Recent Progress in Understanding the Conformational Mechanism of Heterotrimeric G Protein Activation

Nguyen Minh Duc, Hee Ryung Kim and Ka Young Chung*

School of Pharmacy, Sungkyunkwan University, Suwon 16419, Republic of Korea

Abstract

Heterotrimeric G proteins are key intracellular coordinators that receive signals from cells through activation of cognate G protein-coupled receptors (GPCRs). The details of their atomic interactions and structural mechanisms have been described by many biochemical and biophysical studies. Specifically, a framework for understanding conformational changes in the receptor upon ligand binding and associated G protein activation was provided by description of the crystal structure of the β2-adrenoceptor-Gs complex in 2011. This review focused on recent findings in the conformational dynamics of G proteins and GPCRs during activation processes.

Key Words: G protein, G protein-coupled receptor, Structure, Dynamics

OVERVIEW OF HETEROTRIMERIC G PROTEINS

Heterotrimeric guanine nucleotide-binding proteins (G proteins) are signal transducers that play a crucial role in mediating downstream signal transduction of G protein-coupled receptors (GPCRs) (Ross and Gilman, 1980). G proteins contain three subunits, α, β, and γ. In their inactive state, the Gα subunit binds to guanosine diphosphate (GDP) to form a stable complex with Gβγ partners. When agonist-activated GPCRs couple to the GDP-bound form of G proteins, GDP is replaced by guanosine triphosphate (GTP), which induces dissociation of the Gα subunit from GPCR and Gβγ subunits (Fig. 1A). The GTP-bound Gα subunit or the Gβγ subunits transduce signals through interaction with downstream effectors (Hamm, 1998). Finally, the signal is terminated by the intrinsic GTPase catalytic activity of the Gα subunit, which hydrolyzes GTP to GDP, enabling recruitment of Gβγ subunits to form inactive heterotrimers (Fig. 1A).

There are 21 known isoforms encoded by 16 Gα subunit genes, 6 documented Gβ subunits encoded by 5 genes, and 12 reported Gγ subunits in human (Simon et al., 1991; Downes and Gautam, 1999). These subunits can thus combine to form approximately 700 potential Gαβγ heterotrimers, which contributes to the selectivity as well as the specificity of both GPCRs and effector systems (Fletcher et al., 1998; Richardson and Robishaw, 1999). Despite this, G proteins are typically grouped into only four main classes on the basis of sequence similarity of the Gα subunit: Gαs, Gαi/o, Gαq/11, and Gα12/13 (Moreira, 2014). This classification also defines both the specificity and selectivity of effectors and receptors.

The Gs family includes 2 isoforms, Gs and Golf, which signal via stimulation of second messengers such as cAMP, as well as Src tyrosine kinase and protein kinase A (Neves et al., 2002). The Gi/o family is the largest subgroup, consisting of 8 members including Gi1, Gi2, Gi3, GoA, GoB, Gz, and Gt, which inhibit adenyl cyclase activity resulting in a decrease in intracellular cAMP levels (Neer, 1995). The Gq/11 family is composed of 5 isoforms: Qq, G11, G14, G15, and G16. The most important effector for Gq signaling is phospholipase C-β (PLC-β); this pathway produces the intracellular messengers inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 subsequently induces the release of calcium from the intracellular reservoir, while DAG recruits and activates protein kinase C. The G12/13 subgroup contains only 2 members, G12 and G13, which mediate activation of RhoGTPase nucleotide exchange factors (Siehler, 2009).

OVERVIEW OF G PROTEIN STRUCTURES

In the 1990s, the structures of Gα subunits in GTP- and GDP-bound forms were described as either a monomer or a...
Fig. 1. G protein structure and activation process. (A) The GPCR-mediated G protein activation cycle. (B and C) The representative structures of GDP-bound inactive state of G\textsubscript{ai} subunit (green, PDB: 1TAG) (B) and GTP-bound active state of G\textsubscript{ai} subunit (yellow, PDB: 1TND) (C). The GDP and GTP\textsubscript{GS} are shown as sticks. (D) Structural comparison near the nucleotide-binding pocket between GDP-bound inactive (green) and GTP-bound active (yellow) states.

