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Receptor-Mediated Activation of Heterotrimeric G-proteins: Current Structural Insights *

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Running Title: Structural Determinants of G-protein Activation by GPCRs

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192 words in Introduction

ABBREVIATIONS: 7TM, seven transmembrane-domain; GAP, GTPase-accelerating protein; GDI, guanine nucleotide dissociation inhibitor; GDP, guanosine diphosphate; GEF, guanine nucleotide exchange factor; GPCR, G protein-coupled receptor; G-protein, guanine nucleotide-binding protein; GTP, guanosine triphosphate; GTPase, guanosine triphosphatase; RGS, regulator of G-protein signaling; TM, transmembrane domain.

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ABSTRACT

G protein-coupled receptors (GPCRs) serve as catalytic activators of heterotrimeric G-proteins (Gαβγ) by exchanging GTP for the bound GDP on the Gα subunit. This guanine nucleotide exchange factor (GEF) activity of GPCRs is the initial step in the G-protein cycle and determines the onset of various intracellular signaling pathways that govern critical physiological responses to extracellular cues. Although the structural basis for many steps in the G-protein nucleotide cycle have been made clear over the past decade, the precise mechanism for receptor-mediated G-protein activation remains incompletely defined. As these receptors have historically represented a set of rich drug targets, more complete understanding of their mechanism of action should provide further avenues for drug discovery. Currently, several models have been proposed to explain the communication between activated GPCRs and Gαβγ leading to the structural changes required for guanine nucleotide exchange. This review is focused on the structural biology of G-protein signal transduction with an emphasis on the current hypotheses regarding Gαβγ activation. We highlight several recent results shedding new light on the structural changes in Gα that may underlie GDP release.

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Many key extracellular signals, including hormones, neurotransmitters, growth factors, and sensory stimuli, relay information intracellularly by activation of plasma membrane-bound receptors. The largest class of such receptors is the superfamily of heptahelical G protein-coupled receptors (GPCRs). In many genomes, GPCRs are encoded by the largest gene family; in humans, >1% of the genome is dedicated to producing hundreds of these critical signal detectors (Fredriksson et al., 2003; Takeda et al., 2002). Genetic studies have highlighted the physiological importance of GPCRs, with knockout models revealing pathological phenotypes involving the cardiovascular, nervous, endocrine, and sensory systems (Karasinska et al., 2003; Rohrer and Kobilka, 1998; Yang et al., 2002). Several hereditary diseases have also been linked to mutations within the genes encoding specific GPCRs (Spiegel and Weinstein, 2004). Indeed, GPCRs represent a major therapeutic target giving rise to the largest single fraction of the prescription drug market with annual sales of several billion dollars (Overington et al., 2006). Therefore, a complete mechanistic understanding of how GPCRs communicate extracellular signals into the cell would be extremely valuable for the continued development of novel therapeutics that target this family of receptors and the signaling cascades they modulate.

G-PROTEIN SIGNALING AND THE GUANINE NUCLEOTIDE CYCLE

GPCRs transduce signals by activating heterotrimeric G-proteins that normally exist in an inactive state of Gα·GDP bound to Gβγ subunits (Figure 1). Agonist activation of GPCRs induces a conformational change within the receptor, which subsequently catalyzes the exchange of GDP for GTP on the Gα subunit (Gilman, 1987). By this means, GPCRs serve as guanine nucleotide exchange factors (GEFs) for Gα·GDP/Gβγ complexes (Figure 1). Although the exact mechanism by which GPCRs exert their GEF activity remains to be fully elucidated, this action

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is critical to the commencement of G protein signaling, as GDP release is the rate-limiting step of the Gα guanine nucleotide cycle (Ferguson et al., 1986). The subsequent binding of GTP induces a conformational change in three flexible 'switch regions' of the Gα subunit, leading to dissociation of the Gβγ dimer and adoption of the conformation capable of interacting with effectors (Hamm, 1998). Activated Gα·GTP and liberated Gβγ each relay signals to several downstream effectors including ion channels, adenylyl cyclases, phosphodiesterases, and phospholipases, giving rise to respective second messenger molecules intimately involved in regulating physiological processes (McCudden et al., 2005; Offermanns, 2003) (Figure 1). Based on their sequence homology and differential regulation of effectors, G-proteins are grouped in four classes: $G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_q$, and $G\alpha_{12/13}$ (Offermanns, 2003). GPCRs have the ability to couple selectively to members of one or more of these G-protein subfamilies, thus allowing selective modulation of signaling cascades by particular GPCR ligands. Deactivation of G-protein signaling occurs by the intrinsic hydrolysis of GTP to GDP by the Gα subunit, which occurs at a rate that varies among the G-protein subfamilies. Hydrolysis rates can be dramatically enhanced by members of a superfamily of "regulators of G-protein signaling" (RGS) proteins (Ross and Wilkie, 2000; Siderovski et al., 1996; Siderovski and Willard, 2005) that serve as GTPase-accelerating proteins (or "GAPs") (Figure 1). The inactivated, GDP-bound Gα subsequently reassociates with Gβγ to complete the cycle. Because this represents a true *cycle* of activation (by nucleotide exchange and subunit dissocation) and deactivation (by GTP hydrolysis and subunit reassociation), heterotrimeric G-proteins serve as molecular switches and are critical to defining the spatial and temporal aspects of cellular responses to external stimuli.

Biochemical and structural analyses over the past two decades have advanced our understanding of the mechanics underlying G-protein regulation and the guanine nucleotide

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cycle (Sprang, 1997). Despite these extensive and formidable efforts, the precise molecular details of how GPCRs activate Gα subunits remain elusive. The remainder of this review therefore focuses on G-protein structure and current perspectives regarding receptor-mediated activation of heterotrimeric G-proteins to highlight recent findings that are helping to shape a contemporary structural view of this process.

G-PROTEIN AND RECEPTOR STRUCTURE

Structures of GDP-bound G-proteins (both as isolated Gα and Gαβγ), as well as GTPγS-bound and transition-state GDP·AlF_4^--bound G-proteins (Table 1), have detailed the secondary structures (Figure 2) and tertiary structures of both Gα and Gβγ, how the heterotrimeric complex is formed, the conformational changes induced by GTP binding, and the mechanism of intrinsic GTP hydrolysis (Coleman et al., 1994; Lambright et al., 1994; Lambright et al., 1996; Mixon et al., 1995; Noel et al., 1993; Sondek et al., 1994; Sunahara et al., 1997; Wall et al., 1995). Subsequent analyses have defined the structural basis for engagement of several Gα subunits with their specific downstream effectors (Chen et al., 2005; Slep et al., 2001; Tesmer et al., 1997b; Tesmer et al., 2005). Additionally, the interaction of Gα with RGS proteins and the mechanism of GAP activity have been extensively characterized structurally (Chen et al., 2005; Slep et al., 2001; Tesmer et al., 1997a). Finally, recent efforts have resulted in structures of dark-adapted (inactive) and light-activated rhodopsin, the archetypical GPCR of visual phototransduction most amenable to structural interrogation (Palczewski et al., 2000; Salom et al., 2006). Outlined below are aspects of this impressive collection of work especially germane to our understanding of Gα activation and deactivation.

