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Regulation, Signaling and Physiological Functions of G-proteins

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Abstract

Heterotrimeric G-proteins mainly relay the information from G-protein-coupled receptors (GPCRs) on the plasma membrane to the inside of cells to regulate various biochemical functions. Depending on the targeted cell types, tissues and organs, these signals modulate diverse physiological functions. The basic schemes of heterotrimeric G-proteins have been outlined. In this review we briefly summarize what is known about the regulation, signaling and physiological functions of G-proteins. We then focus on a few less explored areas such as regulation of G-proteins by non-GPCRs, and the physiological functions of G-proteins that can not be easily explained by the known G-protein signaling pathways. There are new signaling pathways and physiological functions for G-proteins to be discovered and further interrogated. With the advancements in structural and computational biological techniques, we are closer to having a better understanding of how G-proteins are regulated, and the specificity of G-protein interactions with their regulators.

Graphical abstract

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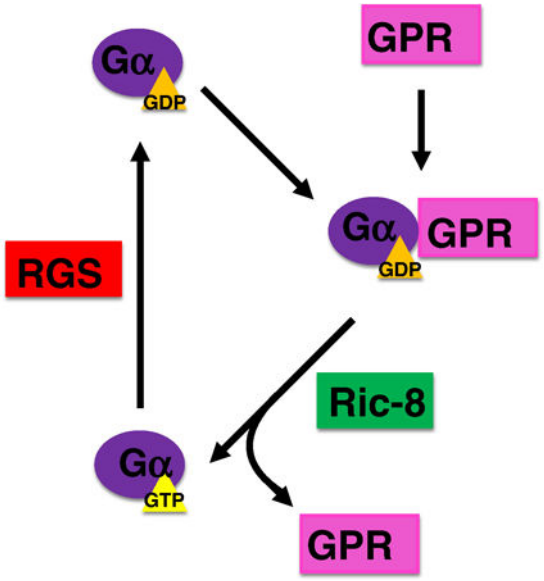

1. Introduction

A structurally diverse repertoire of ligands, from photons to many hormones and neurotransmitters, activate G-protein-coupled receptors (GPCRs) to elicit their physiological functions [1, 2]. GPCRs comprise a large and diverse superfamily, and family members have been identified in organisms as evolutionarily distant as yeast and humans. Heterotrimeric guanine nucleotide-binding regulatory proteins (G-proteins) directly relay the signals from GPCRs [3-5]. These G-proteins are composed of α, β, and γ subunits. The β and γ subunits are tightly associated and can be regarded as one functional unit. G-proteins function as molecular binary switches with their biological activity determined by the bound nucleotide [3-5]. Upon agonist binding, GPCRs increase the exchange of GDP bound on the Gα subunit with GTP. This leads to the dissociation of Gα subunit from Gβγ dimer resulting in two functional subunits (Gα and Gβγ). Both Gα and Gβγ subunits signal to various cellular pathways.

G-proteins are identified by their Gα subunits. Based on the sequence and functional similarities, Gα proteins are grouped into four families: $G\alpha_s$, $G\alpha_i$, $G\alpha_q$, and $G\alpha_{12}$ (Figure 1). In the $G\alpha_s$ family, there are two members: $G\alpha_s$ and $G\alpha_{olf}$. While $G\alpha_s$ (*s* stands for *s*timulation) is expressed in most types of cells, $G\alpha_{olf}$ (*olf* stands for *olf*action) is specifically expressed in the olfactory sensory neurons. $G\alpha_i$ family is the largest and most diverse family, including $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_t$, $G\alpha_g$ and $G\alpha_z$. $G\alpha_i$ proteins (*i* stands for *i*nhibition) have been detected in most types of cells. $G\alpha_o$ is highly expressed in neurons and has two spliced variants: $G\alpha_{oA}$ and $G\alpha_{oB}$. $G\alpha_t$ (*t* stands for *t*ransducin) has two isoforms. $G\alpha_{t1}$ is expressed in the rod cells in the eye, while $G\alpha_{t2}$ is in the cone cells of the eye. $G\alpha_g$ (*g* stands for *g*ustducin) is found in taste receptor cells. $G\alpha_z$ is expressed in neuronal tissues and in platelets. In humans, the $G\alpha_q$ family consists of $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{16}$ (The mouse equivalent is $G\alpha_{15}$). $G\alpha_q$ and $G\alpha_{11}$ are ubiquitously expressed, while $G\alpha_{14}$ and $G\alpha_{15/16}$ expression is more restricted. $G\alpha_{14}$ is mainly found in the kidney, lung

and liver, and $G\alpha_{15/16}$ is specifically expressed in hematopoietic cells. In the $G\alpha_{12}$ family, there are $G\alpha_{12}$ and $G\alpha_{13}$, which are expressed in most types of cells.

In addition to these Gα subunits, heterotrimeric G-proteins contain Gβγ subunits. There are 5 Gβ and 12 Gγ genes in the human and mouse genomes (Figures 2 and 3). $G\beta_1$, $G\beta_2$, $G\beta_3$ and $G\beta_4$ share high sequence similarities (between 80 to 90%), while $G\beta_5$ is ~50% similar to other Gβ subunits (Figure 2). While $G\beta_5$ is mainly found in the brain, other Gβ subunits are widely distributed. Gγ subunits are more diverse and share sequence similarities ranging from 20% to 80% (Figure 3). Although purified Gβγ subunits with different Gβ and Gγ isoform combinations generally have similar biochemical activities in *in vitro* assays, gene-deletion experiments in mice show that at least some Gβ and Gγ genes have different physiological functions. These might reflect the different distributions and expression levels of Gβ and Gγ genes.

The crystal structures of several Gα, Gβγ, and Gαβγ have been solved (Figure 4). The structure of a Gα subunit consists of two domains: a Ras-like GTPase domain and an α-helical domain [6] (Figure 4a). These two domains are linked by Linker 1 and Linker 2 (Figure 4a). Between these two domains lies a deep cleft within which GDP or GTP is tightly bound (Figure 4a). The nucleotide is essentially occluded from the bulk solvent, leading to the proposal that the helical domain is the inhibitory barrier and provides the regulatory entry point by GPCRs or Gβγ subunits [7-10]. The structure of a Gβγ subunit shows that Gβ folds into a β-propeller with 7 blades (Figure 4b). Each blade consists of four-stranded β-sheets. The N-terminal α-helical segment of Gβ forms a tight coiled-coil interaction with the Gγ subunit (Figure 4b). The crystal structure of a Gαβγ heterotrimer illustrates that the two domains of Gα interact with different regions of Gβ (Figure 4 c and d). The Gα N-terminal α helix interacts with the side of the Gβ propeller. The Gα switch region II region interacts with the top of the Gβ propeller (Figure 4 c and d).

Great progress has been made in understanding the mechanisms by which heterotrimeric G-proteins regulate their downstream targets [6, 11]. Recently a series of crystal structures of GPCRs in the inactive and active states, bound with antagonists, inverse agonists or agonists, have elucidated the structural basis for the modulation and activation of GPCRs by ligands [1, 12-14]. A crystal structure of the complex of β_2-adrenergic receptor (β_2-AR) and Gs has revealed the structural changes in β_2-AR and in Gs, as well as the interacting regions and residues between a GPCR and a G protein [15-17].

In this review, we summarize the activation of G-proteins by GPCRs, and regulation of G-proteins by non-GPCR proteins, such as Ric-8 protein, GPR-domain containing proteins, GBA-motif containing proteins, and RGS-domain containing proteins. Furthermore, we list some G-protein interacting proteins, in addition to the well-established G-protein effectors such as adenylyl cyclases and phospholipase C, although the physiological functions of many of these interactions are not clear. Finally, we describe two examples of non-canonical signaling events that deserve more attention: one is the role of G-protein signaling in cell division, and the other is the role of G-proteins in receptor tyrosine kinase signaling. This review does not intend to be complete, but rather illustrates that there is much more exciting work to be done in the G-protein signaling field.

2. Regulation of G-Proteins

a). General features of G-protein regulation

G-proteins biochemically function in a GTPase cycle (Figure 5). In the inactive form, GDP-bound Gα subunits bind tightly to the obligate heterodimer of Gβγ [1]. Upon agonist binding to a GPCR, downstream signaling is initiated. GPCRs act as guanine nucleotide exchange factors (GEFs), promoting the release of bound GDP from Gα [18, 19]. Nucleotide-free Gα then binds GTP, resulting in the dissociation of Gβγ. Both GTP-bound Gα and free Gβγ are capable of initiating signals by interacting with downstream effector proteins. Gα subunit signaling is terminated by the intrinsic GTPase activity of Gα which hydrolyzes the bound GTP to GDP. GTPase activating proteins (GAPs) (such as RGS proteins) bind with Gα to accelerate the intrinsically low GTPase activity of Gα subunit. The Gβγ signaling, on the other hand, is terminated by re-association with Gα·GDP. This process represents a G-protein cycle.

The nucleotide-binding pocket of G-proteins is highly conserved, and binding of GDP or GTP proceeds through a mechanism which is conserved. Generally described as a “switch” mechanism, guanine nucleotide loading and unloading take place in the Ras-like domain of G-proteins which serves as a platform for both guanine nucleotide binding as well as in GTP hydrolysis (Figure 4a) [20]. This Ras-like domain is a typical α/β nucleotide-binding fold, and consists of a six-stranded β-sheet with five helices located on both sides. Lining the binding pocket are four to five signature sequence elements attributable to G-proteins, two of which are the most important for guanine-base binding specificity. The first is a N/TKXD motif [where X is any amino acid] whose aspartic acid is thought to form a bifurcated H bond with the guanine base. The second is the highly conserved P-loop region which contains the sequence pattern GXXXXGKS/T, also known as a Walker A motif [21]. G-proteins possessing this motif belong to the “P-loop containing nucleotide triphosphate hydrolase superfamily”.

