation of the receptor and **R*** to refer to the conformation capable of activating G proteins.

Agonist-induced Conformational Changes

Is Dimerization Involved in Receptor Activation?—Agonist-induced receptor dimerization is required for signal transduction for several non-GPCR receptor families having single membrane-spanning domains (such as receptor tyrosine kinases and the growth hormone receptor family (21)). Several recent reports provide evidence that GPCRs can also form dimers, raising the possibility that dimerization is part of the activation process (22–24). However, different mechanisms of dimer formation were observed for different receptors (β_2 adrenergic receptor (22), the δ -opioid receptor (23), and the metabotropic glutamate receptor (24)) suggesting that receptor dimerization is not essential for G protein activation but may play a role in other receptor functions such as subtype-specific receptor regulation.

Evidence for Protonation as a Key Element in Receptor Activation—The conserved DRY motif at the cytoplasmic side of TM3 (Fig. 1, orange) is highly conserved in members of the rhodopsin GPCR family (25). The invariably conserved arginine (ArgIII:26) in this motif has been hypothesized to be constrained in a hydrophilic pocket formed by conserved polar residues in TM1, TM2, and TM7 (Fig. 1, yellow) (14, 26). It has been proposed that receptor activation involves protonation of AspIII:25 causing ArgIII:26 to shift out of the polar pocket leading to cytoplasmic exposure of buried sequences in the second and third intracellular loops. The hypothesis is supported both by computational simulations and by the observation that mutating AspIII:25 results in increased agonist-independent receptor activity of both the α_{1b} adrenergic receptor (14, 26) and the β_2 adrenergic receptor.² Similarly, mutation of the corresponding GluIII:25 in rhodopsin also causes constitutive receptor activation (27, 28), and it has been demonstrated that proton uptake by GluIII:25 in rhodopsin is an important event in the formation of the activated metarhodopsin II intermediate (29).

GPCRs Are Maintained in an Inactive Conformation by Stabilizing Intramolecular Interactions—In several receptors it has been demonstrated that discrete mutations in the C-terminal region of the third intracellular loop can cause dramatic increases in agonist-independent receptor activity (reviewed in Ref. 30). Replacement of Ala²⁹³ in the α_{1b} receptor (Fig. 1, green) with any other residue resulted in higher constitutive activity (31). It was therefore proposed that important conformational constraints maintain

² S. Rasmussen, P. Ghanouni, A. D. Jensen, and U. Gether, manuscript in preparation.

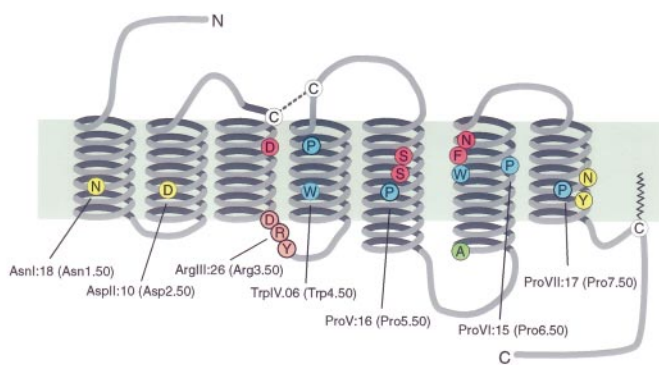


FIG. 1. Two-dimensional model of the β_2 receptor illustrating the key structural features of a GPCR belonging to the “rhodopsin-like” subfamily. The most conserved residue in each transmembrane segment is indicated both using the Schwartz nomenclature (59) and the Ballesteros-Weinstein nomenclature (15). In the Schwartz nomenclature the most conserved residue in each helix had been given a generic number according to its position in the helix. In the Ballesteros-Weinstein nomenclature the most conserved residue in each helix had been given the number 50. A series of conserved tryptophans and prolines are indicated in *blue*. The kink that may be caused by ProVI:15 has been suggested to be critical for the conformational changes involved in receptor activation (49). The almost invariable disulfide bridge between extracellular loops 2 and 3 and the conserved palmitoylation site in the C-terminal tail are indicated in *white*. Residues AspIII:08, SerV:12, SerV:16, PheVI:17, and AsnVI:20 shown to be involved in binding of epinephrine to the β_2 adrenergic receptor are indicated in *red* (3, 60). Mutations at Ala²⁹³ (*green*) in the α_{1D} receptor lead to agonist-independent activation (31). Other residues are discussed in the text.