Fig. 2. The structure of GPCR-G protein complex. (A) Structural comparison between β2AR-bound nucleotide-free G\textsubscript{ai}s subunit (PDB: 3SN6) and the GTP\textsubscript{GS}-bound G\textsubscript{ai}s subunit (1AZT). β2AR is colored as cyan, nucleotide-free G\textsubscript{ai}s as orange, and GTP\textsubscript{GS}-bound G\textsubscript{ai}s as green, respectively. GTP\textsubscript{GS} is shown as stick. The superimposition of Ras domains from each states shows the displacement of AH domain. (B) GPCR and G protein interfaces. The binding regions of G\textsubscript{ai} subunit with receptor are shown in red which constitute of αN/β1 hinge, α4/β6 loop, and C-terminus of α5 helix.
A heterotrimer (Noel et al., 1993; Coleman et al., 1994; Lambright et al., 1994; Sondek et al., 1994; Mixon et al., 1995; Wall et al., 1995; Lambright et al., 1996; Lutz et al., 2007; Nishimura et al., 2010). These studies revealed that the nucleotide-binding pocket is tightly sandwiched between two domains of the Gα subunit, the Ras-like (Ras) domain and the α-helical (AH) domain (Fig. 1B, 1C). In its inactive state, GDP is stabilized by interactions between the guanine ring of GDP and the P-loop, the α1 helix, and switch 1 of the Gα subunit (Fig. 1D). In the GTP-bound active state, the γ-phosphate group dynamically contacts the switches 1, 2, and 3, which helps to stabilize these highly flexible regions (Jones et al., 2012) (Fig. 1D).

Various biochemical and biophysical studies have investigated GPCR-mediated G protein activation (Preininger et al., 2013; Moreira, 2014; Duc et al., 2015). In 2011, the high-resolution X-ray crystal structure of the GPCR-G protein complex was first revealed using the β2-adrenoceptor-Gs complex (β2AR-Gs) as a model system (Rasmussen et al., 2011b). This structure provided fundamental information about the structural mechanism of GPCR-mediated G protein activation. It showed that the nucleotide-binding pocket opens via movement of the AH domain of the Gα subunit, and, furthermore, revealed the interfaces between GPCRs and G proteins (Fig. 2A). There are three major contact sites between β2AR and Gs: 1) the C-terminus of Gαs contacting transmembranes (TM3, 5, and 6) and the intracellular loop (ICL) 2 of β2AR, 2) the hydrophobic region surrounded by the αN/β1 hinge and β2/β3 loops of Gαs contacting ICL2 of β2AR, and 3) part of the α4 helix, α4/β6 loop, and β6 of Gαs contacting ICL3 of β2AR (Fig. 2B). Knowledge regarding the structure of β2AR-Gs heralded a new era in G protein studies and accelerated other biochemical and biophysical studies on the conformational mechanisms of G protein regulation. This review focuses on recent progress in this field with two main topics: 1) GPCR-mediated allosteric conformational changes of G proteins, 2) G protein-mediated allosteric modulation of GPCRs.