GEFs work to weaken the affinity for the nucleotide [22]. G-proteins have a high affinity for GDP (pmol in some cases) that causes the GDP disassociation process to be slow. To overcome this barrier, G-proteins employ the action of GEFs which reduce the affinity for bound GDP, and subsequently enable binding of the triphosphate moiety, due to its higher cellular concentration (Figure 5). While the interaction between G-proteins and either GDP or GTP is very high, affinity of the GEF for G-protein in either nucleotide-bound state is much lower. GEFs cause perturbations in the two switch regions of G-proteins. Bound GDP is encompassed by two loop regions called Switch I and Switch II, the latter of which is the location of the conserved N/TKXD motif. The switch regions together with the P-loop interact with the phosphates of the bound nucleotide as well as a coordinating Mg^{2+} ion. In the exchange reaction of a Ras-superfamily GTPase, a GEF interfaces with the GDP-bound GTPase in such a way that the nucleotide is partially competed from its binding site. More specifically, the Mg^{2+} ion is displaced by residues residing on the GTPase, causing disruption in the interactions between the P-loop region and the nucleotide phosphates. This produces a “push-pull” effect between the switch regions and the nucleotide. The affinity of the GTPase for the nucleotide is decreased as it is “loosened” from its binding site, and the

nucleotide is eventually released. Finally a high affinity binary complex is formed between the GEF and GTPase, which is free to bind GTP. Not much structural information is available for the ternary complexes or the intermediate conformational states, and therefore it remains to be elucidated how the GEFs approach the G-protein, how the nucleotide is released from the binding site and how GTP is recruited.

b). Activation of G-proteins by GPCRs

The main physiological functions of G-proteins are to relay the signals from GPCRs which function as GEFs for G-proteins. Binding with exogenous or endogenous agonists induces GPCRs into an active conformational state which, in turn, influences intracellular binding of G-proteins or arrestin proteins [23, 24]. This superfamily of receptors shares seven transmembrane (TM) helical domains (TM1 to TM7) connected by alternating three intra- and three extra- cellular loops (ICL1, ECL1, ICL2, ECL2, ICL3, and ECL3) (Figure 6). GPCR targeted ligands are classified into agonists, inverse agonists, and antagonists. Agonists binding to GPCRs promote an active conformation, which in turn increases the signaling effect. Conversely inverse agonists inhibit spontaneous basal signaling activity by stabilizing an inactive conformation of GPCRs. Antagonists have no effect on the dynamic equilibrium between the active and inactive conformations of GPCRs, but prevent binding of both agonists and inverse agonists.

Various studies have suggested that ICL2, and the N- and C-terminal regions of ICL3 are G-protein interacting sites on GPCRs [25]. Additionally, the ICL1 and some C-terminal residues might contribute to the coupling of G-proteins [18]. Binding of a ligand at the extracellular region induces a structural rearrangement in the transmembrane core region which further leads to a conformational change in the intracellular region at the cytoplasmic side. This facilitates interaction with intracellular effectors such as G-proteins. Agonist binding alone may not be sufficient to stabilize a fully active conformation of GPCRs which might require the additional binding of an intracellular protein, such as a G-protein or arrestin, at the cytoplasmic side [15, 26].

For the interaction between GPCRs and G-proteins, it was originally proposed that GPCRs and G-proteins interact by collision. Both GPCRs and G-proteins diffuse freely within the plasma membrane, and that only activated (agonist-bound) GPCRs couple to and activate G-proteins [27]. However, some GPCRs and G-proteins were reported to form a preassembled (or precoupled) complex in the absence of any ligands [27, 28]. Agonist binding activates the GPCR which causing the conformational changes in the preassembled GPCR/G-protein complex, resulting in G-protein activation. The structure of the preassembled GPCR/G-protein complex is proposed to be different from that of the activated GPCR/G-protein complex. Collision coupling or preassembly can yield different kinetics of activation of G-proteins, and may underlie the different constitutive activities of GPCRs. These different GPCR/G-protein interaction modes depend on the specific GPCR/G-protein pair. Further investigations are needed to provide the structural basis for these different GPCR/G-protein interactions.

In the crystal structure of a complex of β_2-AR and Gs, a large β_2-AR-Gα_s protein interface is formed by ICL2 and transmembrane helices TM5 and TM6 of the receptor, as well as by

the α4- and α5-helices, the αN–β1 junction, and the β3-strand of Ras-like domain of $G\alpha_s$ [15] (Figure 6). Major interactions between β_2-AR and $G\alpha_s$ protein occur along the carboxyl-terminal end of TM5 of the receptor. Here, residues Glu225, Gln229, and Lys232 belonging to TM5 of β_2-AR are involved in strong electrostatic interactions with Asp381, Gln384, and Arg385 located at the carboxyl-terminal end of the α5-helix of $G\alpha_s$ (Figure 6b). Main chain oxygens of residues Thr136 and Ile135 at the carboxyl-terminus of TM3 are also involved in polar interactions with Arg380 and Gln384 of the same α5-helix of the Ras-domain of $G\alpha_s$. When the structure of $G\alpha_s$ alone (cyan color in Figure 6d) is superimposed on top of its crystal structure in complex with β_2–AR (in orange color), it shows that the α-helical domain of $G\alpha_s$ in the complex was rotated by about 127° towards the receptor relative to its apo-structure. The closed and open states of the cleft (the GDP/GTP binding site) are determined by the relative pivoting of the Ras-like domain and the α-helical domain at Linker 2. Given this pivot model, the two domains of Gα could be 'clam-shell' opening or 'rolling-top' expansion including a relative rotation of the Ras-like domain and the helical domain around an axis through the linkers [29]. In addition to the established role for α5-helix of Gα in connecting GPCRs to the guanine-nucleotide binding pocket, an extended Linker 2 connects GPCRs to the nucleotide binding pocket as well as the α-helical domain of Gα [29]. Many more structures in different states and with different GPCRs/G-proteins are needed to fully understand the biochemistry of the G-protein activation cycle.

c). GPCR dimers and oligomers

GPCRs can exist and function as dimers or oligomers [30-34]. Dimerization and oligomerization modulate various GPCR functions such as cell surface targeting, cooperativity, activation, G-protein coupling, signaling and internalization[33-35]. The requirement of dimers or tetramers of GPCRs for G-protein activation has not been firmly established. However, for the class-C family of GPCRs, the dimerization of two protomers (either heterodimers or homodimers) is essential for its biological function and G-protein activation.

In the crystal structure of β_1-adrenergic receptor (β_1-AR) oligomers in a membrane-like environment, there are two dimer interfaces: one involves TM1/TM2/H8 (H8: helix 8) and the other engages TM4/TM5/ICL2 [14] (Figure 7). These are the two major dimer interfaces observed in crystal structures of various GPCRs [36-39]. In the TM1/TM2/H8 dimer interface, interacting residues are mainly from TM1 (including Gln38, Gln39, Ala42, Leu46, Ala49, Leu50, Val52, Leu53, and Leu54) [14]. Residues from other parts of the receptor also contribute to this dimer interface, including residues from TM2 (Pro96, Ala99, Thr100 and Val103), the extracellular loop 1 (Thr106, Leu108, and Trp109), and the C-terminal H8 (Arg351, Lys354, Arg355 and Leu356) [14]. In addition to these hydrophobic and van der Waals interactions, Ser45 in one TM1 forms a hydrogen bond with Ser45 from another TM1. Glu41 in TM1 from one monomer forms a salt bridge with Arg104 in TM2 from the second monomer [14]. This dimer interface is similar to the one observed in the dimer of rhodopsin in the active state which uses TM1 and H8 as an interface [37, 38].

In the second TM4/TM5/ICL2 dimer interface, residues from both TM4 (including Leu171) and TM5 (including Arg205, Ala206, Ala210, Ile218 and Arg229) contribute to the

hydrophobic interaction [14]. ICL2 also plays a significant role in this dimer interaction (including residues Tyr140, Leu141, Thr144, Ser145, Phe147, Arg148, Ser151, and Leu152) [14]. Two residues (Trp181 and Arg183) from extracellular loop 2 also participate in this interaction [14]. There is a variation of the TM4/TM5 dimer interface that uses TM5/TM6 as observed in the crystal structures of several GPCRs [40, 41]. ICL2 is critical for interacting with G-proteins based on the structural model of the β_2-AR-G_s complex [15]. Participation of ICL2 in this dimer interface may prevent G-protein coupling to the dimer formed through TM4/TM5/ICL2 interface, or G-protein binding may disrupt this dimer interface. Therefore, if the signaling unit is a pentamer (two GPCRs and one trimeric G protein), the GPCR dimer interface in this signaling unit is likely TM1/TM2/H8. In this model, only one GPCR contacts with the G-protein trimer, and the other receptor is "spared" or could function through trans-protomer allosteric regulation.