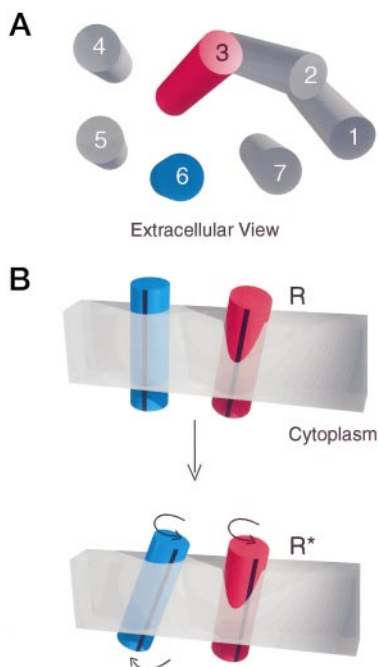


FIG. 2. A, arrangement of transmembrane domains of a prototypical G protein-coupled receptor as viewed from the extracellular surface of the membrane (based on the projection maps from two-dimensional crystals of rhodopsin (7)). **B,** proposed conformational changes in TM3 and TM6 following agonist activation based on studies of rhodopsin and the β_2 adrenergic receptor.

the receptor preferentially in an inactive conformation and that these constraint(s) are released upon activation (or by specific mutations) causing key sequences to be exposed to the G protein (30, 31). Recently, this hypothesis has been supported by the observation that a mutation causing constitutive activation of the β_2 receptor is associated with a marked structural instability and enhanced conformational flexibility (32).

There is compelling experimental evidence from several different receptors supporting the existence of a stabilizing network of intramolecular interactions constraining the receptor in the inactive conformation (14, 33–36). In rhodopsin for example, disruption of a presumed salt bridge between TM3 and TM7 results in constitutive activation of opsin in the absence of chromophore (33). Experi-

ments on a series of chimeric luteinizing hormone/follicle-stimulating hormone receptors provide evidence that important stabilizing interactions between TM5 and TM6 are responsible for the resistance of the follicle-stimulating hormone receptor to constitutively activating mutations observed in the highly homologous luteinizing hormone receptor (35).

Javitch and co-workers (37) obtained evidence for a conformational rearrangement of TM6 in a constitutively activated β_2 adrenergic receptor, providing additional support for an important role of TM6 in the network of conformational constraints required to maintain the receptor in the inactive state. A cysteine in TM6, which is not accessible to modification by a charged hydrophilic sulfhydryl-specific reagent in the wild type β_2 receptor, becomes accessible in the constitutively active mutant (37). Further support for the importance of the orientation of TM6 relative to TM5 and TM3 in receptor activation comes from studies using engineered metal ion binding sites. Histidine substitutions can be used to generate a zinc-binding site between two TM domains. Zinc binding to engineered metal ion binding sites linking TM6 and TM5 in the NK-1 (substance P) (38) and TM6 and TM3 in rhodopsin (39) prevented receptor activation. A similar result was obtained by intramolecular disulfide cross-linking between engineered pairs of cysteines in rhodopsin (40, 41).

Dissecting Specific Conformational Changes Involved in Receptor Activation—Rhodopsin activation has been analyzed by different forms of spectroscopy including Fourier transform infrared resonance spectroscopy (42, 43), surface plasmon resonance spectroscopy (44), tryptophan UV absorbance spectroscopy (45), and EPR spectroscopy (41, 46, 47). All approaches have consistently provided evidence for significant conformational rearrangements accompanying transition of rhodopsin to metarhodopsin. Using tryptophan UV absorbance spectroscopy Sakmar and co-workers (45) were able to obtain evidence that photoactivation involves movement of TM3 relative to TM6. Further insight into the character of conformational changes in rhodopsin has been obtained by Khorana, Hubbell and co-workers (41, 46, 47) using EPR spectroscopy in combination with multiple cysteine substitutions. By site-selective incorporation of pairs of sulfhydryl-reactive spin labels in a series of double cysteine mutants they were able to measure changes in relative distance between TM3 and TM6 (41). The movement of TM3 was interpreted as being relatively small whereas the data pointed to a significant rigid-body movement of TM6 in a counterclockwise direction (when the TM is viewed from the extracellular surface of the receptor) and a movement of the cytoplasmic end of TM6 away from TM3 (41).