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THE Gα SUBUNIT. The nucleotide-binding pocket of the Gα subunit resides between two distinct domains: a Ras-like domain (named given its structural resemblance to the Ras superfamily of monomeric GTPases) and an additional, all α-helical domain composed of a structurally distinct six-helix bundle (Figures 2A and 3A). Binding of GTP causes a structural rearrangement within three segments of Gα, called "switch" regions (I-III), resulting from favorable interactions with the γ-phosphate of the newly bound GTP (Lambright et al., 1994; Wall et al., 1998). Switch I serves as one of two connections between the Ras-like and α-helical domains. Switch II assumes a partially helical conformation in the active state and governs many of the interactions of Gα with Gβγ, effectors, RGS proteins, GoLoco motifs, and other nucleotide-state-selective binding partners (e.g., (Johnston et al., 2006; Johnston et al., 2005; Kimple et al., 2002)). Switch III assumes a loop structure found ordered only in the active conformation of Gα.

The structural conformations adopted by switches I-III upon GTP binding allows the Gα subunit to specifically recognize downstream effectors. Structures of Gα/effector complexes ($G\alpha_s$/adenylyl cyclase, $G\alpha_t$/PDEγ, $G\alpha_{13}$/p115-RhoGEF, and $G\alpha_q$/GRK2: (Chen et al., 2005; Slep et al., 2001; Tesmer et al., 1997b; Tesmer et al., 2005)) have revealed that a highly conserved hydrophobic cleft within GTP-bound Gα, formed by the α2 and α3 helices (Figure 3A), serves as a universal site for effector engagement (reviewed in (Johnston et al., 2006)). Additional effector-binding regions are formed by the α2/β4 and α3/β5 loops of Gα (Itoh and Gilman, 1991; Slep et al., 2001; Tesmer et al., 1997b). However, the precise nature of effector specificity remains unclear (Johnston et al., 2006; Tesmer et al., 2005).

The mechanism of intrinsic GTP hydrolysis, and RGS protein-mediated acceleration of this activity, have been delineated from structures of the GTPase transition state ($G\alpha \cdot GDP \cdot AlF_4^-$)

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in isolation (Figure 3A) and bound to RGS proteins (Coleman et al., 1994; Slep et al., 2001; Sondek et al., 1994; Tesmer et al., 1997a), as well as reaction intermediates such as Gα bound to GppNHp or GDP plus inorganic phosphate (Coleman and Sprang, 1999; Raw et al., 1997). Intrinsic GTP hydrolysis is mediated by a triad of conserved Gα residues (numbered as in $G\alpha_{i1}$; Figure 2A): threonine-181 in switch I coordinates a Mg^{2+} ion that helps stabilize the γ-phosphate ion; arginine-178 in switch I also aids in the stabilization of the leaving γ-phosphate ion; and glutamine-204 in switch II coordinates the critical nucleophilic water molecule responsible for hydrolysis of the γ-phosphate (Coleman et al., 1994; Sondek et al., 1994) (Figure 3A). As these residues are both necessary and sufficient for GTP hydrolysis, RGS protein binding does not introduce additional catalytic residues, but rather stabilizes the transition state conformation, thus lowering the activational free energy required for the hydrolysis reaction (Berman et al., 1996; Srinivasa et al., 1998; Tesmer et al., 1997a).

THE Gβγ SUBUNIT. Dimerization between the Gβ and Gγ subunits of the G-protein heterotrimer is obligate in nature, with Gβ requiring Gγ to fold properly (Higgins and Casey, 1994); the Gβγ dimer only dissociates under denaturing conditions (Schmidt et al., 1992). The Gβ subunit begins with an extended N-terminal α-helix and mainly comprises a β-propeller fold (Figure 3B), a structural motif found in many other proteins unrelated to the Gβ family (Li and Roberts, 2001; Neer et al., 1994). The β-propeller domain of Gβ is formed by seven individual segments of a ~43-amino acid sequence known as a WD repeat motif (Figure 2B). An arrangement of seven four-stranded antiparallel β sheets forms the β-propeller; however, a given WD repeat motif does not correspond exactly to any one blade. The β-propeller is completed by the connection of strands from the first and seventh WD repeat (Figure 3B), with hydrophobic packing between blades contributing to the overall architecture. The Gγ subunit is an extended

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stretch of two α-helices joined by an intervening loop (Figure 2C). Assuming no significant tertiary structure on its own, the N-terminus of Gγ forms a coiled-coil interaction with the N-terminal α-helix of Gβ (Figure 3B,C); much of the remainder of Gγ binds along the outer edge of the Gβ toroid (Sondek et al., 1996; Wall et al., 1995). More recently, structures of Gβγ bound to phosducin (a regulatory protein), GRK2 (an effector), and SIRK (a non-natural peptide capable of disrupting effector activation) have defined the mode of Gβγ/effector interactions (Davis et al., 2005; Gaudet et al., 1996; Lodowski et al., 2003). Interestingly, the effector-binding site on Gβγ overlaps significantly with the region responsible for binding switch II of Gα near the central pore of the Gβ toroid (Figure 3B). Additional interaction sites exist for each specific complex (Davis et al., 2005; Gaudet et al., 1996; Lodowski et al., 2003).

THE Gαβγ HETEROTRIMER. The G-protein heterotrimer is formed by two principal sites of interaction between Gα and Gβγ (Figure 3C). First, extensive burial of the β3/α2 loop and α2 helix (switch II) of Gα within six of the seven WD repeats (specifically the DA and BC loops) of Gβ, with a majority of interactions governed by a hydrophobic core centered around tryptophan-211 of Gα (positioned deep within the α2/α3 cleft) and tryptophan-99 of Gβ (numbered as in $G\beta_1$) (Figure 3B). This interaction buries about 1800 $Å^2$ of solvent-accessible surface and forms the basis for Gβγ-mediated guanine nucleotide dissociation inhibitor (GDI) activity (Higashijima et al., 1987) and competition for Gβγ binding between Gα·GDP and Gβγ-effectors. The structures of Gβγ bound to the effector-competing, non-natural peptide, SIRK, and a *bona fide* effector, GRK2, have now firmly established this region of Gβ as critical to effector recognition (Davis et al., 2005; Lodowski et al., 2003). Second, a Gα/Gβ interaction surface occurs between the side of the first β-propeller blade of Gβ (WD1 and 2; specifically the D

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strands and CD loops) and the extended N-terminal helix of Gα (Figure 3B,C), the latter being normally disordered in structures of isolated Gα subunits (Lambright et al., 1996; Wall et al., 1995; Wall et al., 1998). This interaction buries an additional ~900 $Å^2$ of solvent-accessible surface. Figure 3C illustrates the structure of $G\alpha_{i1}\beta_1\gamma_2$ in its predicted membrane orientation with the Gα N-terminus and Gγ C-terminus, both sites of critical lipid modifications (Manahan et al., 2000; Wedegaertner et al., 1995), juxtaposed to the plasma membrane.