A dimer interface involving TM1 was shown to be insensitive to ligand binding and the receptor activation state as shown for dopamine D2 receptors and serotonin 5HT2c receptors [42, 43]. The similarity of TM1/TM2/H8 dimer interface in the inactive β_1-AR and in the active rhodopsin is consistent with the notion that this TM1/TM2/H8 dimer interface does not undergo significant conformational changes from inactive to active states of GPCRs. On the other hand, the TM4/TM5/ICL2 dimer interface makes structural rearrangement during the GPCR activation process, at least in the cases of dopamine D2 receptors and serotonin 5HT2c receptors [42, 44]. Based on the structures of active GPCRs, the intracellular end of TM5 moves away from the TM bundle core [15]. Therefore it is possible that, upon the agonist binding, the configuration change at the TM4/TM5/ICL2 dimer interface is part of the receptor activation process.

d). Regulation of G-proteins by non-GPCRs

In addition to GPCRs, other regulatory proteins exist for heterotrimeric G-proteins [45-49]. These regulators include Ric-8, GPR-domain containing proteins, GBA motif-containing proteins, and RGS-domain containing proteins. Although there might be other non-GPCR regulators, we will focus on a few as examples of G-protein regulation by non-GPCR proteins.

(i) Regulation of G-proteins by Ric-8—Ric-8 (synembryn) was originally identified in *C. elegans* through genetic analysis [50]. Ric-8 functions upstream of $G\alpha_q$ in regulating neurotransmitter secretion [50]. Ric-8 also acts upstream of $G\alpha_o$ and GPA16 (another $G\alpha$ subunit in *C. elegans*) during asymmetric cell division of one-cell stage *C. elegans* embryos [51-53]. In *Drosophila*, Ric-8 is required for $G\alpha$-mediated spindle orientation and cell polarity during asymmetric cell division [54-56]. In Ric-8 mutants, $G\alpha_i$ failed to localize at the cell cortex [54-56]. Ric-8 has also been genetically shown to play a role in gastrulation and is involved in the *fog-concertina* pathway [55]. *Concertina* is the *Drosophila* homolog of $G\alpha_{13}$ [57]. *Fog* (*folded gastrulation*) is an extracellular polypeptide growth factor [57]. Thus, Ric-8 has been genetically demonstrated to be involved in $G\alpha_{13}$-mediated signaling in *Drosophila*.

There are two distinct mammalian Ric-8-like genes, Ric-8A and Ric-8B. Ric-8A was identified in yeast two-hybrid screens of a rat brain embryonic cDNA library as a protein that interacted with a $G\alpha_o$ bait [58]. The Ric-8A prey clone interacted with $G\alpha_o$, $G\alpha_{i1}$, $G\alpha_q$ and $G\alpha_{13}$, but not $G\alpha_s$ baits in pair-wise two-hybrid interaction studies. *In vitro* biochemical studies have shown that Ric-8A is a GEF for $G\alpha_q$, $G\alpha_i$, $G\alpha_o$, $G\alpha_{12}$, $G\alpha_{13}$ but not $G\alpha_s$ [58, 59]. On the other hand, Ric-8B interacts with $G\alpha_s$ and $G\alpha_q$ [58, 59]. Mechanistically, Ric-8A binds to GDP-bound Gα proteins, promotes rapid GDP release and forms a stable nucleotide free transition state complex with Gα that is disrupted upon GTP binding, thus leading to the formation of Gα-GTP. Ric-8 differs from GPCRs in that GPCRs work on the inactive Gα-GDP/Gβγ heterotrimer while Ric-8 acts only on the Gα-GDP monomer. Ric-8 could also work on Gα-GDP in complex with GPR proteins (see below). While Ric-8A mRNA is expressed in a variety of tissues, Ric-8B mRNA is mainly expressed in the olfactory epithelium [60]. Moreover, Ric-8A$^{-/-}$ mouse embryos died in the early stages of embryonic development [61].

Although the biochemical mechanism of activation of Gα by Ric-8 is not understood, it has been shown that Ric-8A induces structural changes in the nucleotide binding site of Gα which reduces its affinity for GDP, and subsequently releases the nucleotide and exchanges it for GTP [58] (Figure 8). A mechanistic study of the guanine nucleotide-exchange of GDP for GTP by Ric-8A using SDSL and DEER spectroscopy shed light on some of the structural features of this exchange [62]. Ric-8A binding induces structural displacements in $G\alpha_i$, resulting in the separation of the α-helical and Ras-like domains. Thermostability studies of $G\alpha_i$ showed that not only do $G\alpha_i$ proteins exhibit characteristics of an inherently disordered protein without GDP bound, but that GDP-bound as well as Ric-8A-bound $G\alpha_i$ are thermodynamically more stable than the non-bound protein. Locally, Ric-8A is thought to instigate structural changes within the nucleotide binding site, producing a series of intermediate states which affect Switch I and Switch II regions, and decreases the affinity of $G\alpha_i$ for the bound GDP nucleotide. Ultimately these perturbations result in a solvent exposed, structurally heterogeneous binding site. These structural rearrangements lead to a viable entry and escape pathway for nucleotides. Though extensive functional studies have been carried out to study the interaction between Ric-8A and Gα, the lack of a crystal structure precludes any concrete determination of the exact mechanism of Ric-8A GEF activity toward Gα proteins.

In addition to its GEF activity, Ric-8 proteins have multiple other functions [58, 63]. They could act as a chaperone for the Gα subunit by regulating Gα folding and processing during its biosynthesis [64] [65]. They could regulate the membrane translocation of Gα subunits. In *C. elegans*, Ric-8 directly interacts with GPA-16, but has no GEF activity towards GPA-16 [66]. Instead, Ric-8 is required for the plasma membrane localization of GPA-16 [66]. In *Drosophila*, Ric-8 directly interacts with $G\alpha_i$ and is required for the plasma membrane localization of $G\alpha_i$ (in this case, Ric-8 also functions as a GEF for this $G\alpha_i$) [54-56]. There are still many unanswered questions regarding functions and mechanisms of Ric-8 proteins.

(ii) Regulation of G-proteins by GRP proteins—The second group of non-GPCR regulators of G-proteins is the GPR-domain containing proteins. GPR (*G-p*rotein *r*egulator)

domains are ~25 amino acid segments (also called the GoLoco domain) [67, 68]. GPR domains have been shown biochemically to bind the inactive GDP-bound $G\alpha_{i/o}$. These GPR proteins inhibit nucleotide exchange in Gα, and compete with Gβγ for Gα binding [46, 48, 69]. Furthermore, Ric-8A can activate Gα by promoting exchange of GDP for GTP when Gα is bound to a GPR domain [70, 71], but not when Gα is bound to Gβγ [58] (Figure 8). Although *in vitro* biochemical studies implied that GPR domain-containing proteins could function as a GDI (guanine nucleotide dissociation inhibitor) and thus inhibit G-protein signaling, genetic studies in *C. elegans* showed that GPR domain-containing proteins promote G-protein signaling by either sequestering Gα from Gβγ so that free Gβγ can signal, and/or keeping Gα in a form that can be activated by Ric-8A to prolong the G-protein signaling (Figure 8).

Some AGS proteins (for *a*ctivator of *G* protein *s*ignaling) are GPR-domain-containing proteins. In a yeast expression screen for GPCR-independent activators of Gβγ-dependent signaling, several AGS proteins were identified [46, 72]. One of these AGS proteins, AGS3, contains four GPR domains, and selectively binds to the GDP-bound form of the $G\alpha_i$ family, as well as acts as a guanine nucleotide dissociation inhibitor [46].

Additional GPR domain-containing proteins include the human proteins LGN, Pcp2, G181b, and *Drosophila* protein PINS (*p*artner of *ins*cuteable), as well as a related protein (F32A6.4) from *C. elegans* [46, 67, 73]. These proteins have been proposed to participate in GPCR-independent G-protein regulated events such as asymmetric cell division, mitotic spindle formation, and planar polarity of developing sensory organ precursor cells [46, 74, 75].

(iii) Regulation of G-proteins by GBA motif-containing proteins—The third group of non-GPCR activators of G-proteins is the GBA motif-containing proteins [76, 77]. GBA stands for *G*α-*b*inding and *a*ctivating. GBA motif-containing proteins include Gα-Interacting-Vesicle-associated protein (GIV/Girdin), Daple, NUCB1, NUCB2 and GBAS-1. GIV was discovered as a Gα-interacting protein which could enhance Akt activation [76]. GIV could bind robustly to the $G\alpha_i$ family members and to a lesser extent to $G\alpha_s$ [78]. A series of *in vitro* studies with purified components demonstrated that GIV accelerates the rate of nucleotide exchange, leading to Gα subunit activation [77]. Further, GIV could not bind Gα subunits in the active GTP-bound conformation. This feature of GEFs ensures that GIV only engages with inactive Gα-GDP and thus promotes Gα signaling. It is not known whether GIV can directly activate a $G\alpha_i$-βγ trimer *in vitro*. However, it was shown that GIV could displace Gβγ from a preformed $G\alpha_i$-βγ trimer *in vitro* and enhance Gβγ-dependent signaling in cells [76].

GBA motif-containing proteins have been coined “molecular rheostats” due to their ability to fine-tune the duration and extent of signal transduction cascades, more specifically in cancer cell migration [79, 80]. Elevated levels of these proteins in circulating tumor cells and their role as G-protein activators suggest that hyperactivation of G-proteins, as opposed to gene mutations in G-proteins and GPCRs, might play a role in tumor metastatic progression.

e). Regulation of G-proteins by RGS proteins

A large family of RGS (*r*egulators of *G*-protein *s*ignaling) proteins has been identified. After the active GTP-bound Gα exerts its effect on downstream effectors, this activation must be highly regulated to maintain the appropriate signal strength and duration. Although Gα subunits contain weak intrinsic GTP hydrolytic activity, the hydrolysis of GTP to GDP and the re-association of Gα with Gβγ subunits are steps in the G-protein deactivation cycle that can be modulated to achieve the desired cellular signal output, and these steps are regulated by RGS proteins [81]. RGS proteins act as GAPs for Gα subunits. Gα selectivity can be attributed to heterogeneity in the RGS domains of different RGS proteins which in turn result in different interactions with the α-helical domains and switch III regions of Gα subunits (Figure 9) [82, 83]. The interaction between the RGS domain and the switch and α-helical regions of Gα is thought to promote the transition state for GTP hydrolysis within the nucleotide binding domain and thus facilitate deactivation of Gα [83].