The use of fluorescence spectroscopic techniques in the β_2 adrenergic receptor has allowed the first direct structural analysis of conformational changes in a ligand-activated GPCR (32, 48, 49). The spectroscopic technique used in these studies takes advantage of the sensitivity of many fluorescent molecules to the polarity of their local molecular environment (48). The sulfhydryl-reactive fluorophore *N,N'*-dimethyl-*N*-(iodoacetyl)-*N'*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-ethylenediamine (NBD) was used to label free cysteine residues in purified, detergent-solubilized β_2 adrenergic receptor (48). Both the quantum yield of the emission spectrum and the limited accessibility to hydrophilic quenchers strongly suggested that one or more of the naturally occurring transmembrane cysteines was labeled with the fluorophore. Exposure of NBD-labeled receptor to agonist led to a reversible and dose-dependent decrease in fluorescence emission consistent with movements of the fluorophore to a more hydrophilic environment following binding of the full agonist isoproterenol (48). Interestingly, exposure of the NBD-labeled receptor to inverse agonists led to an apparent increase in fluorescence, suggesting that not only agonists but also inverse agonists can promote structural changes in a GPCR (48).

Spectral analysis of a series of mutant β_2 receptors having only one, two, or three of the natural cysteines available for fluorophore labeling showed that NBD bound to Cys¹²⁵ in TM3 and to Cys²⁸⁵ in TM6 was responsible for the observed agonist-induced changes in fluorescence (49). This suggests that movements of TM3 and TM6 occur during receptor activation (49). In a molecular model of the β_2 receptor based on the projection map of rhodopsin, the NBD attached to Cys¹²⁵ is bounded by the lipid bilayer and the interface of TM3 and TM4, whereas the NBD attached to Cys²⁸⁵ is bounded by

TM6 and TM7 and the lipid bilayer (49). In the framework of this model the change in fluorescence of NBD-labeled β_2 receptor can best be explained by a counterclockwise rotation (when viewed from the extracellular side of the membrane) of both TM3 and TM6, which would move the NBD molecules from the nonpolar lipid environment to the more polar interior of the protein (49).

In summary, both biophysical and mutagenesis studies of rhodopsin and a number of ligand-activated GPCRs provide evidence that the formation of the R^* state involves movements of TM3 and TM6. Fig. 2*B* illustrates the type of movement that would be predicted from the spectroscopic studies of rhodopsin and the β_2 adrenergic receptor (41, 49). It should be noted that these studies do not exclude the possibility that other TMs or cytoplasmic domains undergo significant movement during activation. Further investigation will be required to provide a more detailed map of changes in GPCR structure during activation.

Mechanism of Agonist Activation

The Mode of Activation of Rhodopsin Is Unique among GPCRs—As discussed above, experimental evidence suggests that the conformational changes associated with activation of rhodopsin are similar to changes associated with activation of ligand-activated GPCRs such as the β_2 adrenergic receptor. However, the remarkable structural diversity among GPCR agonists (small molecules, peptides, and proteins) and the apparent lack of a common agonist-binding site suggest that the mechanism by which agonists induce the activating conformational changes in GPCRs may differ significantly for different receptors (5). Rhodopsin is unique among the GPCRs in that its ligand is covalently bound to the receptor as an inverse agonist and upon absorption of a photon isomerizes to an agonist within the binding pocket (reviewed by Sakmar (50)). Thus, the process of ligand binding is not part of the activation process. This specialized mechanism of activation may be necessary to facilitate the very rapid response of rhodopsin to light. Conversion from the inverse agonist *cis*-retinal to the full agonist *trans*-retinal occurs within femtoseconds of photon activation. Rhodopsin then rapidly undergoes a series of conformational changes that have been characterized spectroscopically (rhodopsin > bathorhodopsin > lumirhodopsin > metarhodopsin I > metarhodopsin II). The structural changes associated with the formation of metarhodopsin II, the R^* form of rhodopsin, are observed within microseconds of photoactivation even in detergent solution in the absence of transducin (46). Metarhodopsin II then undergoes a slow ($t_{1/2} \sim 6$ min) transition to the inactive metarhodopsin III (46). This inactivating transition is associated with the hydrolysis and release of *trans*-retinal from the binding pocket (51). It is interesting that free *trans*-retinal is not a very effective agonist for opsin (52, 53), producing only $\sim 14\%$ of the response observed for light-activated rhodopsin (52). Thus, the efficient activation of rhodopsin by *trans*-retinal requires that the agonist be rapidly generated by photoisomerization of the prebound inverse agonist *cis*-retinal. The less efficient activation of opsin by free *trans*-retinal may more closely reflect the process of activation of other GPCRs.