### GPCR-MEDIATED ALLOSTERIC CONFORMATIONAL CHANGES OF G PROTEINS

The distance between GPCR-G protein contact sites and the nucleotide-binding pocket is approximately 30 Å (Fig. 2A), and therefore an allosterical regulation induced by GPCRs

---

**Table 1. Summary of published papers described in this review**

| Authors          | Journal                      | Method                      | Main findings                                                                 |
|------------------|------------------------------|-----------------------------|-------------------------------------------------------------------------------|
| Shim et al.      | 2013, J. Biol. Chem. 288,    | Computer simulation         | This study described a structural model of cannabinoid CB1 receptor and G protein complex showing the important role of the α5 helix in G protein signaling. |
|                  | 32449                        |                             |                                                                               |
| Yao and Grant    | 2013, Biophys. J. 105, L08   | MD simulation               | This study revealed the intrinsic flexibility of the AH domain in the nucleotide-free state of Gαs. |
| Mnpotra et al.   | 2014, J. Biol. Chem. 289,    | Cross-linking and simulations | This study presented a model of the complex between cannabinoid CB2 receptor and G protein. |
|                  | 20259                        |                             |                                                                               |
| Alexander et al. | 2014, Nat. Struct. Mol. Biol.   21, 56 | Modeling                   | This study described the formation and structural basis of the Rho-Gi heterotrimer complex and the role of the α5 helix in G protein activation. |
| Kaya et al.      | 2014, J. Biol. Chem. 289,    | Crystallography, binding    | This study described the role of hydrophobic interactions between the α5 helix, β2-β3 strands, and α1 helix. |
|                  | 24475                        | assay                       |                                                                               |
| Dror et al.      | 2015, Science 348, 1361      | MD simulation, DEER          | This study found that the AH domain is spontaneously separated in the native state of GDP-bound Gαs, as well as investigated the important role of the α5 helix, subsequently β6/α5, in G protein activation. |
| Flock et al.     | 2015, Nature 524, 173        | Analysis                    | This study provided a universal mechanism for Gα activation by GPCRs.           |
| Sun et al.       | 2015, Nat. Struct. Mol. Biol.  22, 686 | Alanine-scanning mutagenesis | This study described the role of distinct residues in the stability of GDP, GTP, and receptor-bound states of G protein. |
| Yao et al.       | 2016, J. Biol. Chem. 291,    | Computer simulation         | This study provided the role of the β1 strand in activation of G proteins by GPCRs. |
|                  | 4742                         |                             |                                                                               |
| Goricanec et al. | 2016, Proc. Natl. Acad. Sci. U.S.A. 113, E3629 | NMR, SAXS, and MD simulations | This study indicated the highly dynamic flexibility of apo and GDP-bound states relating to activation by GPCR. |
| DeVree et al.    | 2016, Nature 535, 182        | Radio-ligand binding assay  | This study suggested that coupling to G proteins allosterically stabilized the ligand-binding pocket of a GPCR. |
| Pachov et al.    | 2016, J. Chem. Theory Comput. 12, 946 | Computer simulation        | This study mentioned that interactions between αN with ICL2 facilitates nucleotide exchange by weakening a salt bridge between the P-loop and Switch 1 through β1 strand. |
should be existed to transform signal from the binding sites to the nucleotide-binding pocket to trigger the release of GDP from Gα subunit. A number of recent studies sought to define the allosteric conformational changes in G proteins upon GPCR binding using in silico, biochemical, and biophysical approaches (Table 1).

The C-terminus of the Gα subunit is the major GPCR contact site (Fig. 2B), and therefore the interaction between a GPCR and the C-terminus of the Gα subunit may induce conformational changes allosterically in Gα through the α5 helix. Recent modeling and experimental studies predicted the critical role of the α5 helix in G protein activation by GPCRs. Using a combination of mutagenesis and MD simulation, Shim and colleagues first described the molecular basis of cannabinoid CB1 receptor coupling to heterotrimeric Gαiβγ proteins (Shim et al., 2013). This study described tight interactions between CB1 receptor and the C-terminal of the α5 helix of Gai, as well as emphasized the crucial role of these interactions in G protein activation. Alexander et al. (2014) applied Rosetta-based sampling and energy analysis to provide a structural mechanism for rhodopsin-mediated GDP release from Gi, and observed a 5.7-Å translation and 63° rotation of the α5 helix. More recently, a long time-scale MD simulation by Dror et al. also observed a 60° rotation of the α5 helix with the removal of GDP (Dror et al., 2015).