RHODOPSIN. A crystal structure of a prototypical GPCR, bovine rhodopsin, was first determined in its inactive, dark-adapted state (Palczewski et al., 2000). This structure provided the first glimpse into the arrangement of the seven transmembrane (TM) architecture of GPCRs and has provided an excellent tool for homology model-based studies involving other GPCRs (*e.g.*, (Mehler et al., 2006; Zhang et al., 2006)). The observed orientation of the TM helices within rhodopsin positions specific residues, previously identified as important for ligand binding in other GPCRs (*e.g.*, the β2-adrenergic receptor), towards the central core of the 7TM topology. The highly conserved E/DRY motif, involved in the activation mechanism of many rhodopsin-like (class A) GPCRs (reviewed in (Flanagan, 2005; Rovati et al., 2006)), was found within the rhodopsin structure to be engaged in an ion pair interaction between glutamate-134 and arginine-135 residues, supporting the hypothesis that disruption of this bond and movement of TM6 is involved in receptor activation (Ballesteros et al., 2001; Palczewski et al., 2000). The remainder of the intramolecular interactions within the 7TM core of rhodopsin are dominated by hydrophobic interactions. Biophysical studies have suggested that this network of interactions must rearrange (likely involving movements of TM3, TM5, and TM6) during activation to allow G-protein coupling (Swaminath et al., 2005). However, the structure of light-activated rhodopsin has now been solved and, contrary to previous thought (Altenbach et al., 2001; Ghanouni et al.,

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2001), demonstrates only minor conformational changes within the TM helices (Salom et al., 2006). In contrast, alterations in the cytoplasmic loops that contact the rhodopsin-specific G-protein (transducin; $G\alpha_t\beta_1\gamma_1$) were noticed, most notably with the paths of ic2 and ic3 which become largely disordered upon activation. These results suggest that receptor activation leads to a relaxation within the intracellular loops allowing for an induced fit with the G-protein heterotrimer. It must be stressed, however, that the low resolution in these structures prevents a precise definition of the molecular determinants for G-protein coupling and activation (Salom et al., 2006); furthermore, neither the dark-adapted nor light-activated rhodopsin structures (Palczewski et al., 2000; Salom et al., 2006) were obtained in the presence of G-protein heterotrimer. Thus, hypothetical models based on these and other experimental results have been put forth to describe the molecular mechanism of receptor-mediated G-protein activation. Below, we detail two of these prevailing hypotheses and recent evidence in their favor.

MECHANISM OF RECEPTOR-MEDIATED G-PROTEIN ACTIVATION

Despite the immense efforts and resounding successes described above in discerning the structural aspects of G-protein signal transduction, the structural basis for heterotrimeric G-protein activation by GPCRs remains largely unknown. This deficit has arisen mostly from the inherent obstacles to purification and crystallization of receptors and receptor/Gαβγ complexes (reviewed in (Sarramegn et al., 2006)). In lieu of such structural insights, biochemical approaches such as site-directed mutagenesis and the use of synthetic peptides and protein chimera have been used to identify regions within both receptors and Gαβγ heterotrimers critical to the activation process. Although these studies have mapped the receptor contact interface to the N-terminus, C-terminus, and the α4/β6 loop of Gα (Grishina and Berlot, 2000; Hamm et al.,

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1988; Onrust et al., 1997) along with the C-termini of both Gβ and Gγ (Hou et al., 2000; Hou et al., 2001) (Figure 4), they have provided little direct evidence for the actual mechanism of receptor-mediated activation of Gαβγ. Thus, these studies have led to hypothetical, and somewhat conflicting, models for receptor-mediated G-protein activation (Cherfils and Chabre, 2003; Johnston et al., 2005; Rondard et al., 2001; Van Eps et al., 2006). It is clear from the structures of dark-adapted (inactive) rhodopsin and Gαβγ, and their predicted orientations at the plasma membrane, that the receptor must act 'at a distance' to invoke GDP release by communicating structural changes *through* the G-protein toward its GDP-binding pocket that resides ~30 Å from the intracellular surface of the receptor (Bourne, 1997). To date, three distinct models have been proposed to describe how this process of long-range structural changes might occur. Below, we discuss two of these models: the 'C-terminal latch' and 'Gβγ lever' models (Nanoff et al., 2006; Rondard et al., 2001). We detail the foundations of each model and how each proposes distinct regions of the G-protein to be critical to activation. Finally, with recent results from our laboratory, we remark on the potential that these two models are indeed complementary with one another, rather than competing, in assembling the overall mechanism of receptor action. Notably, the third proposed model, termed the 'gear-shift' model (Cherfils and Chabre, 2003), will not be discussed in detail here, as significant experimental results in its favor are currently lacking.

C-TERMINUS 'LATCH' HYPOTHESIS. One of the first regions within Gα identified as being critical to receptor-promoted activation is the extreme C-terminus. Hamm and colleagues first demonstrated that synthetic peptides corresponding to the C-terminus of $G\alpha_t$ could block rhodopsin-promoted activation, suggesting that the C-terminus of Gα is a critical receptor-binding site (Hamm et al., 1988). Additional peptides corresponding to the α4/β6 loop

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region of Gα_t resulted in a similar attenuation, suggesting multiple sites of Gα/receptor contact. Alanine-scanning experiments confirmed these two regions (*i.e.*, C-terminus/α5 helix and α4/β6 loop) were essential for rhodopsin-promoted activation of Gα_t (Onrust et al., 1997) (Figure 4). Several subsequent studies have suggested that the extreme C-terminus communicates through the extended α5 helix of Gα to invoke the structural changes necessary for GDP release. Marin and colleagues have shown that mutations to several residues in an inward-facing, buried surface of the α5 helix cause a dramatic increase in the basal and receptor-promoted nucleotide exchange rates of Gα_t (Marin et al., 2001). Moreover, disruption of the α5 helix (via insertional and deletional mutagenesis) results in a loss of rhodopsin-mediated activation of Gα_t (Marin et al., 2002). For example, insertion of a five-glycine flexible repeat sequence in Gα between the α5 helix and extreme C-terminus dramatically reduces receptor-promoted activation with little effect on receptor coupling (Natochin et al., 2001). Electron paramagnetic resonance (EPR) studies have suggested that the Gα C-terminus moves into a more hydrophobic environment following AlF_4^--mediated activation, perhaps resulting in an interaction with the α2/β4 loop (Yang et al., 1999). Together, these results suggest that activated receptor uses critical contacts with the C-terminal tail of Gα to elicit conformational changes in the α5 helix during nucleotide exchange (Figure 5).

The α5 helix extends to the nucleotide binding pocket and connects with the β6 strand through the β6/α5 loop – a loop which makes several contacts to the guanine ring of the bound GDP molecule (Bohm et al., 1997; Sprang, 1997) (Figure 4A). Within the β6/α5 loop resides a conserved threonine-cysteine-alanine-threonine (TCAT) motif that mediates key contacts with GDP that are thought to stabilize the binding of GDP within Gα. Indeed, mutations within this

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region (Gα_s-A366S, Gα_i-A326S, Gα_o-C325S) result in dramatically enhanced spontaneous nucleotide exchange rates (Iiri et al., 1994; Posner et al., 1998; Thomas et al., 1993), and are clinically manifested in pseudohypoparathyroidism and gonadotropin-independent precocious puberty in the case of Gα_s-A366S (Iiri et al., 1998; Iiri et al., 1994). The effect of the alanine-to-serine mutation on nucleotide exchange is thought to result from introduction of steric clash between the extended side chain of serine and the guanine ring of GDP. Thus, the TCAT motif within the β6/α5 loop may serve as a conserved regulator of nucleotide exchange. Overall, this prevailing model suggests that the receptor contacts the Gα C-terminus and communicates structural changes through the α5 helix to modulate the conformation of the β6/α5 loop and its TCAT motif, ultimately resulting in the release of GDP via an exit route thought to be *away from* the 'Gβγ face' of Gα (Kisselev et al., 1998; Oldham et al., 2006) (Figures 5 and 6).