In contrast to the extensive characterization of retinal photoisomerization and the subsequent effects on the conformation of rhodopsin, very little is known about the mechanism by which binding of diffusible agonists such as catecholamines, peptides, and glycoprotein hormones leads to the formation of the R^* state. Recently it has been possible to study agonist-induced conformational changes in the human β_2 receptor in real time by fluorescence spectroscopy (32, 48, 49). These studies were done under conditions that are similar to those used to study conformational changes in rhodopsin (in the detergent β -dodecyl maltoside). In contrast to the rapid activation and slow inactivation kinetics of rhodopsin, the agonist-induced conformational changes observed in purified β_2 adrenergic receptor are slow ($t_{1/2} \sim 3$ min) (48), even at agonist concentrations that would be predicted to ensure saturation of binding sites in less than 1 min (54). Reversal of the agonist-induced conformation by antagonists is relatively rapid ($t_{1/2} \sim 30$ s) (48). The differences in activation kinetics between rhodopsin and the β_2 receptor may be influenced somewhat by the different methods used to monitor conformational changes but most likely reflect fundamental differences in the mechanism of activation by a covalently bound agonist (*trans*-retinal) and a diffusible agonist

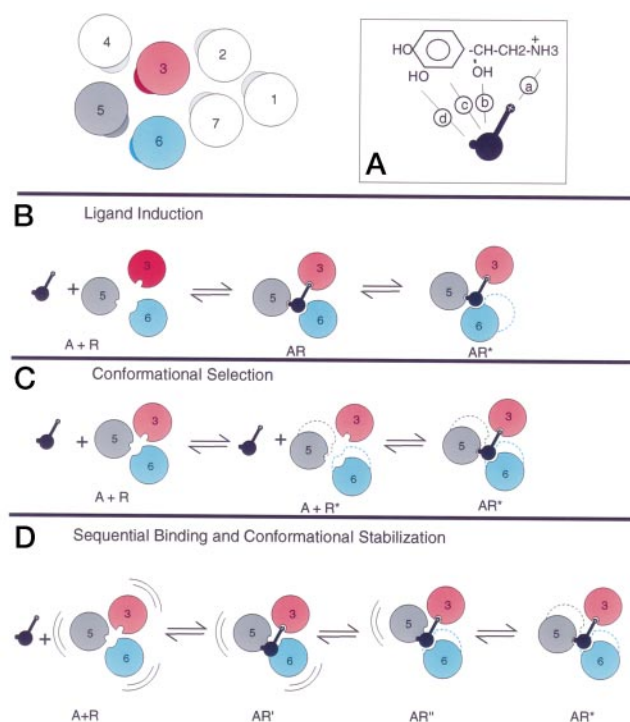


FIG. 3. *A*, sites of interaction between a catecholamine agonist and the β_2 adrenergic receptor (reviewed in Ref. 3). The amine (*a*) of agonists and antagonists interacts with an aspartate in TM3 through a salt bridge. The aromatic catechol ring (*c*) is thought to interact with a Phe²⁹⁰ in TM6. There is also evidence for a functional interaction between the β -hydroxyl (*b*) and Tyr²⁹⁶ in TM6 of the β_2 adrenergic receptor (60). Hydroxyl groups on the catechol ring (*d*) have been shown to interact with serines on TM5. *B–D*, models for the mechanism of agonist activation of the β_2 adrenergic receptor are discussed in the text (*B*, ligand induction; *C*, conformational selection; *D*, sequential binding and conformational stabilization). Only TMs 3, 5, and 6 are shown to simplify the illustration.

(isoproterenol).