The displacement or rotation of the α5 helix appears to be linked to the perturbation of intramolecular interactions in the Gα subunit, which would facilitate GDP release (Fig. 3A). Dror et al. (2015)’s study suggested that α5 displacement upon receptor binding increases the flexibility of the guanine ring-contacting the β6/α5 loop, thus perturbing contact between GDP and the Ras domain. Alexander et al. (2014)’s study also suggested that G protein activation is associated with rearrangement of the intramolecular interaction between the α5 helix, β6/α5 loop, α1 helix, and αG helix. Subsequent experimental mutagenesis studies of the same group indicated that residue F336 in the α5 helix of Gai1 is crucially important in G protein activation because its mutation increases the rate of spontaneous GDP release (Kaya et al., 2014). The proposed mechanism involves F336 acting as a relay transmitting conformational changes from the C-terminus via a hydrophobic interaction with strands β2, β3 and α1 helix (Fig. 3A). More recently, Sun et al. (2015) revealed the particular importance of this residue and its surrounding contacts with the α1 helix, β1, and β2 strands in GPCR-G protein complex formation, as well as its role in the stability of GDP-bound Gi1.

A comprehensive analysis of available Gα crystal structures further emphasized the role of the α5 helix as a bridge for GPCR-mediated allosteric GDP release and suggested the α1 helix as a “hub”; the α1 helix links various important functional regions of Gα including the N-terminus of the α5 helix, AH domain, and GDP through universally conserved residues (Flock et al., 2015) (Fig. 3B). In this model, the mechanism of allosteric activation is triggered by movement of the α5 helix, subsequently breaking the contacts between the α5 and α1 helices leading to an increased flexibility in the α1 helix. The contacts between the α1 helix and AH domain as well as GDP are disrupted, thereby GDP affinity is weakened, which promotes GDP release together with AH domain separation. Importantly, this study indicated that the residues involved in these contacts are highly conserved across all Gα proteins suggesting that the above-mentioned mechanism is likely to be universal throughout Gα proteins. An experimental study also suggested the crucial role of the interaction between the α1 and α5 helices in GPCR-mediated G protein activation since cross-linking between these two helices, which restricts free movement or translocation of the α5 helix, impeded G protein coupling to the receptor (Kaya et al., 2014).

The hydrophobic region surrounded by the αN/β1 hinge and the β2/β3 loop of Gαs is another major contact site with
receptors (Fig. 3A), and the interaction of receptors with this region may induce allosteric conformational changes at the nucleotide-binding pocket through the β1 strand. A crosslinking study together with MD simulation data indicated the high conservation of Phe139 in ICL2 of cannabinoid CB2 receptor anchors in a hydrophobic triad formed by residues from the αN/β1 hinge, β2/β3 loop, and α5 helix of Gu1 (Mnptona et al., 2014). The authors also suggested that the interactions between ICL2 of CB2 receptor and the hydrophobic pocket in the Gu1 act as the key “registration” for complex formation. Recent MD simulations demonstrate new evidence for an “alternate allosteric route” through the β1 strand (Fig. 3A) (Yao et al., 2016). The role of the β1 strand is involved in the paths from receptor to the Ras-AH interface which expressed different favored routes in distinct states of Gα (apo, GDP-, and GTP-bound form). Indeed, the MD simulations of L32A of Go1, a highly conserved residue in the β1 strand, displayed an enhanced domain displacement and increased nucleotide exchange rate as well as G protein activation suggesting the functional relevance of this allosteric mutation. Furthermore, analysis of the structural dynamic of mutations in these regions manifests the novel role of the β1 strand together with β2, β3, P-loop, and Switch 1 in the modulation of domain opening that is critical for nucleotide exchange. Most recently, Pachov et al. (2016) also suggests that ICL2 of the receptor interacts with the N terminus of β1, subsequently weakening the interaction of the P loop with the nucleotide.