A recent study by Hamm and colleagues has added further support for the 'latch' hypothesis and the involvement of the α5 helix in transmitting structural changes to the GDP binding pocket (Oldham et al., 2006). By examining the dynamics of an EPR probe systematically attached to several individual Gα residues, the authors demonstrated that labeled residues within the α5 helix undergo specific receptor-mediated changes in EPR spectra, indicative of a perturbation in the conformation of this helix. The authors suggest that the receptor induces a rigid-body movement, specifically a rotation-translation function, in the α5 helix that preserves its overall helical structure (Oldham et al., 2006) (Figure 5A). Further experiments suggested this effect constitutes a ~5Å change in the distance distribution in the α5 helix. Moreover, insertion of a flexible glycine linker between the α5 helix and C-terminus reduced the receptor-mediated changes in EPR spectra of specific α5 helix residues (Oldham et al., 2006). These results strongly suggest the receptor uses contacts with the extreme C-terminus

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to communicate structural changes through the α5 helix presumably to the β6/α5 loop to induce the release of GDP. However, these studies also revealed changes in the EPR spectra of labeled residues in the β2/β3 loop and the β6 strand, suggesting these regions are mobile during receptor activation and thus may also play a crucial role in receptor-mediated activation of Gα (Figure 5A).

Beyond the α5 helix, several studies have implicated other regions of the Gα subunit in transmitting the necessary conformational changes to the β6/α5 loop for GDP release. The α3 helix, which connects the α3/β5 loop to switch III, was found by Berlot and colleagues to be important for receptor activation of $G\alpha_s$ (Grishina and Berlot, 2000; Marsh et al., 1998). A network of β-strands within Gα (β1, β2, β3; Figure 4A), which connect the N-terminus, P-loop, and switch I-II regions, has been shown to regulate the intrinsically slow rate of spontaneous nucleotide exchange in $G\alpha_t$ (Thomas et al., 2001). Finally, the β6 strand, through results of mutational studies (Onrust et al., 1997), is also considered an essential component of rhodopsin-mediated activation of $G\alpha_t$ (Figures 4 – 6).

Recent results from our laboratory have added more direct evidence for the involvement of the β6 strand in receptor-mediated Gα activation. We determined the structure of $G\alpha_{i1}$ bound to a peptide (D2N) corresponding to the N-terminal portion of the third intracellular loop (ic3) of the dopamine D2-receptor (Johnston and Siderovski, 2007). D2N, in common with several other receptor loop peptides, exhibits modest GEF activity on Gα subunits *in vitro* with a selectivity profile analogous to the cognate full-length receptor (Nanoff et al., 2006). We found that D2N binds to the α4/β6 loop region of Gα, previously identified as a critical receptor contact site important for Gα-coupling selectivity (Hamm et al., 1988; Oldham et al., 2006; Onrust et al., 1997; Slessareva et al., 2003) (Figures 4 and 6). Binding of D2N results in a displacement of the

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β6 strand compared to the native $G\alpha_{i1}$ structure (Johnston and Siderovski, 2007). These results suggest that the receptor uses the β6 strand, perhaps in combination with the α5 helix, to communicate structural changes to the β6/α5 loop and thereby disrupt contacts to GDP, resulting in nucleotide release (Figure 5A).

Gβγ 'LEVER' HYPOTHESIS. Whereas the above model of receptor-catalyzed nucleotide exchange relies solely on receptor/Gα contacts, an alternative model has been proposed that evokes Gβγ as an active participant in the exchange reaction (Iiri et al., 1998; Johnston and Siderovski, 2007; Johnston et al., 2005; Rondard et al., 2001). In the previously considered 'latch' model, Gβγ may serve merely to aid in heterotrimer association with the plasma membrane (via Gγ prenylation (Iniguez-Lluhi et al., 1992; Muntz et al., 1992)) and/or direct interaction with the receptor (Kisselev et al., 1999), thus playing only a passive role in the actual activation event. However, several observations would suggest that Gβγ indeed has a more active role in the activation mechanism. The requirement of Gβγ for proper receptor coupling and Gα activation has been long established (Fung, 1983; Fung and Nash, 1983). Receptor contacts established with both Gα and Gβγ (Figures 4 and 6) could be used to transmit conformational changes in both subunits relative to one another to establish a GDP exit route.

As detailed above, several regions of the Gα subunit have been proposed to directly contact receptor (Figure 4A). Similarly, efforts have been made to determine direct interactions between receptor and the Gβγ subunit. Intracellular regions of GPCRs, namely the third intracellular loop (ic3) and C-terminal tail, have been implicated in direct interaction with Gβγ (Mahon et al., 2006; Taylor et al., 1996; Wu et al., 1998), and may engage the N-terminus and sixth WD repeat segment of Gβ (Figure 4B), both of which are located on the outer surface and

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contained within the inferred receptor contact face (Hou et al., 2001). In this way, the activated receptor would undergo a conformational change that, in turn, would use contacts with Gβγ as a 'lever' to indirectly induce conformational changes in Gα (Figure 5B). Mutational experiments using $G\alpha_t$ have demonstrated that alanine substitution at several Gα/Gβγ contact sites in the switch II/Gβ interface attenuate rhodopsin-promoted activation without affecting Gαβγ heterotrimer formation (Ford et al., 1998). Additionally, disruption of a salt bridge mediated by lysine-206 (Gα switch II) and aspartate-228 (Gβ) completely abolishes β-adrenergic-mediated activation of $G\alpha_s$ without disrupting heterotrimer formation (Rondard et al., 2001). Together, these results suggest that an activation model evoking Gβγ may indeed apply universally to all Gα families. Finally, the established mechanism of action of GEFs for monomeric GTPases involves direct reorientation of switch I and II to establish a feasible GDP exit route (Cherfils and Chardin, 1999; Kawashima et al., 1996; Rossman et al., 2005). As the receptor cannot rationally be in direct contact with the Gα switch regions while Gα is ensconced within the heterotrimer (Figure 6), Gβγ would have to serve as a surrogate contact site for receptor-mediated manipulation of these key regions within Gα. The model described herein has been referred to as the 'Gβγ lever' hypothesis in which the receptor actively uses Gβγ as an 'adjuvant catalyst' in the nucleotide exchange reaction (Rondard et al., 2001). Specifically, Gβ contacts with Gα switch II (Figure 3B) are proposed to lever open switch II away from the GDP binding site following a receptor-mediated tilt of Gβγ away from Gα. Reorientation of switch II (α2 helix) in this way would, by necessity, also reorient the β3/α2 loop thought to serve as an 'occlusive lip' normally preventing GDP release (Iiri et al., 1994). GDP would, in turn, be more efficiently released, likely with an ejection path *toward* the 'Gβγ face' of Gα (Figure 5B).

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Previous work from our laboratory using a phage display-derived peptide (KB-752) with inherent GEF activity on isolated $G\alpha_i$ subunits provided direct biochemical and structural evidence for the 'Gβγ lever' hypothesis. KB-752, originally identified as a GDP-selective Gα-binding peptide, binds within a hydrophobic cleft created by the α2 and α3 helices of Gα, the same site used for binding Gβγ (Johnston et al., 2005). Binding of KB-752 causes a dramatic displacement of switch II as compared to its orientation with the heterotrimeric complex (Johnston et al., 2005). Furthermore, this movement in switch II results in a concurrent displacement of the 'occlusive' β3/α2 loop *away* from the GDP binding pocket – a displacement which is stabilized by contacts between this loop and KB-752. Thus, the KB-752 GEF peptide appears to serve as a Gβγ 'surrogate' in mimicking the proposed Gβγ-mediated displacement of the β3/α2 loop upon receptor activation (Johnston and Siderovski, 2007; Johnston et al., 2005).

Studies from Hamm and colleagues, again using EPR analysis, have recently added further support to structural changes within the Gα-Gβγ interface (*i.e.*, β3/α2 loop, α2 helix, α2/β4 loop) underlying receptor-mediated activation (Van Eps et al., 2006). When complexed with activated rhodopsin, spin-labeled residues within Gα at the interface with Gβγ underwent dramatic rotational changes, suggesting this region of Gα is conformationally altered during receptor-mediated activation. The most dramatic changes were seen in the α2/β4 loop, whereas more moderate changes occurred in the β3/α2 loop and switch II (α2) helix (Van Eps et al., 2006). As these regions on Gα are not considered viable receptor contact sites *per se*, rhodopsin-induced changes here suggest an allosteric regulatory mechanism, possibly through the proposed Gβγ 'levering'.