Crystal structures of the complexes of Gα and the RGS domains have highlighted the structural basis for the interaction between RGS proteins and Gα [84]. While these structures locally and globally describe the interacting domains in the context of both proteins, the exact mechanism for how RGS proteins approach and regulate G-protein deactivation is a subject of speculation. RGS proteins have been proposed as attractive targets for drug development [85].

3. Signaling by G-Proteins

a). Well-defined G-protein signaling pathways

Accumulated data have established several well-defined signaling effectors for G-proteins (Table 1). Both $G\alpha_s$ and $G\alpha_i$ families of G-proteins could regulate adenylyl cyclases. $G\alpha_s$ stimulates adenylyl cyclase to convert ATP into cyclic AMP (cAMP) (Figure 10a) [86]. Elevated cAMP results in the activation of cAMP-regulated proteins, such as protein kinase A, cyclic nucleotide-gated channels, and EPAC (a GEF for Rap small GTP-binding proteins) [86]. $G\alpha_i$ proteins, on the other hand, can inhibit certain isotypes of adenylyl cyclases, leading to reduced intracellular cAMP levels. $G\alpha_q$ family G-proteins activate the β-isoforms of phospholipase C (PLC-β1-4), which cleaves phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P_2] into inositol trisphosphate (IP_3) and membrane-bound diacylglycerol (DAG) [87] (Figure 10b). IP_3 then opens the calcium channel IP_3 receptor on the ER membrane, and DAG activates protein kinase C. Within the $G\alpha_{12/13}$ family, $G\alpha_{13}$ (but not $G\alpha_{12}$) is able to directly increase the activity of p115RhoGEF and related RhoGEF proteins [PDZ-RhoGEF and leukemia-associated RhoGEF (LARG)] by membrane recruitment and direct interaction [88]. However, one should be aware that other G-proteins, such as $G\alpha_q$, could also increase the activity of other RhoGEFs, such as p63RhoGEF, linked to RhoA activation [89, 90] (Figure 10c). Activation of RhoA does not imply a $G\alpha_{13}$-dependent signaling pathway. In endothelial cells, RhoA activation is normal in $G\alpha_{13}$-knockout cells, but is impaired in $G\alpha_q$-knockout cells [91]. For $G\alpha_{12}$, several proteins were reported to interact with $G\alpha_{12}$. These proteins include Btk-family tyrosine kinases, Gap1, rasGAP, cadherins, α-SNAP, and p120-caterin [92-95]. Similarly, several proteins (such as Btk-family tyrosine

kinase, cadherins, radixin, Hax-1, and Integrin $\alpha_{iib}\beta_3$) were reported to interact with $G\alpha_{13}$ [93, 96-100] (Table 1).

In addition to Gα proteins, Gβγ can also signal to downstream effectors. Gβγ can regulate adenylyl cyclases, phospholipase Cβ, inwardly rectifying K^+ channel, and voltage-gated Ca^{2+} channels [101] (Table 2). Due to the relatively higher amounts of G_i families of G-proteins in cells, G_i activation is thought to be the primary source for Gβγ-mediated signaling processes.

b) Non-canonical G-protein signaling pathways

Other than the well-established downstream effectors, such as adenylyl cyclase and phospholipase Cβ, there are many proteins reported to interact with G-proteins (Tables 1 and 2). Although the physiological significances of these interactions are currently not well established, these interactions might underlie cell type-specific G-protein functions, and need to be further explored.

4. Physiological Functions of G-Proteins

a). Physiological functions of G-proteins as observed in gene-knockout mice

There are many physiological functions described for various G-protein signaling pathways. Here we focus on the physiological studies in G-protein gene knockout mice.

G_s and G_i pathways contribute to cardiac functions such as contractility [102]. Studies from null mutation of the $G\alpha_s$ maternal and paternal alleles suggest that $G\alpha_s$ gene is imprinted [103-105] (Table 3). Further studies showed that $G\alpha_s$ gene is imprinted in a tissue-specific matter, being primarily expressed from the maternal allele in renal proximal tubules, thyroid, pituitary and ovary.

Each gene encoding for individual $G\alpha_i$-proteins has been knocked out in mice, and these studies revealed that each knockout has a specific set of deficiencies and abnormalities [106] (Table 4). Heart tissues analyzed from $G\alpha_i$-deficient mice suggest that deletion of one $G\alpha_i$ isoform might be accompanied by an increased protein expression for the non-targeted $G\alpha_i$ subunit in the heart [107]. $G\alpha_{i2}$ and $G\alpha_{i3}$ show isoform-specific roles in the regulation of macrophage migration and hepatic autophagy but exhibit redundant functions in other processes such as macrophage activation [108].

Mice lacking $G\alpha_q$ and $G\alpha_{11}$ genes have multiple defects (Table 5). These include impaired motor coordination associated with cerebellar development, defective platelet activation, cardiac malformation and craniofacial defects associated with embryonic cardiomyocyte proliferation and craniofacial development, and hyperparathyroidism [86]. Overall, the G_q family of G-proteins collectively regulate a wide range of cell- and tissue-specific responses via coupling to PLC β-isoforms. Furthermore, $G\alpha_q$ family members can induce Rho-mediated responses including activation of RhoA in smooth muscle cells via p63RhoGEF, and acetylcholine vesicle release at neuromuscular junctions in C *elegans* as well as regulation of pharynx pumping, speed of locomotion and egg laying via TrioC (a Rho GEF) [109, 110].

While $G\alpha_{12}$ gene-deleted mice were normal, $G\alpha_{13}^{-/-}$ mouse embryos died at E9.5 [111, 112] (Table 6). $G\alpha_{13}$ is essential for blood vessel formation, and highly expressed in endothelial cells [113]. The $G\alpha_{13}^{-/-}$ mouse embryos have defective vascular systems that show no blood vessels [111]. Therefore, deletion of $G\alpha_{13}$ gene impaired the ability of endothelial cells to develop into an organized vascular system, resulting in intrauterine death. Endothelial cell-specific $G\alpha_{13}$ knockout embryos also died at ~E9.5, similar to the above mentioned global $G\alpha_{13}^{-/-}$ mice [113]. Although it is clear that $G\alpha_{13}$ is essential for blood vessel formation, the biochemical signaling mechanisms of $G\alpha_{13}$ in endothelial cells are not completely understood. Furthermore, ablation of the $G\alpha_{12}$ and $G\alpha_{13}$ genes in the nervous system results in neuronal ectopia of the cerebral and cerebellar cortices, indicating that both G-proteins are required for proper positioning of migrating cortical plate neurons and Purkinje cells during development [114].

b). Physiological functions without well-defined signaling pathways

There are many reports of physiological and pathological functions of G-proteins that can not be easily explained by the currently well-defined G-protein signaling effectors [115]. Here we will focus on two examples, one of which is the role of G-proteins in cell division, and the other is the role of G-proteins in mediating signals from receptor tyrosine kinases (RTKs).

(i) Role of G-proteins in cell division—Genetic studies in *C. elegans* and *Drosophila* demonstrate that G-protein subunits function in asymmetric cell division [75, 116-120]. In one-cell *C. elegans* embryo, the Par3/Par6/aPKC complex localizes to the anterior cortex, and a complex of $G\alpha$ and GPR domain-containing protein localizes to the posterior cortex before the first cleavage, thus setting up a series of events that result in the generation of an anterior daughter cell that is larger than its posterior sister (Figure 11a). The difference in sibling size is the result of a shift in the cleavage plane toward the posterior pole, caused by pulling of the posterior spindle pole toward that direction. Depletion of two $G\alpha_i$ family members in *C. elegans*, GOA-1 and GPA-16, produces a loss of asymmetry characterized by equal but greatly decreased pulling forces on both anterior and posterior spindle poles [53, 75]. $G\beta\gamma$ signaling is also necessary for proper asymmetric cell division. Loss of GPB-1, one of the two *C. elegans* $G\beta$ subunits, or GPC-2, one of the two *C. elegans* $G\gamma$ subunits, results in incorrect positioning of the mitotic spindle axes. This improper orientation of cell cleavage results in developmental defects in the embryo [75, 116]. However, the biochemical mechanisms of how G-proteins are regulated during this process are not clearly defined. Nevertheless, numerous studies provide a possible model for how G-proteins function to regulate microtubule pulling forces in cell division (Figure 11b). The key element that comprises this non-canonical pathway is that the GDP bound form of $G\alpha$, rather than the GTP bound form, is the important form in the force generation mechanism. $G\alpha_i$- GDP binds directly to GPR-1/2 through the GRP-domain [118, 119]. At the same time, GPR-1/2 binds to the coiled-coiled protein LIN-5. Deletion of either GPR-1/2 or LIN-5 in *C. elegans* embryos leads to defects in asymmetry cell division (Figure 11b) [118]. Furthermore, the key microtubule motor protein dynein forms a complex with GPR-1/2 and LIN-5. A decrease or loss of function of dynein resulted in decreased pulling forces during asymmetric cell division [121]. Thus, the current model for how G-proteins function in

asymmetric cell division in *C. elegans* involves plasma membrane Gα-GDP forming a complex with GPR1/2 and LIN-5 at the cell cortex which recruits dynein that generates the force necessary to control spindle pole positioning (Figure 11b).