When GDP or GTP is bound to G proteins, Ras and AH domains are in the “close state” based on the X-ray crystal structures (Fig. 1). The interface between the two domains is comprised of interactions between the α1, αA, and αF helices and the linker 1 (α1/αA loop) and between the αG and αA helices, the β4/β3 loop, the αD/αE loop, and the switch 1 (Fig. 1D). It is noteworthy that the residues responsible for the interdomain interactions are highly conserved in all G protein family proteins (Flock et al., 2015; Sun et al., 2015). Once activated by GPCRs, they turn to the “open state” or nucleotide-free state by movement of the AH domain away from the Ras domain (Rasmussen et al., 2011b) (Fig. 2A). It has not been clear until recently if the domain displacement is cause or consequence of nucleotide release.

Surprisingly, Dror et al. (2015)’s simulation study showed that the AH domain fluctuates spontaneously between closed and open states relative to the Ras domain in the microsecond time scale even in the absence of GPCRs while GDP is still bound to the G protein. The spontaneous motion of the AH domain in the ensemble of native states was also mentioned in previous MD simulations (Jonesa et al., 2012; Yao and Grant, 2013). The contacts of GDP with the AH domain were occasionally disrupted and reformed, which leads to highly dynamic movement of this domain even in the receptor-free or GDP-bound inactive state, however, this spontaneous separation is not sufficient to trigger GDP release (Dror et al., 2015). More recently, Goricanec et al. (2016) indicated that the opening of the nucleotide binding pocket is more populated in the GDP-bound state compared to the GTP-bound state by a combination of NMR, small angle X-ray scattering (SAXS), circular dichroism (CD), and fluorescence spectroscopy. The data also showed that GDP binds to the Ras domain, but does not dock into the AH domain at the same time indicating weak contacts between the GDP and AH domain. GDP binding to the Ras domain has also been observed in a simulation with deletion of the AH domain (Markby et al., 1993). The simulations mimicking the α5 distal C-terminus in the receptor-bound conformation showed that GDP is dissociated from binding sites within microseconds; this study indicated that activation of G protein by GPCR triggers GDP release primarily by reducing GDP affinity with the Ras domain rather than by Ras-AH domain separation (Dror et al., 2015). These studies suggest that, upon GPCR binding, the weakening between GDP and the Ras domain is the major factor in GDP release.

Taken together, these combined studies show structural dynamics and conformational relevance of distinct states containing GDP, GTP, and receptor-bound Go, allowing us to gain insight into the activation process of G proteins which start from the GDP-bound form and progress to the nucleotide-free state or receptor bound form and finally to the GTP bound form or active state (Fig. 1A). The activation mechanism suggests the involvement of receptor induced allosteric conformational changes in the Go subunit through two major interactive sites, which was clearly identified previously (Duc et al., 2015). This includes the relocation and binding of the C terminus of Go to the receptor or formation of interactions between the αN/β1 hinge and the receptor, and the respective signal is subsequently transmitted either via a combination of the α5 helix, β6/α5 loop, and α1 helix, or via strand β1 to P-loop to destabilize the nucleotide-binding site and allow GDP release, as well as displacement of the helical domain.

G PROTEIN-MEDIATED ALLOSTERIC MODULATION OF GPCRS

It has been known that agonist-bound receptors adopt multiple conformations equilibrated between inactive and active states (Nyagaard et al., 2013; Manglik et al., 2015). In addition, biophysical and pharmacological studies suggest that the active conformation in intracellular regions of GPCRs is stabilized by nucleotide-free Go or by G protein mimetic nanobodies (Rasmussen et al., 2011a, 2011b; Kruse et al., 2013; Huang et al., 2015). Recently, it has been newly proposed that G protein coupling or nanobody interaction at the cytoplasmic side allosterically induces conformational changes in the orthosteric ligand-binding sites leading to the enhancement of agonist-binding affinity, which results in the stable ternary complex formation of agonist, receptor, and G proteins (DeVree et al., 2016; Staus et al., 2016).