Models of Activation by Diffusible Agonists—The binding site for catecholamines in the β_2 adrenergic receptor is remarkably similar to the binding site for retinal in rhodopsin (3, 50). The essential sites of interaction between catechol agonists and the β_2 adrenergic receptor are illustrated in Fig. 3*A*. Several models for the interaction of ligands with their receptors have evolved from the study of receptors, enzymes, and ligand-gated ion channels (55). The β_2 adrenergic receptor is used to illustrate these models in Fig. 3, *B–D*. “Ligand induction” (56), shown mechanistically in Fig. 3*B*, predicts that transition from the inactive to the active state is extremely rare in the absence of agonist because of the energy barriers between R and R^* . The free energy of agonist binding to R is used to overcome the energy barrier and facilitates (or induces) the transition to R^* . This model is consistent with the mechanism of activation of rhodopsin in that a photon rapidly converts the inverse agonist *cis*-retinal to the agonist *trans*-retinal, thereby inducing a rapid conformational change in the protein. The model could also be used to explain the slow agonist-induced conformational change observed for the β_2 receptor by proposing a rapid association rate for agonist binding to R and a slow rate for the transition from AR to AR^* . However, the model is inconsistent with the high basal activity observed for many ligand-activated GPCRs, suggesting that the energy barrier between R and R^* is surmountable in the absence of agonist.

The model shown in Fig. 3*C* will be referred to as “conformational selection” and is based on the model proposed by Koshland and Neet (57) and the extended ternary complex model for GPCRs (17). Transitions between R and R^* can occur in the absence of agonists. Agonists bind preferentially to the R^* conformation and thereby shift the equilibrium and increase the proportion of receptor in R^* . Inverse agonists bind preferentially to R and, therefore, reduce the population of receptor in R^* . This model can account for the basal activity of GPCRs in the absence of agonist and explains the action of inverse agonists. However, if the affinity of a full agonist for R is

assumed to be negligible, the model does not readily accommodate the observation that the association rate for agonist binding is very rapid whereas the kinetics of agonist-induced conformational change in the absence of G protein is slow for the β_2 adrenergic receptor. The model would predict that the association rate for agonist binding is limited by the rate of transition from **R** to **R***, because agonist would only bind to **R***.

In limiting the number of conformational states to two (**R** and **R***), these models (Fig. 3, *B* and *C*) fail to predict the observation that both agonists and inverse agonists protect the β_2 adrenergic receptor against thermal denaturation (32) and proteolysis (58). These experiments suggest that the unliganded receptor represents a distinct state that is susceptible to denaturation and proteolysis, whereas the conformations of the inverse agonist-bound state and the agonist-bound state are both resistant.

Based on these observations a third model can be formulated (Fig. 3*D*). This model predicts that the unliganded receptor exists in a unique state **R** that can undergo transitions to at least two other states, **R°** and **R***. **R°** is stabilized by inverse agonists, and **R*** is stabilized by agonists. Mechanistically, this could be explained by proposing that the unliganded receptor (**R**) is not highly constrained by stabilizing intramolecular interactions and is therefore more susceptible to thermal denaturation and proteolysis. Moreover, **R** may undergo spontaneous transitions to the **R*** state, explaining the high basal activity observed for some GPCRs. As shown in Fig. 3*D*, binding of agonist domains occurs sequentially, resulting in a series of conformational states that are intermediates (**R'** and **R''**) between **R** and **R***. As discussed above, results from mutagenesis experiments indicate that β_2 receptor agonists have several functionally important sites of interaction with the receptor. Binding may involve an initial interaction between receptor and one structural group of the agonist. Following the initial binding of one structural group, binding of the remaining groups occurs in a sequential manner as a result of random and spontaneous movements of TM domains to positions that permit interaction with the functional groups. Each interaction between the receptor and the agonist stabilizes one or more TM domains until the receptor has been stabilized in the active **R*** state. A similar mode of binding can be envisioned for inverse agonists resulting in stabilization of the **R°** state. Partial agonists may stabilize one of the intermediate states (**R'** or **R''**), thereby increasing the chance of spontaneous isomerization to **R***; or they may stabilize unique conformational states having lower affinity for the G protein. This model would be consistent both with a rapid association rate for agonists (formation of **AR'**) and the relatively slow rate of conformational change observed spectroscopically (formation of **AR***). G proteins may interact with the receptor to stabilize it in one of the intermediate states (**R'** or **R''**) and thereby influence both agonist binding affinity and the kinetics of the conformational change.