This G-protein signaling does not require GPCR involvement, but the nucleotide bound states of Gα are shown to be regulated by other regulator proteins such as RGS-7 and Ric-8. Loss of RGS-7 resulted in increased force at the anterior spindle pole [52], while loss of Ric-8 leads to decreased pulling forces on both anterior and posterior spindle poles [53-56, 66, 121-123]. Hence, Gα needs to cycle between GDP/GTP bound states to carry out its function in asymmetric cell division.

(ii) Roles of G-proteins in mediating signals from receptor tyrosine kinases (RTKs)—Another example of non-canonical functions for G-proteins is their roles in RTK signaling. There are many reports that G-proteins could function downstream of RTKs [77, 124-136]. However, the mechanisms are not well defined. It is not clear whether GPCRs are involved in all these pathways. In some cases, trans-activation of GPCRs by RTKs has been proposed. These areas deserve more biochemical and genetic investigations. Here we will use the role of $G\alpha_{13}$ in RTK-induced actin cytoskeletal reorganization as one example.

The earliest ultra-structural changes of cells treated with growth factors are the intensive bursts of ruffling of the dorsal surface plasma membranes as seen under the phase-contrast microscope [137-139]. The physiological functions of dorsal ruffles, including macropinocytosis, cell migration and invasion, are continually expanding [140-143]. It has been suggested that one major function of dorsal ruffles is to reorganize the actin cytoskeleton to prepare a static cell for motility [144]. In serum-starved fibroblasts, platelet-derived growth factor (PDGF) induces at least two types of membrane ruffles: peripheral membrane ruffles (or lamellipodia) and dorsal ruffles [145]. In addition to fibroblast cells, dorsal ruffle formation in response to growth factors has been reported in glial cells, endothelial cells, hippocampal neurons, kidney epithelial cells and tumor cells [137, 138, 144, 146, 147]. Dorsal ruffles are dynamic structures, and they form and disassemble rapidly [148, 149]. Intensive investigations have revealed several critical regulators of the dorsal ruffle formation in response to growth factors such as Rac, PAK1 and WAVE1 [145].

$G\alpha_{13}$ is essential for RTK-induced cell migration and actin cytoskeletal reorganization in fibroblasts and endothelial cells [150]. $G\alpha_{13}$ has been shown to control actin cytoskeletal reorganization by regulating the disassembly of dorsal ruffles [151]. In the absence of $G\alpha_{13}$, the activity of Rac stays much longer than in normal cells, indicating $G\alpha_{13}$ controls the duration of Rac activation [151]. Re-expression of $G\alpha_{13}$ shortens the duration of Rac activity in cells [151]. Furthermore, Ric-8A is critical in relaying RTK signals to $G\alpha_{13}$ [152]. Down-regulation of Ric-8A in cells impaired PDGF-induced dorsal ruffle turnover and decreased PDGF-initiated cell migration. Deficiency of Ric-8A impaired the translocation of $G\alpha_{13}$ to the cell cortex. Therefore, Ric-8A is involved in the PDGFR-$G\alpha_{13}$ pathway and possibly functions as the GEF for GPCR-independent activation of $G\alpha_{13}$ in this PDGF-initiated pathway. Furthermore, atypical protein kinase C (aPKC) could phosphorylate Ric-8A after PDGF stimulation [153]. aPKC exists in cells as a complex of PAR3/PAR6/aPKC [154]. This complex regulates cell polarity, cell division and cell

migration. The main mechanism by which this PAR3/PAR6/aPKC complex regulates cell function is to modulate the subcellular localization of downstream proteins. Unphosphorylated Ric-8A is observed mainly in the nucleus whereas phosphorylated Ric-8A is observed mainly in the cytoplasm [153]. Hence, RTKs likely signal through aPKC to regulate the subcellular localization of Ric-8A, which in turn controls the subcellular translocation of $G\alpha_{13}$. Further investigations of the molecular mechanisms by which G-proteins are regulated by RTKs will help us understand the physiological functions of G-proteins in general.

5. Perspectives

In this review, we have briefly summarized the regulation, signaling and physiological functions of heterotrimeric G-proteins. We reviewed the regulation of G-proteins by its main activator (the GPCRs) and by some non-GPCR activators (such as GPR proteins and Ric-8A) as well as its negative regulation by RGS proteins. We reviewed the well-studied cellular signaling pathways controlled by G-proteins. In addition, we mentioned additional G-protein interacting proteins although their physiological importance is not clear or well established at the present time. We have also described some well-known physiological functions of G-proteins, and pointed out that there are many more physiological and pathological functions of G-proteins that we don't yet understand.

We still have a long way to go to fully understand how GPCRs and non-GPCR activators regulate G-proteins. In addition to more crystal structures of different GPCRs with various G-proteins, and of non-GPCR activators and G-proteins, to gain insights on the specific coupling between the activators and G-proteins, we also need to investigate the dynamic process of these activation processes since the crystal structures represent snapshots of particular states. Recent uses of cryo-EM structural studies of different states of receptor ion channels illustrate one example of the types of studies that could be utilized to investigate the activation processes of G-proteins, in addition to computational simulation studies.

There are many hints that G-proteins might have other signaling pathways (in addition to the well-known targets and pathways) which could expand the physiological functions of G-proteins. These additional signaling pathways and physiological functions need to be rigorously investigated with open minds.

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Highlights

- Heterotrimeric G-proteins can be regulated by G-protein-coupled receptors (GPCRs), and by non-receptor proteins such as Ric-8, GRP and RGS proteins.
- Many proteins interacting with G-proteins have been identified.
- G-proteins regulate various physiological functions.
- Combinations of structural and computational biological tools can be used to understand the mechanisms of activation of G-proteins by GPCRs and non-GPCR regulators. Multidisciplinary studies are needed to systematically investigate the biochemical mechanisms, cellular effects and physiological functions in processes involving apparently non-canonical G-protein signaling.

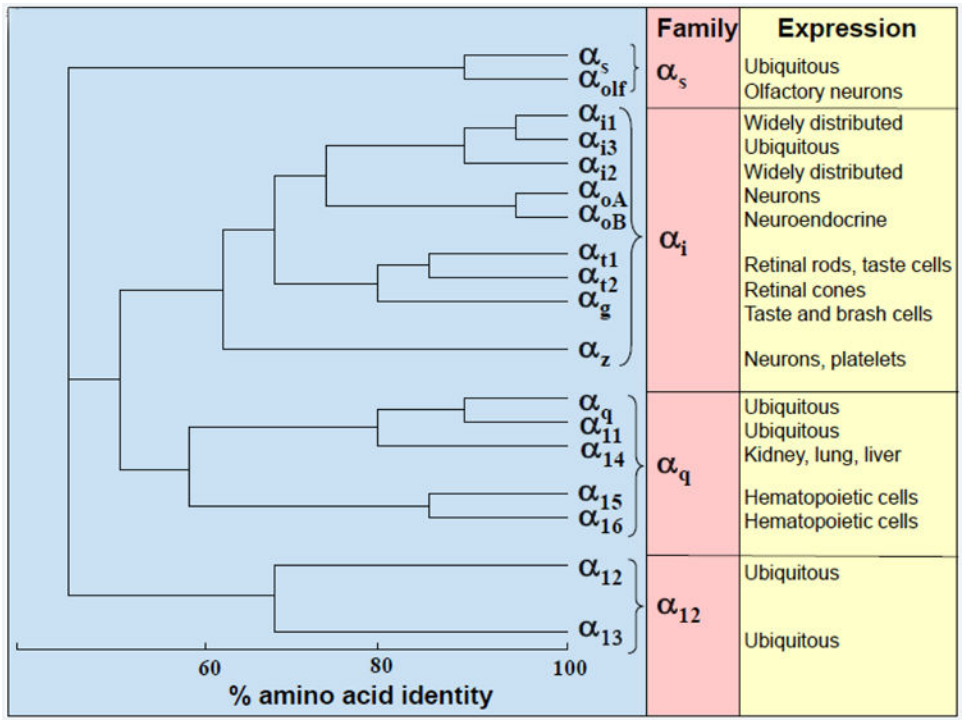

Figure 1.
Phylogenetic relationship of human and mouse Gα subunits and their expression.

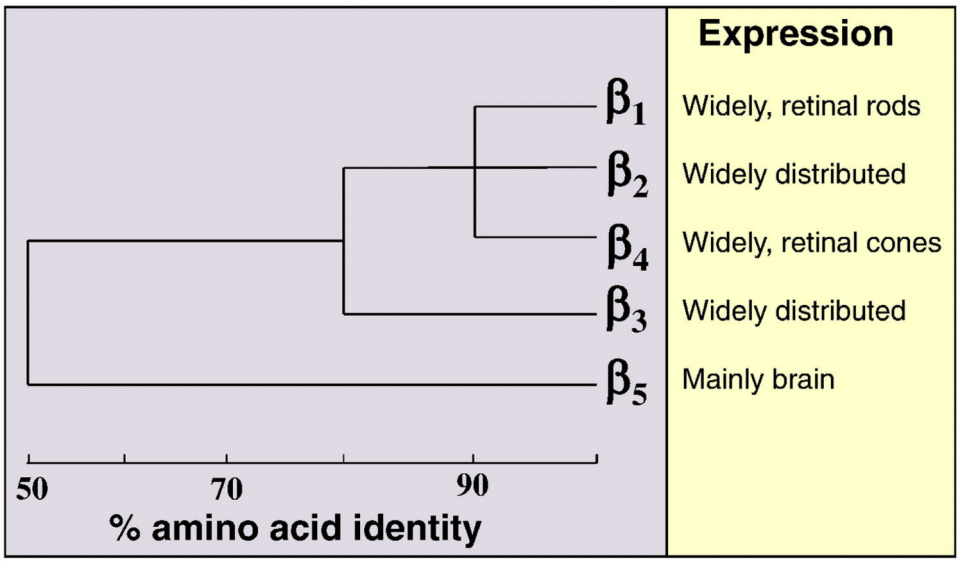

Figure 2.
Phylogenetic relationship of human Gβ subunits and their expression.