The modeling study suggests the existence of an inverse correlation between the ligand binding site and the G protein binding interface (Kolan et al., 2014). This study indicated that, upon coupling, the G protein leads to contraction of the ligand binding pocket which positively correlated to the expansion of the G protein binding cavity. Previously, Louet and colleagues used normal mode analysis (NMA) to predict collective motions of agonist-bound β2AR both in complexes with G proteins and in the uncoupled conformation (Louet et al., 2013). In this model, the receptor adopts only one major motion in the presence of G proteins which relates to an anti-symmetric rotation of both its extra and intra-cellular regions; the authors proposed that the overall dynamic conformations of the β2AR-Gs complex might be controlled by the G protein rather than by the receptor. DeVree et al. (2016) have used a radioligand binding assay to investigate the ligand association and dissociation induced
by G protein engagement in GPCRs. They found that Gs protein can form a complex with β2-AR even in the absence of an agonist, indicating the existence of basal receptor activity. Particularly, nucleotide-free Gs stabilized the conformation of the β2-AR in a “closed state”, in which state the ligand inserting route is closed at the extracellular side and the association with an antagonist, full agonist, as well as partial agonist is restricted (Fig. 4). This study also illustrated that G protein coupling induced the stabilized conformation that restrains the agonist in the binding pocket thus enhancing its initially observed affinity or impairing agonist dissociation from the binding site (Fig. 4). In general, TM domains in the intracellular region undergo an outward movement in active-state GPCRs to open a docking cavity for the C-terminus of Gs. This large displacement of the TM domain is associated with inward structural changes on extracellular regions to form a “lid-like structure” that impairs the dissociation of an agonist from the orthosteric ligand-binding site.

Together, these data suggest that coupling to G protein and subsequent nucleotide release is sufficient to promote stabilization of the active state of the receptor or “a closed receptor conformation”, preventing ligand access to and/or exit from the orthosteric ligand-binding site. Despite structural variance, the stabilization of G proteins in structural changes of GPCRs might be shared throughout GPCRs. Similar findings were observed in several families of GPCRs including the muscarinic receptor, the opioid receptor, and the ghrelin receptor (Mary et al., 2012; DevVee et al., 2016). Previously, Mary et al. (2012) also suggested that heterotrimeric Gq protein coupling to the ghrelin receptor subsequently stabilized receptor conformation. Interestingly, this conformation was not regulated by addition of an inverse agonist suggesting the restriction of the ligand binding site for an inverse agonist (Mary et al., 2012).

**PERSPECTIVES**

The high-resolution crystal structure of β2-AR-Gs provides an excellent model to carry out a large number of computational and biochemical/biophysical studies in order to understand the conformational mechanism of G protein activation. Combined with previous findings over the last thirty years, these studies provide us with more details about the structural mechanism of the G protein activation cycle. However, there are still more questions to be answered to develop a concrete model for the G protein activation processes.

Although many structural and functional assays have been used to indicate several critical regions in either the Gs subunit or GPCR that are responsible for selectivity, understanding how various ligand-induced conformational changes in GPCRs allow recognition of specific cognate G proteins still remains challenging. Several studies have reported models of GPCR-G protein complexes by using β2AR-Gs structures as a model (Shim et al., 2013; Alexander et al., 2014; Mnpotra et al., 2014), which provides more information about novel residues that are extremely important in GPCRs-G protein selectivity. It has been reported that receptor oligomerization can regulate coupling ability and selectivity of GPCRs to G proteins (Moreno et al., 2011; Ellenbroek, 2013; Ferré et al., 2014; Navarro et al., 2016). However, the exact mechanism underlying GPCR/G protein specificity is still ambiguous and will require further investigation.