The model shown in Fig. 3 represents our best efforts to explain the mechanism of GPCR activation with the limited experimental data available. A more complete understanding of the molecular mechanism of GPCR activation will require a high resolution structure, more detailed information about the structural changes induced in the receptor by different classes of ligands, and information from time-resolved studies characterizing the sequence of conformational changes that follow ligand binding.

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REFERENCES

- Ji, T. H., Grossmann, M., and Ji, I. (1998) *J. Biol. Chem.* **273**, 17299–17302
- Wess, J. (1997) *FASEB J.* **11**, 346–354
- Strader, C. D., Fong, T. M., Graziano, M. P., and Tota, M. R. (1995) *FASEB J.* **9**, 745–754
- Beck-Sickinger, A. G. (1996) *Drug Disc. Today* **1**, 501–513
- Schwartz, T. W., and Rosenkilde, M. M. (1996) *Trends Pharmacol. Sci.* **17**, 213–216
- Schertler, G. F., Villa, C., and Henderson, R. (1993) *Nature* **362**, 770–772
- Unger, V. M., Hargrave, P. A., Baldwin, J. M., and Schertler, G. F. (1997) *Nature* **389**, 203–206
- Pittel, Z., and Wess, J. (1994) *Mol. Pharmacol.* **45**, 61–64
- Liu, J., Schoneberg, T., van Rhee, M., and Wess, J. (1995) *J. Biol. Chem.* **270**, 19532–19539
- Zhou, W., Flanagan, C., Ballesteros, J. A., Konvicka, K., Davidson, J. S., Weinstein, H., Millar, R. P., and Sealfon, S. C. (1994) *Mol. Pharmacol.* **45**, 165–170
- Mizobe, T., Maze, M., Lam, V., Suryanarayana, S., and Kobilka, B. K. (1996) *J. Biol. Chem.* **271**, 2387–2389
- Elling, C. E., and Schwartz, T. W. (1996) *EMBO J.* **15**, 6213–6219
- Baldwin, J. M. (1993) *EMBO J.* **12**, 1693–1703
- Scheer, A., Fanelli, F., Costa, T., De Benedetti, P. G., and Cotecchia, S. (1996) *EMBO J.* **15**, 3566–3578
- Ballesteros, J. A., and Weinstein, H. (1995) *Methods Neurosci.* **25**, 366–428
- De Lean, A., Stadel, J. M., and Lefkowitz, R. J. (1980) *J. Biol. Chem.* **255**, 7108–7117
- Samama, P., Cotecchia, S., Costa, T., and Lefkowitz, R. J. (1993) *J. Biol. Chem.* **268**, 4625–4636
- Chidiac, P., Hebert, T. E., Valiquette, M., Dennis, M., and Bouvier, M. (1994) *Mol. Pharmacol.* **45**, 490–499
- Kenakin, T. (1997) *Trends Pharmacol. Sci.* **18**, 416–417
- Kenakin, T. (1995) *Trends Pharmacol. Sci.* **16**, 232–238
- Wells, J. A. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 1–6
- Hebert, T. E., Moffett, S., Morello, J. P., Loisel, T. P., Bichet, D. G., Barret, C., and Bouvier, M. (1996) *J. Biol. Chem.* **271**, 16384–16392
- Cvejić, S., and Devi, L. A. (1997) *J. Biol. Chem.* **272**, 26959–26964
- Romano, C., Yang, W. L., and O'Malley, K. L. (1996) *J. Biol. Chem.* **271**, 28612–28616
- Probst, W. C., Snyder, L. A., Schuster, D. I., Brosius, J., and Sealfon, S. C. (1992) *DNA Cell Biol.* **11**, 1–20
- Scheer, A., and Cotecchia, S. (1997) *J. Recept. Signal Transduct. Res.* **17**, 57–73
- Cohen, G. B., Yang, T., Robinson, P. R., and Oprian, D. D. (1993) *Biochemistry* **32**, 6111–6115
- Fahmy, K., and Sakmar, T. P. (1993) *Biochemistry* **32**, 7229–7236
- Arnis, S., Fahmy, K., Hofmann, K. P., and Sakmar, T. P. (1994) *J. Biol. Chem.* **269**, 23879–23881