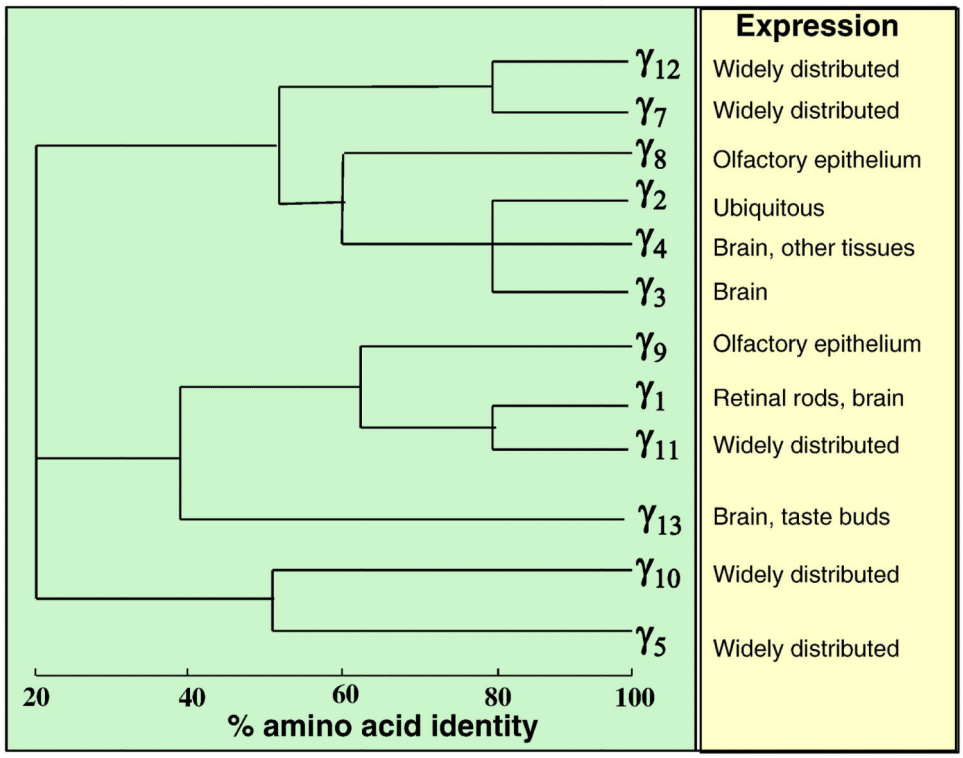

Figure 3.
Phylogenetic relationship of human Gγ subunits and their expression.

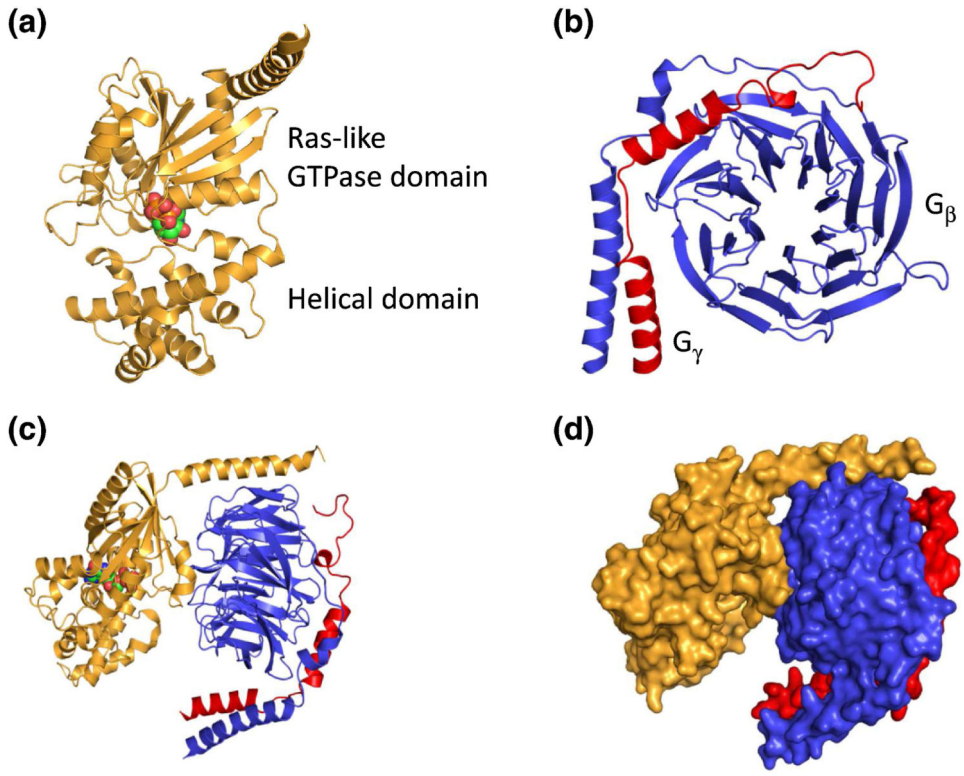

Figure 4.

Crystal structures of G-protein heterotrimer. Cartoon diagrams of $G\alpha_{i1}$ (orange) with GDP in space filling representation (color by atom) (a), $G\beta_1\gamma_2$ dimer where blue is Gβ, and red is $G\gamma_2$ (b), $G\alpha_{i1}\beta_1\gamma_2$ heterotrimer (c), and surface representation of $G\alpha_{i1}\beta_1\gamma_2$ heterotrimer (d).

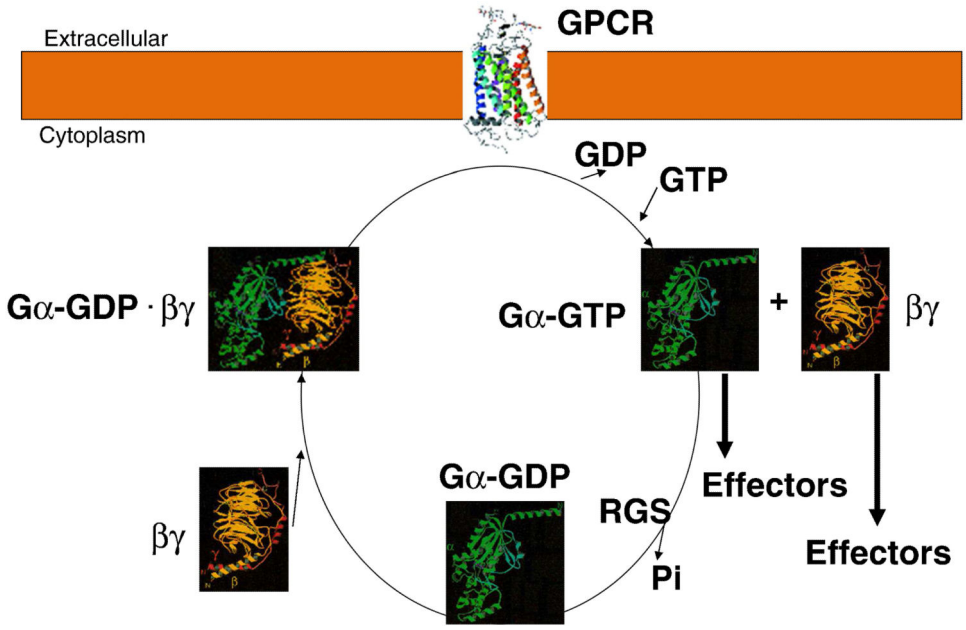

Figure 5.
G-protein cycle.

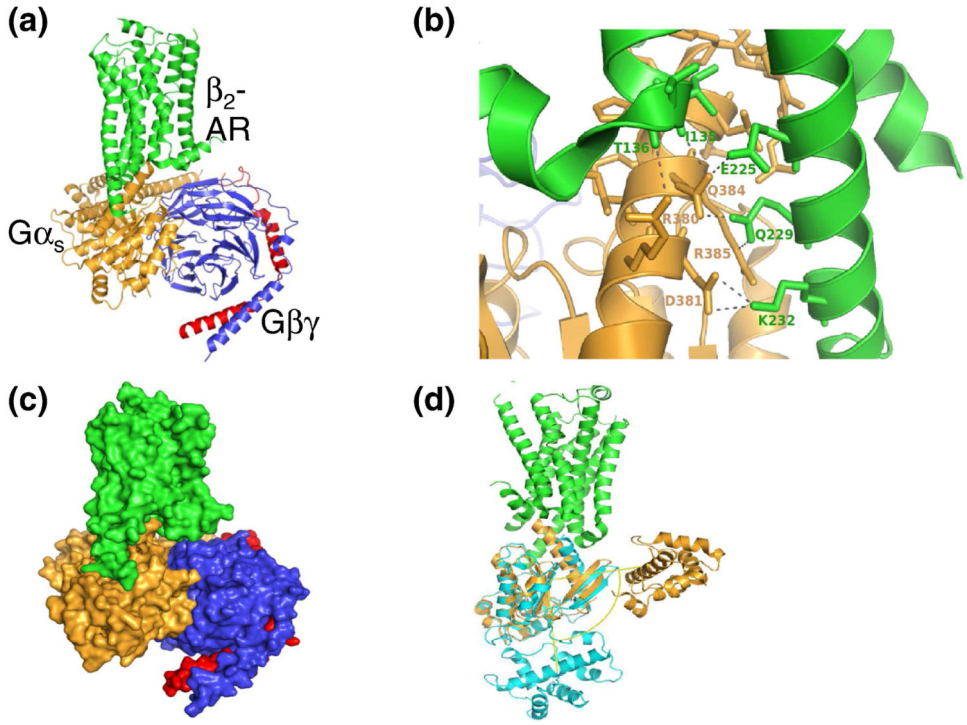

Figure 6.