Another question yet to be answered is the conformational sequence of G protein activation by GPCRs. Two major regions in the Gα subunit are involved in the GPCR interaction: the C-terminus of the α5 helix and αN/β1 hinge as described in this review. However, we still do not have definitive answers on the conformational steps of GPCR-G protein activation or which regions have a major role in the initial release of GDP.

Besides G proteins, arrestins also have important roles in GPCR signaling in relation to G protein-independent signal transduction. Recently, great achievements have been made to understand the structural mechanism of the GPCR-mediated arrestin activation process (Park et al., 2016) increasing our understanding of the structural mechanism of functionally biased GPCR signaling. However, we still do not know the conformational change that distinguishes G protein- or arrestin-dependent GPCR signaling, or how the ligand induces specific conformational changes to selectively recognize G proteins or arrestins. Interestingly, the recent crystal structures of activated rhodopsin bound to the finger loop peptide of arrestin (Szczepek et al., 2014) or visual arrestin (Kang et al., 2015) also indicate the outward movement of TM6 as seen in the β2AR-Gs structure. It is not clear if the structural changes upon arrestin engagement into intracellular regions results in stabilization of the extracellular domain to form a “lid-like structure” over the orthosteric or a closed receptor conformation as described for G proteins. Hopefully, the rapid development of technology and a collaboration of experimental approaches and computational modeling will allow it to be possible to solve
these problems in the near future.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea funded by the Korean government (NRF-2015R1A1A05027473 and NRF-2012R1A5A2A28671860).

REFERENCES

Alexander, N. S., Preininger, A. M., Kaya, A. I., Stein, R. A., Hamm, H. E. and Meiler, J. (2014) Enzymatic analysis of the rhodopsin-G-protein complex links the α5 helix to GDP release. Nat. Struct. Mol. Biol. 21, 56-63.

Coleman, D. E., Berghuis, A. M., Lee, E., Linder, M. E., Gilman, A. G. and Sprang, S. R. (1994) Structures of active conformations of Gi α 1 and the GDP-bound α subunit. Science 265, 1405-1412.

DeVree, B. T., Mahoney, J. P., Vélez-Ruiz, G. A., Rasmussen, S. G., Kusak, A. J., Edwald, E., Fung, J. J., Manglik, A., Mesureel, M., Du, Y., Matt, R. A., Pardon, E., Steyaert, J., Koblika, B. K. and Sunahara, R. K. (2016) Allosteric coupling from G protein to the agonist-binding pocket in GPCRs. Nature 535, 182-186.

Downes, G. B. and Gautam, N. (1999) The G protein subunit gene families. Genomics 62, 544-552.

Dror, R. O., Mildorf, T. J., Hilger, D., Lech, M. T., Hubbell, W. L., Koblika, B. K., Sunahara, R. K. and Shaw, D. E. (2015) Structural basis for nucleotide exchange in heterotrimeric G proteins. Science 348, 1361-1365.

Duc, M. M., Kim, H. R. and Chung, K. Y. (2015) Structural mechanism of G protein activation by G protein-coupled receptor. Eur. J. Pharmacol. 763, 214-222.

Ellenbroek, B. A. (2013) Histamine H₄ receptors, the complex interaction with dopamine and its implications for addiction. Br. J. Pharmacol. 170, 46-57.

Ferré, S., Casadó, V., Devi, L. A., Filizola, M., Jockers, R., Lohse, M. J., Milligan, G., Pin, J. P. and Gautam, N. (2014) X4 G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. Pharmacol. Rev. 66, 413-434.

Fletcher, J. E., Lindorfer, M. A., DeFilippo, J. M., Yasuda, H., Guilmard, C. C., Gmeiner, P., Steyaert, J., Weiss, W. I., Garcia, K. C., Wess, J. and Koblika, B. K. (2013) Activation and allosteric modulation of a muscarinic acetylcholine receptor. Nature 504, 101-106.