- Lefkowitz, R. J., Cotecchia, S., Samama, P., and Costa, T. (1993) *Trends Pharmacol. Sci.* **14**, 303–307
- Kjelsberg, M. A., Cotecchia, S., Ostrowski, J., Caron, M. G., and Lefkowitz, R. J. (1992) *J. Biol. Chem.* **267**, 1430–1433
- Gether, U., Ballesteros, J. A., Seifert, R., Sanders-Bush, E., Weinstein, H., and Kobilka, B. K. (1997) *J. Biol. Chem.* **272**, 2587–2590
- Robinson, P. R., Cohen, G. B., Zhukovsky, E. A., and Oprian, D. D. (1992) *Neuron* **9**, 719–725
- Sealfon, S. C., Chi, L., Ebersole, B. J., Rodic, V., Zhang, D., Ballesteros, J. A., and Weinstein, H. (1995) *J. Biol. Chem.* **270**, 16683–16688
- Kudo, M., Osuga, Y., Kobilka, B. K., and Hsueh, A. J. W. (1996) *J. Biol. Chem.* **271**, 22470–22478
- Groblewski, T., Maigret, B., Larguier, R., Lombard, C., Bonnafous, J. C., and Marie, J. (1997) *J. Biol. Chem.* **272**, 1822–1826
- Javitch, J. A., Fu, D., Liapakis, G., and Chen, J. (1997) *J. Biol. Chem.* **272**, 18546–18549
- Elling, C. E., Nielsen, S. M., and Schwartz, T. W. (1995) *Nature* **374**, 74–77
- Sheikh, S. P., Zvyaga, T. A., Lichtarge, O., Sakmar, T. P., and Bourne, H. R. (1996) *Nature* **383**, 347–350
- Yu, H., Kono, M., McKee, T. D., and Oprian, D. D. (1995) *Biochemistry* **34**, 14963–14969
- Farrens, D. L., Altenbach, C., Yang, K., Hubbell, W. L., and Khorana, H. G. (1996) *Science* **274**, 768–770
- Rothschild, K. J., Cantore, W. A., and Marrero, H. (1983) *Science* **219**, 1333–1335
- Garcia-Quintana, D., Francesch, A., Garriga, P., de Lera, A. R., Padros, E., and Manyosa, J. (1995) *Biophys. J.* **69**, 1077–1082
- Salamon, Z., Wang, Y., Brown, M. F., Macleod, H. A., and Tollin, G. (1994) *Biochemistry* **33**, 13706–13711
- Lin, S. W., and Sakmar, T. P. (1996) *Biochemistry* **35**, 11149–11159
- Farahbakhsh, Z. T., Hideg, K., and Hubbell, W. L. (1993) *Science* **262**, 1416–1419
- Resek, J. F., Farahbakhsh, Z. T., Hubbell, W. L., and Khorana, H. G. (1993) *Biochemistry* **32**, 12025–12032
- Gether, U., Lin, S., and Kobilka, B. K. (1995) *J. Biol. Chem.* **270**, 28268–28275
- Gether, U., Lin, S., Ghanouni, P., Ballesteros, J. A., Weinstein, H., and Kobilka, B. K. (1997) *EMBO J.* **16**, 6737–6747
- Sakmar, T. P. (1998) *Prog. Nucleic Acid Res. Mol. Biol.* **59**, 1–34
- Farrens, D. L., and Khorana, H. G. (1995) *J. Biol. Chem.* **270**, 5073–5076
- Han, M., Lin, S. W., Minkova, M., Smith, S. O., and Sakmar, T. P. (1996) *J. Biol. Chem.* **271**, 32337–32342
- Jager, S., Palczewski, K., and Hofmann, K. P. (1996) *Biochemistry* **35**, 2901–2908
- Williams, L. T., and Lefkowitz, R. J. (1977) *J. Biol. Chem.* **252**, 7207–7213
- Franklin, T. J. (1983) *J. Neural Transm. Suppl.* **18**, 55–60
- Bennett, W. S., Jr., and Steitz, T. A. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75**, 4848–4852
- Koshland, D. E., Jr., and Neet, K. E. (1968) *Annu. Rev. Biochem.* **37**, 359–410
- Kobilka, B. K. (1990) *J. Biol. Chem.* **265**, 7610–7618
- Schwartz, T. W. (1994) *Curr. Opin. Biotechnol.* **5**, 434–444
- Wieland, K., Zuurmond, H. M., Krasel, C., Ijzerman, A. P., and Lohse, M. J. (1996) *Proc. Natl. Acad. Sci. U. S. A.* **93**, 9276–9281