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MODEL CONVERGENCE. The two models detailed above represent the prevailing thoughts regarding the mechanism of receptor-promoted activation of heterotrimeric G-proteins, although other hypotheses have also been presented in the literature (Cherfils and Chabre, 2003; Remmers et al., 1999). While these models are often presented as starkly opposing mechanisms, they may actually not be mutually exclusive. Indeed, Bourne and colleagues, in particular, while championing the idea of the 'Gβγ lever' have suggested that multiple mechanisms may play complementary roles in the overall action of activated receptors (Onrust et al., 1997).

Recent results from our laboratory now lend experimental evidence for such a case of model convergence. As previously mentioned, we determined the structure of $G\alpha_{i1}$ bound to D2N, a receptor-derived peptide with demonstrated GEF activity (Johnston and Siderovski, 2007). This structure also included the KB-752 peptide described above. Interestingly, whereas D2N and KB-752 each possess modest GEF activity alone, a combination of both peptides yields a synergistic GEF activity on $G\alpha_{i1}$ (Johnston and Siderovski, 2007). The D2N peptide binds to and displaces the β6 strand, which connects to the α5 helix via the β6/α5 loop, while simultaneously, KB-752 'pulls' the β3/α2 loop *away* from the GDP binding pocket. These findings suggest that activated receptors could use a similar multi-pronged approach to cause structural changes in several Gα regions acting together to invoke GDP release. In accordance with the overwhelming data supporting a role for the α5 helix in this process, we hypothesize that the receptor uses contacts with both the β6 strand and the α5 helix to ultimately disrupt the critical contacts between the β6/α5 loop and GDP. However, the synergistic GEF activity observed by concomitant application of D2N and KB-752 peptides onto Gα suggests that modulation of the β6/α5 loop alone is insufficient for maximal GEF activity and, therefore, modulation of the β3/α2 loop serves as a second key determinant of GDP release. Thus, we

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suggest that the receptor uses direct contacts with the β6 strand and α5 helix to release guanine base contacts with the β6/α5 loop (Figure 5A), coincident with Gβγ-mediated levering of the β3/α2 loop to remove the occlusive lip blocking GDP release (Figure 5B), thereby causing maximally efficient nucleotide release.

RECEPTOR DIMERIZATION. The precise mechanism of receptor-catalyzed G-protein activation is likely to be quite complex. Another feature of this process that remains intensely debated is the existence of receptor dimerization (Fotiadis et al., 2006; Prinster et al., 2005). Historically, a single receptor was thought sufficient to activate a G-protein heterotrimer. However, determination of the rhodopsin structure, as a dimer, illustrated the G protein-facing surface to be relatively narrow compared to the width of the Gαβγ heterotrimer (Palczewski et al., 2000; Salom et al., 2006) and see Figures 5 and 6), adding support to the argument that a receptor dimer is necessary for efficient G-protein activation (Angers et al., 2002). Clearly, dimerization can be critical for certain aspects of receptor function such as membrane targeting or ligand recognition (e.g.,(Waldhoer et al., 2005; White et al., 1998)); however, it remains controversial whether the receptor dimer is absolutely required for G-protein activation (Chabre and le Maire, 2005). Although far from conclusive, our low-resolution model depicting key Gα and Gβγ interaction sites occurring within the rhodopsin dimer (Figure 6) supports a role for dimerization in proper G-protein coupling and activation. We do not intend to draw conclusions regarding any requirement for both receptor protomers to bind agonist or the allosteric regulation (e.g., cooperativity) that may result (Schwartz and Holst, 2006); rather, we suggest that each receptor protomer underlies specific contacts with Gα and/or Gβγ that may not be achieved by a monomeric receptor and are therefore required for efficient activation.

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CONCLUDING REMARKS AND FUTURE DIRECTIONS

The importance to human physiology of GPCR signaling through heterotrimeric G-proteins cannot be overstated. These receptors and the pathways regulated by activated G-proteins are crucial to a wide variety of cellular responses, underlie the etiology of many serious pathophysiologies, and represent the molecular target for many pharmacotherapeutic agents. Although an amazing amount of work has led to our current understanding the structural basis for much of the G-protein signaling cycle, the precise mechanism of receptor-mediated activation remains incompletely defined. Several models, described herein, have been proposed to depict this event.

Continued efforts should one day yield the 'holy grail' of a high resolution structure of a receptor/Gαβγ complex, ultimately revealing to atomic resolution the structural basis for this ubiquitous event of receptor GEF activity. Such a feat will undoubtedly greatly enhance continued drug discovery and design with GPCR targets, although prospects for achieving such a monumental accomplishment might be limited given the inherent conformational flexibility of these receptors and the dynamic nature of the G-protein activation process. Certain technical "tricks" could enhance efforts to trap the receptor/Gα·GDP/Gβγ complex into a state of non-productive or "stalled" activation that would be more stable and thus suitable for crystallization: application of stabilizing ligands such as inverse agonists (Kenakin, 2004), mutation to the receptor/Gα or receptor/Gβγ interface to increase the affinity of the overall complex, or mutation to Gα (*e.g.*, (Wall et al., 1998)) or Gβγ (*e.g.*, (Rondard et al., 2001)) to limit conformational changes in the heterotrimer while preserving receptor association. Such analyses would likely reveal the structural determinants for complex formation between receptor and heterotrimer,

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allowing one to manipulate one or more aspects of their interface for subsequent studies – perhaps even rational drug design.

However, in order to provide the full picture of the structural rearrangements induced by receptor that provoke GDP release, one would require the structural determinants of the transition-state reaction intermediate as represented by a receptor-bound heterotrimer depleted of nucleotide. As the activation process is undoubtedly highly dynamic in nature, with the transition state likely a highly transient event, capturing such a conformation in a crystal structure may prove more difficult yet. Our work using both non-native and receptor-derived peptide GEFs (Johnston and Siderovski, 2007; Johnston et al., 2005) have been unsuccessful in promoting a crystallization-compatible, nucleotide-free state of the Gα subunit and thus have not yet revealed profound structural alterations within the nucleotide binding pocket *per se*. The crystal structure of a $G\alpha_{i1}$ mutant (A326S) with a dramatically enhanced intrinsic nucleotide exchange rate revealed a state only partially occupied with GDP; however, no significant alterations in the overall nucleotide binding pocket were observed either (Posner et al., 1998). Although these crystallography results suggest that gross alterations in the nucleotide binding pocket may not be required for nucleotide release, recent NMR analysis of a receptor-bound, 'nucleotide empty' Gα (under activation conditions) has revealed significant line broadenings in the obtained spectra (Abdulaev et al., 2006), suggesting that the nucleotide-free form represents a dynamic intermediate state. Interestingly, these structural changes were specific to conditions incorporating activated receptor, highlighting the critical role of receptor-induced conformational changes. Strategies designed to trap stable, receptor-bound and nucleotide-free Gαβγ complexes suitable for crystal formation will thus be crucial to ultimately understand the precise conformational changes induced by activated receptor leading to nucleotide exchange.