Crystal structure of the complex of β_2-adrenergic receptor and Gs. (a) Cartoon representation of β_2-adrenergic receptor (green) and Gs (orange, blue and red), (b) Close view of the β_2-adrenergic receptor and Gs interface, (c) Surface representation of the β_2-adrenergic receptor and Gs. (d) Superposition of the α subunit of Gs from the apo form (cyan) and from its complex form with β_2-adrenergic receptor (orange).

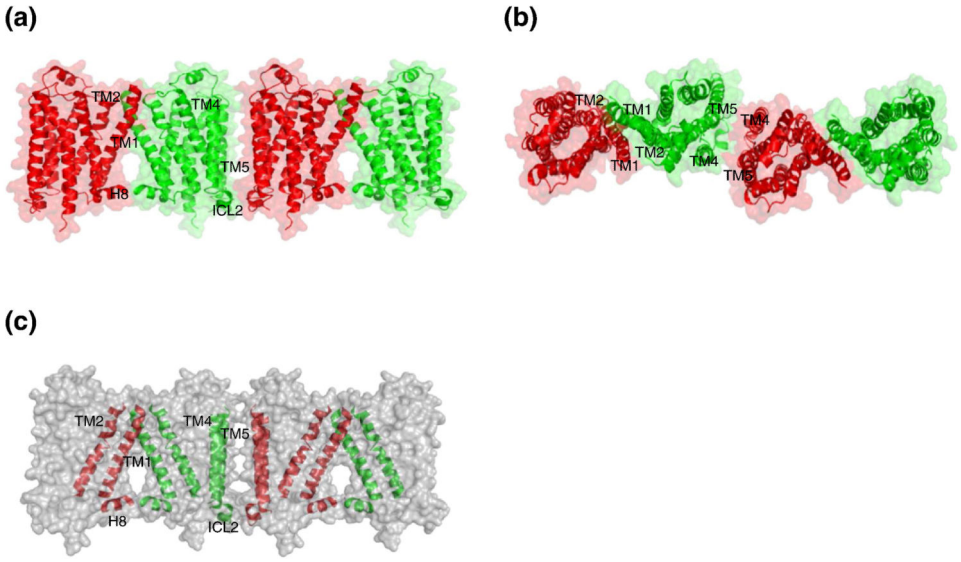

Figure 7.

Crystal structure of the oligomeric β_1-adrenergic receptor in a membrane-like environment. (a) Cartoon representation of β_1-AR as a tetramer in presence of molecular surface highlighting two distinct dimer interfaces. (b) Top view of β_1-AR tetramer down the extracellular surface. (c) Interacting TM1/TM2/H8 and TM4/TM5/ICL2 segments in cartoon diagram in presence of the surface representation of β_1-AR tetramer.

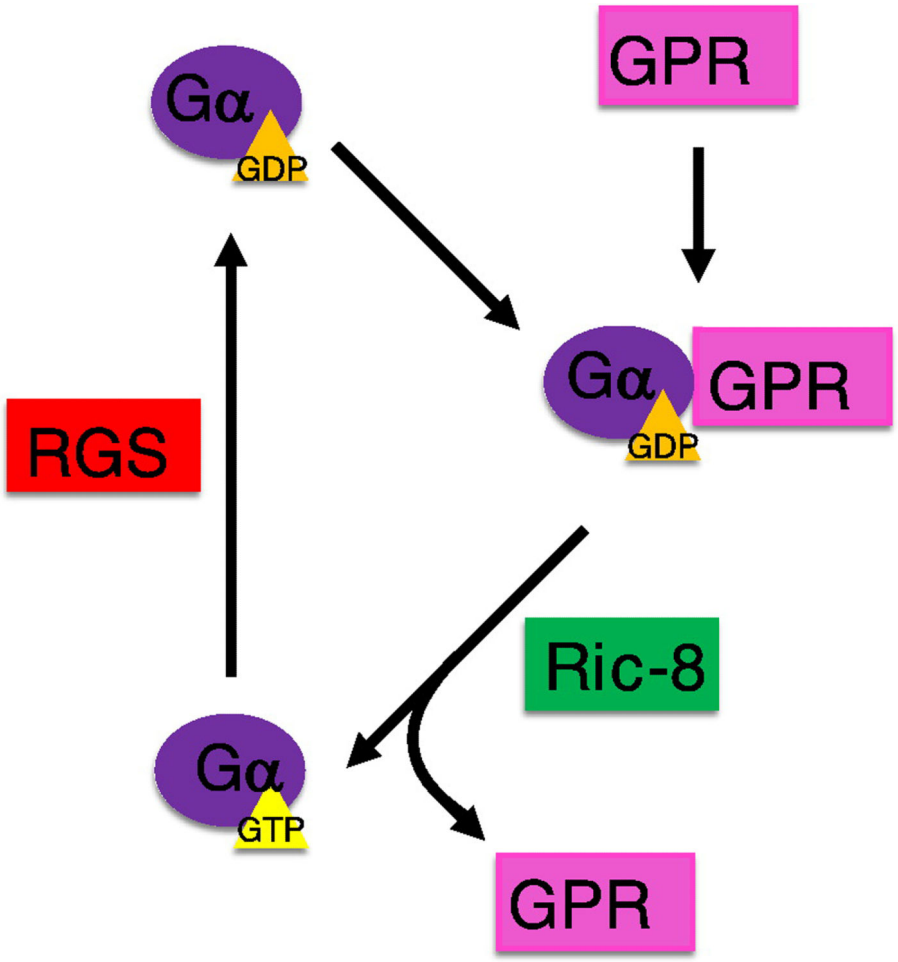

Figure 8.

G-protein activation by Ric-8. A GDP-bound Gα subunit disassociates from the membrane and can interact with a GPR-domain containing protein. This complex can then interact with Ric-8, which facilitates the exchange of GTP for GDP. Once activated, Gα can go on to interact with downstream effectors. The signal is terminated when Gα-GTP interacts with a RGS protein, which promotes the hydrolysis of GTP to GDP.

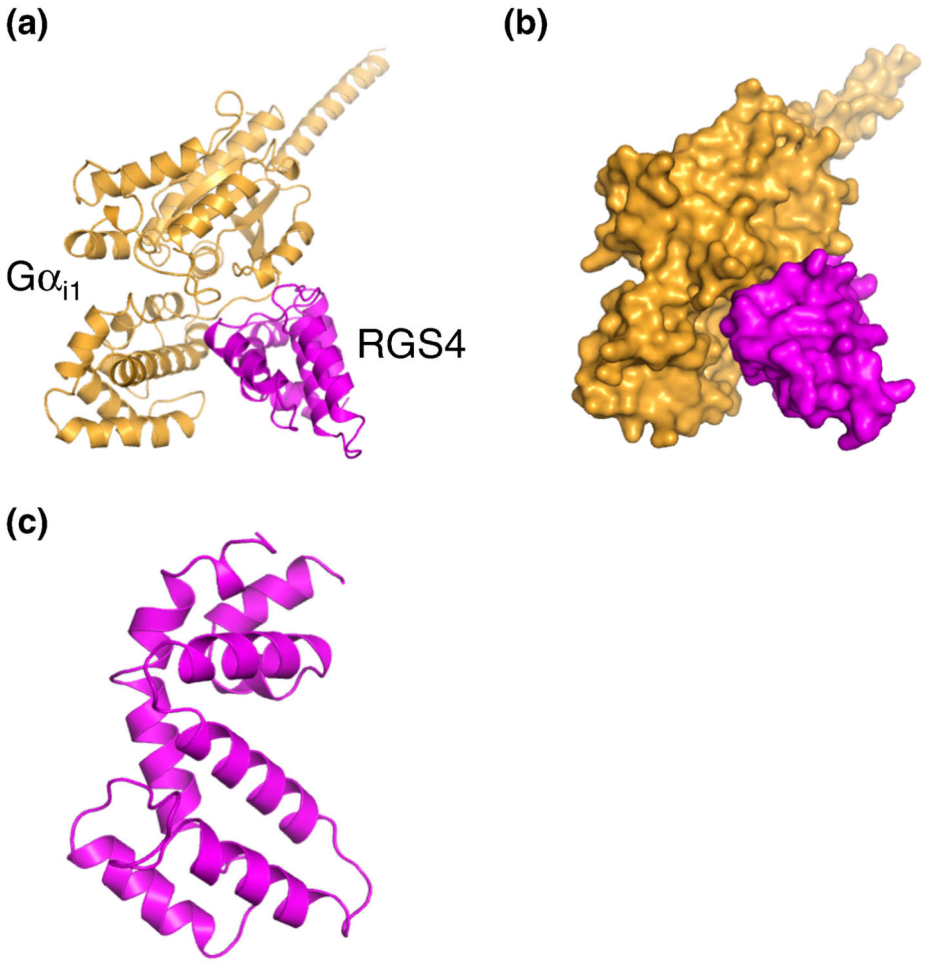

Figure 9.