Kaya, A. I., Lokitis, A. D., Gilbert, J. A., Iverson, T. M., Meiler, J. and Hamm, H. E. (2014) A conserved phenylalanine as a relay between the α5 helix and the GDP binding region of heterotrimeric G protein α subunit. J. Biol. Chem. 289, 24475-24487.

Kolan, D., Fornar, G. and Sams, A. O. (2014) Elastic network normal mode dynamics reveal the GPCR activation mechanism. Proteins 82, 579-586.

Kruse, A. C., Ring, A. M., Manglik, A., Hu, J., Hu, K., Etel, K., Hübner, H., Pardon, E., Valant, C., Sexton, P. M., Christopoulos, A., Felder, C. C., Gmeiner, P., Steyaert, J., Weiss, W. I., Garcia, K. C., Wess, J. and Koblika, B. K. (2013) Activation and allosteric modulation of a muscarinic acetylcholine receptor. Nature 504, 101-106.

Kolb, D. G., Sondek, J., Bohn, A., Skiba, N. P., Hamm, H. E. and Sigler, P. B. (1996) The 2.0 A crystal structure of a heterotrimeric G protein. Nature 379, 311-319.

Louet, M., Karakas, E., Perret, A., Perahia, D., Martinez, J. and Floquet, N. (2013) Conformational restriction of G-proteins Coupled Receptors (GPCRs) upon complexation to G-proteins: a putative activation mode of GPCRs. FEBS Lett. 587, 2656-2661.

Lutz, S., Shankaranarayanan, A., Coco, C., Ridilla, M., Nance, M. R., Vettel, C., Baltus, D., Evelyn, C. R., Neubig, R. R., Wieland, T. and Tesmer, J. J. (2007) Structure of G(a)-p63RhoGEF-RhoA complex reveals a pathway for the activation of RhoA by GPCRs. Science 318, 1923-1927.

Mixon, M. B., Lee, E., Coleman, D. E., Berghuis, A. M., Gilman, A. G. and Sprang, S. R. (1995) Tertiary and quaternary structural changes in Gi α 1 induced by GTP hydrolysis. Science 270, 954-960.

MnPOTRADA, J. S., Qiao, Z., Cai, J., Lynch, D. L., Grossfield, A., Leioatts, N., Hurst, D. P., Pitman, M. C., Song, Z. H. and Reggio, P. H. (2014) Structural basis of G protein-coupled receptor-Gi protein interaction: formation of the cannabinoid CB2 receptor-Gi protein complex. J. Biol. Chem. 289, 20259-20272.

Moreira, I. S. (2014) Structural features of the G-protein/GPCR interaction. Biochim. Biophys. Acts 1840, 16-33.

Moreno, E., Hoffmann, H., Gonzalez-Sepúlveda, M., Navarro, G., Casadó, V., Cortés, A., Mallol, J., Vignes, M., McCormick, P. J., Canela, E. I., Lluis, C., Morisota, R., Ferré, S., Ortiz, J. and Franco, R. (2011) Dopamine D1-histamine H₃ receptor heteromers provide a selective link to MAPK signaling in GABAergic neurons of the direct striatal pathway. J. Biol. Chem. 286, 5846-5854.

Navarro, G., Cordomí, A., Zelman-Femiak, M., Brugarolas, M., Moreno, E., Aguina, D., Perez-Benítez, L., Cortés, A., Casadó, V., Mallol, J., Canela, E. I., Lluis, C., Pardo, L., Garcia-Sáez, A. J., McCormick, P. J. and Franco, R. (2016) Quaternary structure of a G-protein-coupled receptor heterotrimer in complex with Gi and Gs. BMC Biol. 14, 26.

Neer, E. J. (1995) Heterotrimeric G proteins: organizers of transmembrane signals. Cell 80, 249-257.