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FIGURE LEGENDS

Figure 1. Guanine nucleotide cycle of heterotrimeric G-proteins

Seven transmembrane domain, G protein-coupled receptors (GPCRs) bind, via their intracellular loops, to the heterotrimeric G-protein consisting of Gα (with bound GDP) associated with the Gβγ dimer. The isoprenylated Gβγ dimer aids in association of the heterotrimer with the plasma membrane, participates in receptor coupling, and serves as a guanine nucleotide dissociation inhibitor (GDI) preventing spontaneous activation of the Gα subunit. Agonist-bound receptors act as guanine nucleotide exchange factors (GEFs) by provoking conformational changes in Gαβγ resulting in the release of GDP and binding of GTP by Gα. Binding of GTP induces changes in three conformationally-flexible switch regions within Gα, leading to the dissociation of Gβγ. Both Gα·GTP and freed Gβγ can subsequently regulate downstream effector molecules alone or in a coordinated fashion. The system returns to the inactive state by intrinsic GTP hydrolysis activity of the Gα subunit, cleaving the terminal γ-phosphate from GTP (note the loss of inorganic phosphate [Pi]) and rendering Gα again bound to GDP and reassociated with Gβγ, thus mutually terminating the signaling capacity of both subunits. The GTP hydrolysis reaction is greatly enhanced by the "regulator of G-protein signaling" (RGS) family of proteins, which serve as GTPase-accelerating proteins (GAPs).

Figure 2. Heterotrimeric G-protein subunit secondary structure

Amino acid sequence alignments and secondary structure features from high-resolution structures of Gα (**A**), Gβ (**B**), and Gγ (**C**) subunits. Secondary structure assignments (alpha helices, beta-strands) and ruler numbering are derived from (**A**) $G\alpha_{i1}$ (PDB id: 1GFI), (**B**) $G\beta_1$ (PDB id: 1TBG), and (**C**) $G\gamma_1$ (PDB id: 1TBG) and color-coded to match tertiary and quaternary

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structural representations of Figure 3. (**A**) The three, conformationally-flexible switch regions of Gα (including the entire α2 helix within switch II) are indicated by *green* dots within the ruler line; residues in Gα that contact Gβ are marked with *red* dots above the ruler line. Conserved Gα guanine base and phosphate contact positions are highlighted in *purple* (GAGE and DVGGQ motifs) and *red* (TCAT motif); the conserved arginine, threonine, and glutamate residues involved in GTP hydrolysis are highlighted in *orange*. (**B**) The four beta-strands that comprise each of the seven WD repeat segments within Gβ subunits are color-coded to match the tertiary structure of $G\beta_1$ as represented in Figure 3B. Residues in Gβ that contact Gγ or the switch regions of Gα are marked with *gray* or *green* dots, respectively; additional Gα contacts are marked with *blue* dots. (**C**) Residues in Gγ that contact Gβ are marked with *red* dots.

Figure 3. Heterotrimeric G-protein tertiary and quaternary structure

(A) The tertiary structure of $G\alpha_{i1}$ (PDB id 1GFI), comprised of a Ras-like domain (*blue*) and an all alpha-helical domain (*yellow*), is shown in a transition-state mimetic form bound to a molecule of GDP (*magenta*), magnesium ion (*red*), and tetrafluoroaluminate (AlF_4^-) ion (*grey/blue* sticks). The three critical switch regions (numbered SI to SIII) are colored *green*. Three essential catalytic residues that participate in the water-mediated GTP hydrolysis reaction are shown in *orange*: arginine-178 (R178) and threonine-181 (T181) in switch I as well as glutamine-204 (Q204) in switch II. **(B)** The structure of $G\beta_1\gamma_2$ (PDB id 1GP2) reveals the prototypical β-propeller fold of the $G\beta_1$ subunit and the partially α-helical nature of the $G\gamma_2$ peptide (*black*); note the coil-coiled interaction between the N-termini of these subunits. The $G\beta_1$ subunit is colored according to the seven WD repeat segments (N-terminal helix, *red*; WD1, *brown*; WD2, *yellow*; WD3, *magenta*; WD4, *teal*; WD5, *orange*; WD6, *grey*; WD7, *wheat*).

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Under this color scheme, notice that each 4-bladed propeller segment is comprised of 3 blades from one WD repeat and 1 blade from the preceding WD repeat. The tryptophan (W99) in WD2 critical for interaction with $G\alpha_{i1}$ is shown in sticks. Also shown are the two significant contact regions from $G\alpha_{i1}$: the $G\alpha_{i1}$ N-terminal α-helix ("N-end Gα"; *blue*) interacts along the outer edge of the $G\beta_1$ toroid, whereas the switch II helix (*green*; W211 shown in sticks) interacts in the center and makes critical contacts with 6 of the 7 WD repeats. **(C)** The structure of the $G\alpha_{i1}$·GDP/$G\beta_1\gamma_2$ (PDB id 1GP2) illustrates the molecular basis for the formation of a heterotrimeric G-protein. The heterotrimer is depicted in its proposed membrane-bound orientation with the $G\beta_1\gamma_2$ heterodimer. $G\alpha_{i1}$ is colored as in panel A, with bound GDP in *magenta* and several key switch II residues that interact with $G\beta_1$ represented as sticks (K209, W211, I212, and F215; *green*). The $G\beta_1$ subunit is shown in *red* and the $G\gamma_2$ in *grey*. Key $G\beta_1$ residues interacting with the $G\alpha_{i1}$ switch II helix are represented in sticks (W99, D228, and D246; *red*). The C-terminus of $G\gamma_2$ terminates with a CAAX motif that is isoprenylated (depicted as saw-tooth line) to increase association with plasma membrane.

Figure 4. Regions within the G-protein heterotrimer critical to the receptor-mediated activation process.

Several regions within both Gα and Gβγ have been implicated in receptor coupling and the activation process. The $G\alpha_{i1}$·GDP/$G\beta_1\gamma_2$ structure (PDB id 1GP2) is depicted in the proposed membrane-bound, receptor-associated conformation from two different perspectives. **(A)** Lateral view of the heterotrimer highlights critical $G\alpha_{i1}$ regions discussed and referenced in detail in the text. The coloring scheme is as follows: Gα N-terminus (*teal*), β1-β3 strands including the β2/β3

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loop (*orange*), α3/β5 loop (*cyan*), α4/β6 loop (*yellow*), β6 strand (*blue*), β6/α5 loop (*green*), and α5 helix (*red*). A molecule of GDP is depicted as sticks (*magenta*). **(B)** Rotation 90° about the y-axis reveals additional regions, including potential receptor contacts within $G\beta_1\gamma_2$. The $G\alpha_{i1}$ switch II and β3/α2 and α2/β4 loops are colored *brown*. Within $G\beta_1\gamma_2$, proposed receptor contact sites of $G\beta_1$ are colored *salmon* (including residues 31-39 in the N-terminus as well as residues 280-340 of WD6 and WD7). The C-terminus of $G\gamma_2$ (residues 60-71) is colored *pink*.

Figure 5. Proposed receptor-induced conformational changes in the G-protein heterotrimer leading to GDP release.