Crystal structure of the complex of $G\alpha_{i1}$ and RGS4. (a) Cartoon representation of $G\alpha_{i1}$ (orange) in complex with RGS4 (magenta). (b) Surface representation of the complex. (c) Cartoon diagram of RGS4.

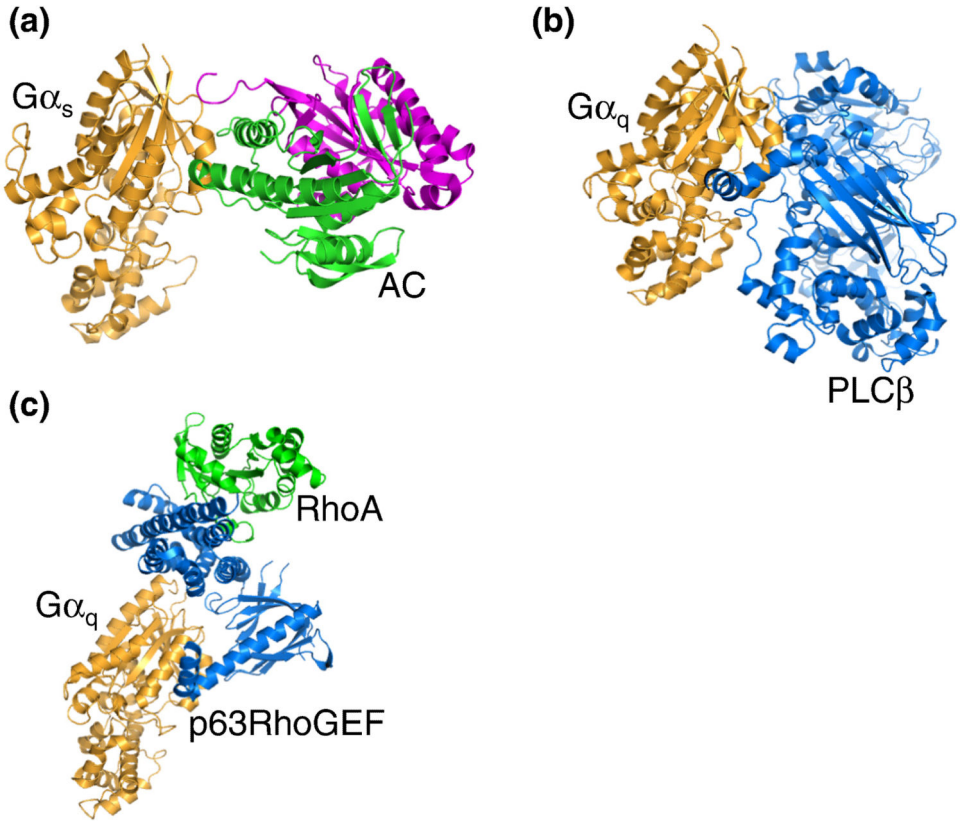

Figure 10.

Crystal structures of Gα in complex with different downstream effectors. (a) Cartoon representation of $G\alpha_s$ (orange) with adenylyl cyclase (AC) C1A (magenta) and C2A domains (green). (b) Representation of $G\alpha_q$ (orange) with phospholipase $C\beta_3$(blue). (c) Representation of $G\alpha_q$ (orange) with p63RhoGEF (blue) and RhoA (green).

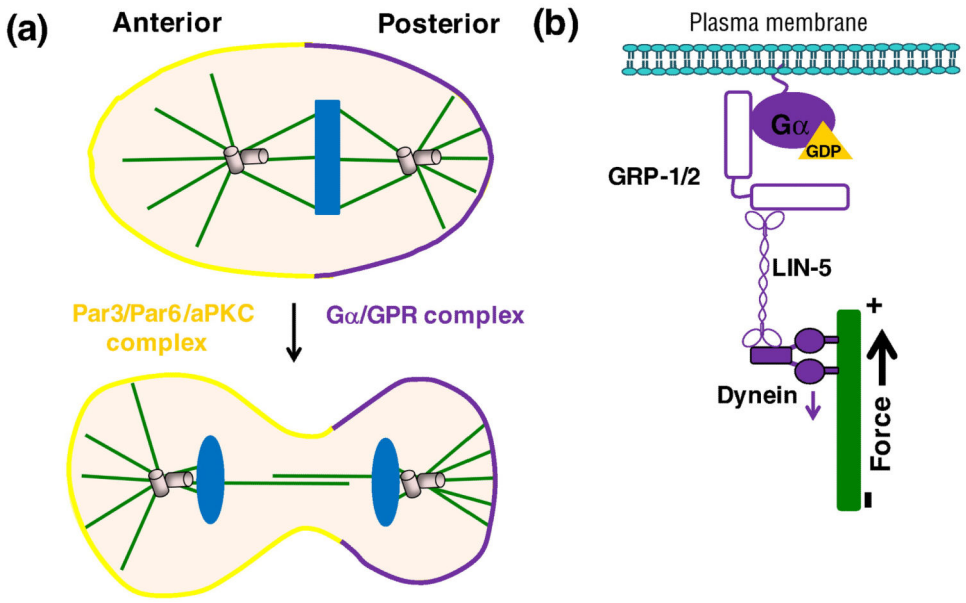

Figure 11.

An example of the non-canonical roles of G-protein signaling. In the control of embryonic spindle positioning in *C. eiegans* fertilized eggs, the Par3/Par6/aPKC complex is localized at the anterior while the Gα and GPR complex is in the posterior (a). The Gα/GPR complex is linked to the spindle through LIN-5 and dynein proteins (b).

Table 1

G*α* subunit interacting proteins

Gα subunit	**Well-defined G-protein effectors**	**Other G-protein interacting proteins**
$G\alpha_s$, $G\alpha_{olf}$	Adenylate cyclase (+)	Tubulin, Calnuc, Src tyrosine kinase, **axin**
$G\alpha_o$, $G\alpha_{i1-3}$, $G\alpha_{t1,2}$, $G\alpha_g$, $G\alpha_z$	Adenylate cyclase (-), cGMP phosphodiesterase (+)	Rap1Gapll, Calnuc, Src tyrosine kinase, nucleobindin 2 (NUCB2), Tubulin, Pins, Pcp1, LGN, GRIN1, Eya2, Pcp2
$G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$, $G\alpha_{15/16}$	Phospholipase C-β (+), p63RhoGEF	GRK2, actin, tubulin, PI3K, TPR1, Btk tyrosine kinase, Phospholipase C-ε, TRPM8
$G\alpha_{12}$, $G\alpha_{13}$	p115RhoGEF, LARG and PDZ-RhoGEF	Gap1, rasGap, Btk tyrosine kinase, Radixin, Hax-1, Cadherins, α-SNAP, p120caterin, Integrin $\alpha_{IIb}\beta_3$

(+) indicates stimulation. (-) represents inhibition.

Table 2

Gβγ subunit effectors

Well-defined Gβγ effectors	Other Gβγ interacting proteins
Adenylate cyclase (+), Phospholipase C-β (+), Phosphoinositide 3 Kinases, G protein-coupled receptor kinases, K^+ and Ca^{2+} channels	Btk-family tyrosine kinase, IP_3 receptors, Raf kinase, Protein kinase D, Histone deacetylase 5 (HDAC5), Tubulin, F-actin, Vinculin, ElmoE, Rab11, mitofusinl, Radil, activator protein 1, TFE3, TRPM1

Table 3

Physiological and Pathological functions of $G\alpha_s$ family

Physiological functions which could be explained by the well-studied cellular signaling pathways	Functions without well-established G-protein signaling pathways
Myocardial hypertrophy, increase in hydrolysis of triglyceride, decrease in amino acid uptake, increase in the conversion of glycogen to glucose, inhibition of synthesis of glycogen, increase in synthesis of estrogen and progesterone, increase in synthesis of aldosterone and Cortisol, increase in excitation/contraction and sympathetic cardiac activation and hypertrophy, iodide organification, secretion of thyroxine, thyroid cell mitogenesis, increase in reabsorption of calcium from bone, fluid secretion, inhibition of platelet aggregation and secretion	Mg^{2+} uptake, hematopoietic stem cell engraftment in bone marrow

Table 4

Physiological and Pathological functions of $G\alpha_i$ family

Physiological functions which could be explained by the well-studied cellular signaling pathways	Functions without well-established G-protein signaling pathways
Vision, taste, vomeronasal function, Cardiac activation (contractility), regulation of cardiac L-type Ca2+ channels, leukaryote activation and migration, hepatic authophagy, developmental processes, lipid metabolism, regulation of immune cells, renal function, platelet activation, chemokine-induced lymphocyte migration	Transformation of fibroblast, spindle positioning during cell division, regulation of diacylglycerol kinase (DGK), neurotransmitter release in synapses, cell migration

Table 5

Physiological and Pathological functions of $G\alpha_q$ family

Physiological functions which could be explained by the well-studied cellular signaling pathways	Functions without well-established G-protein signaling pathways
Myocardial hypertrophy, smooth muscle tone, platelet activation, hormone release in anterior pituitary, synaptic transmission at Purkinje cell synapse	Insulin secretion by β-cells, leukocyte migration and activation, embryonic myocardial growth, neural crest development, transformation of fibroblast

Table 6

Physiological and Pathological functions of $G\alpha_{12}$ family

Physiological functions which could be explained by the well-studied cellular signaling pathways	Functions without well-established G-protein signaling pathways
Platelet activation, smooth muscle contraction, leukocyte migration, and neuronal axon guidance	Leukocyte activation and proliferation, lymphocyte development, embryonic development of blood vessels and angiogenesis, transformation of fibroblast, cancer cell invasion and metastasis