Although the precise mechanism of receptor-catalyzed activation of heterotrimeric G-proteins remains incompletely resolved, several conformational changes within the G-protein have been proposed to occur as the result of agonist activation of the receptor. As detailed in the text, these conformational changes are thought to result in the release of GDP, the rate-limiting step in the activation process. Panels A and B each represent a component movement, with the critical regions of Gα engaged in each movement highlighted in *salmon* and *red*. **(A)** Lateral view of the $G\alpha_{i1}\cdot$GDP/$G\beta_1\gamma_2$ heterotrimer (PDB id 1GP2) highlighting the $G\alpha_{i1}$ subunit, which is colored similarly to Figure 3. The critical β6 strand and α5 helix are colored *salmon*, and the β6/α5 loop connecting them is colored *red*. The bound GDP (sticks) is colored *magenta*. Green arrows indicate the proposed receptor-induced conformational changes in these regions of Gα. Work from our laboratory suggests a displacement in the β6 strand upon binding of a receptor-derived peptide with GEF activity (Johnston and Siderovski, 2007). Work from several laboratories suggests a receptor-induced rotation in the α5 helix (Kisselev et al., 1998; Marin et al., 2002;

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Oldham et al., 2006). Both of these conformational changes are thought to propagate into a movement of the β6/α5 loop, thus weakening the stabilizing bonds it makes with GDP and resulting in a decreased affinity of the heterotrimer for nucleotide. Through direct contacts with the α5 helix, the β2/β3 loop (*cyan*) may serve as a critical regulator of the basal state of the α5 helix (Marin et al., 2001). **(B)** Rotation of the heterotrimer 90° about the y-axis reveals another proposed mechanism of receptor-mediated activation. In this view, the switch II (α2) helix is colored *salmon* and the β3/α2 loop *red*. The $G\beta_1$ subunit is colored *grey* and the $G\gamma_2$ *black*. As the receptor is thought to make contacts with both Gα and Gβγ (see Figure 4), conformational changes in the receptor are proposed to translate into a tilting (*orange arrow*) of the Gβγ subunit relative to Gα (Iiri et al., 1994; Rondard et al., 2001). This tilting (or translation) of G-protein subunits relative to one another may displace switch II and result in the β3/α2 loop being pulled away (*green arrow*) from the GDP-binding pocket (Johnston et al., 2005). As the β3/α2 loop is thought to serve as an occlusive barrier or 'lip' to the release of GDP, this conformational change would create a more feasible exit route for the nucleotide in a direction that is toward the Gβγ subunit (Johnston et al., 2005; Rondard et al., 2001). The two proposed mechanisms depicted in these two panels may work together (and perhaps synergistically) to regulate nucleotide exchange by Gα: the mechanism shown in panel A would weaken the affinity for bound GDP, and that described in panel B would create a pathway for GDP release (Johnston and Siderovski, 2007; Onrust et al., 1997; Van Eps et al., 2006).

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Figure 6. Relative orientation and interactions of the transducin G-protein heterotrimer with a rhodopsin dimer.

A recently determined structure of dark-adapted rhodopsin in a trigonal crystal form (PDB id 2I36) revealed a dimeric interface proposed to be a physiologically functional entity (Salom et al., 2006). This structure is depicted with protomer 1 colored *white* and protomer 2 colored *grey*. The third intracellular loop (ic3), universally critical to receptor activity (Bourne, 1997), is colored *purple* in both protomers. Peptides isolated from the ic3 loop of several receptors have been found to directly interact with the Gα subunit and elicit modest activation (Johnston and Siderovski, 2007; Nanoff et al., 2006). The ic3 loop has also been demonstrated to interact with the Gβγ dimer (Taylor et al., 1996; Wu et al., 2000). The C-terminal tail (including "helix 8" [H8] of rhodopsin [*brown*]) has recently been implicated in direct interaction with the Gβ subunit (Mahon et al., 2006). The $G\alpha_t\beta_1\gamma_1$ heterotrimer (PDB id 1GOT) has been positioned in its presumed orientation at the receptor/membrane interface. The color scheme for the transducin heterotrimer follows that of Figure 4 with slight modifications: the Gα subunit is colored *green-cyan* (with its bound GDP as *orange*), the Gβ is *marine-blue* (with its proposed receptor-interaction site colored *salmon*), and the Gγ is *teal*. Additionally, switch II and the β3/α2 loop are colored *black* and the α4/β6 loop is colored *yellow*. Although this 'docking' is mostly artistic in nature, its position is loosely constrained by our recent identification of molecular determinants within the N-terminus of the dopamine D2-receptor ic3 loop binding to the α4/β6 region of $G\alpha_{i1}$ (Johnston and Siderovski, 2007). Interestingly, when constrained by this particular interaction site, our rendition of the GPCR/G-protein interaction positions the heterotrimer in a way that satisfies many of the previous results discussed in the text. Notably, the α5 helix and C-terminus of Gα (*red*) are positioned within reasonable distance to the ERY

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motif (*copper* sticks) of the rhodopsin protomer 1 that is essential for receptor activity (Palczewski et al., 2000; Rovati et al., 2006). The proposed Gβ contact site (*salmon*) (Hou et al., 2001; Taylor et al., 1996) is positioned within bonding distance to the two receptor regions previously proposed to directly interact with Gβγ: the ic3 loop and the C-terminal region (Mahon et al., 2006; Wade et al., 1996; Wu et al., 1998). Also, in this orientation, the lipid modifications (not depicted) on the Gα N-terminus (myristol and/or palmitoyl groups) and the Gγ C-terminus (isoprenyl group) are positioned to be inserted within the plasma membrane.

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Table 1:
Milestone GPCR/heterotrimer structures obtained by crystallography

PDB Code [a,b]	Description	References
1GDD; 1TAG	$G\alpha_{i1}$ and $G\alpha_t$ bound to GDP; inactive conformation	(Lambright et al., 1994; Mixon et al., 1995)
1GFI; 1TAD	$G\alpha_{i1}$ and $G\alpha_t$ bound to $GDP{\cdot}AlF_4^-$; transition-state mimetic conformation revealing basis for GTP hydrolysis	(Coleman et al., 1994; Sondek et al., 1994)
1GIA; 1TND	$G\alpha_{i1}$ and $G\alpha_t$ bound to GTPγS; active conformation	(Coleman et al., 1994; Noel et al., 1993)
1TBG; 2TRC	$G\beta_1\gamma_1$ uncomplexed and bound to phosducin	(Gaudet et al., 1996; Sondek et al., 1996)
1GP2; 1GOT	$G\alpha_{i1}\beta_1\gamma_2$ and $G\alpha_t\beta_1\gamma_1$ bound to GDP; basis for heterotrimer assembly	(Lambright et al., 1996; Wall et al., 1995)
1AZS; 1FQJ	Activated $G\alpha_s$ bound to adenylyl cyclase and activated $G\alpha_t$ bound to RGS9/PDEγ; basis for Gα/effector interactions	(Slep et al., 2001; Tesmer et al., 1997b)
1AGR	$G\alpha_{i1}$ bound to $GDP{\cdot}AlF_4^-$ and RGS4; basis for RGS protein GTPase-accelerating activity	(Tesmer et al., 1997a)
1KJY	$G\alpha_{i1}$ bound to GDP and RGS14 GoLoco motif; basis for GoLoco-mediated GDI activity	(Kimple et al., 2002)
1OMW	$G\beta_1\gamma_2$ bound to G-protein coupled receptor kinase-2; basis for GRK2 regulation by Gβγ	(Lodowski et al., 2003)
1Y3A	$G\alpha_{i1}$ bound to KB-752 phage-display peptide; basis for KB-752-mediated GEF activity and role of β3/α2 loop in Gα GDP release	(Johnston et al., 2005)
2HLB	$G\alpha_{i1}$ bound to KB-752 and D2N peptides; basis for dopamine D2-receptor ic3 loop interaction in modulating β6 strand during Gα GDP release	(Johnston and Siderovski, 2007)
1F88	Dark-adapted rhodopsin; inactive conformation with bound *cis*-retinal	(Palczewski et al., 2000)
2I37	Light-activated rhodopsin; active conformation with bound *trans*-retinal (physiologically-compatible dimeric orientation)	(Salom et al., 2006)

[a] Accessible via http://www.pdb.org/ or via http://www.ncbi.nlm.nih.gov/Structure/
[b] Viewable using PyMol from http://pymol.sourceforge.net/ (highly recommended) or other molecular visualization freeware from http://www.umass.edu/microbio/rasmol/

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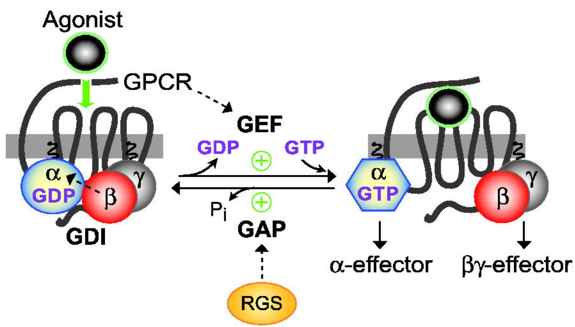

Figure 1

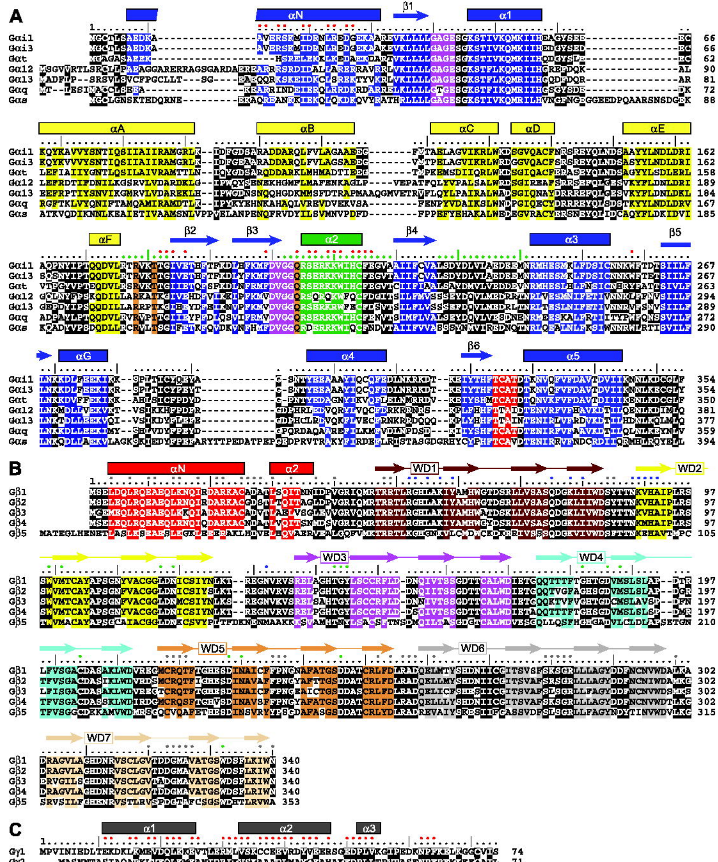

Figure 2

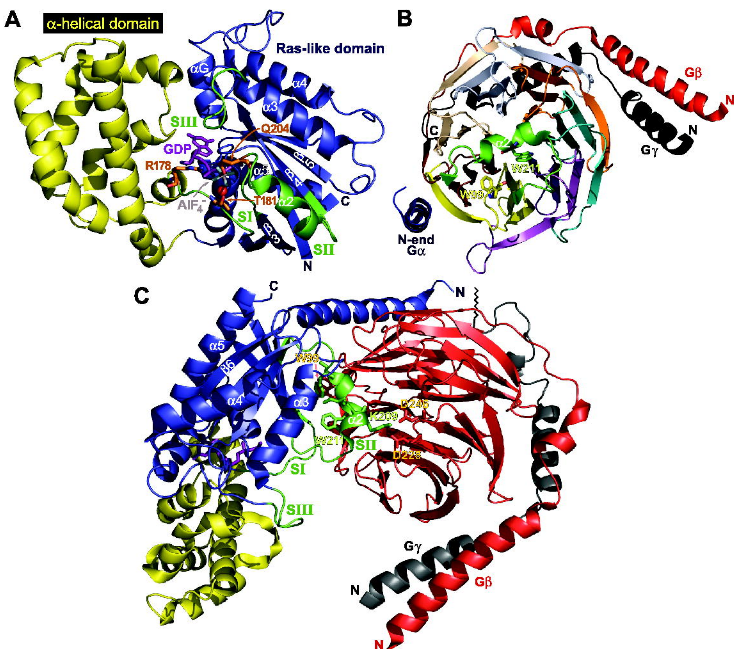

Figure 3

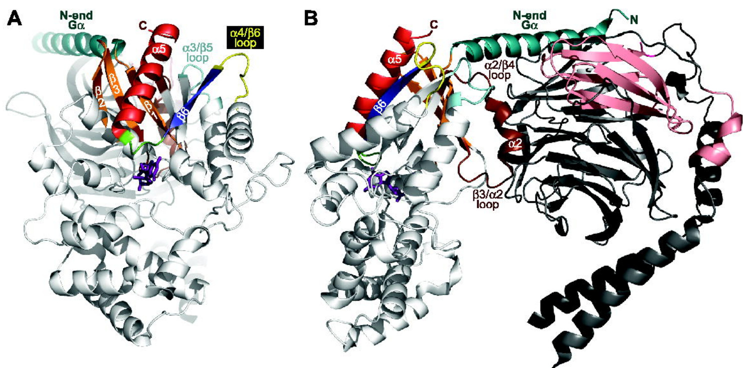

Figure 4

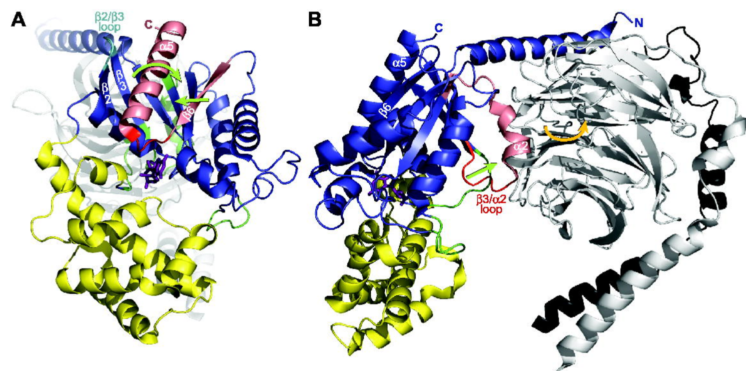

Figure 5

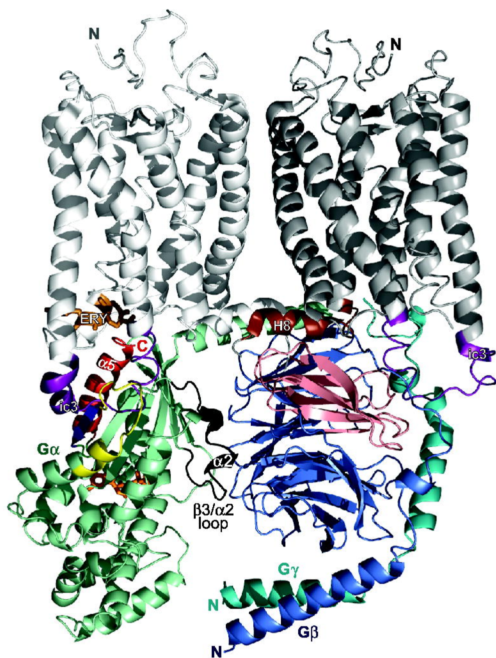

Figure 6