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G PROTEIN $\beta\gamma$ SUBUNITS

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ABSTRACT

Guanine nucleotide binding (G) proteins relay extracellular signals encoded in light, small molecules, peptides, and proteins to activate or inhibit intracellular enzymes and ion channels. The larger G proteins, made up of $G_{\alpha\beta\gamma}$ heterotrimers, dissociate into G_α and $G_{\beta\gamma}$ subunits that separately activate intracellular effector molecules. Only recently has the $G_{\beta\gamma}$ subunit been recognized as a signal transduction molecule in its own right; $G_{\beta\gamma}$ is now known to directly regulate as many different protein targets as the G_α subunit. Recent X-ray crystallography of G_α, $G_{\beta\gamma}$, and $G_{\alpha\beta\gamma}$ subunits will guide the investigation of structure-function relationships.

INTRODUCTION

The cell border is defined by a lipid bilayer that separates the soluble, organized, proteinaceous intracellular space from the chaotic extracellular world. In order for a cell to interact with its surroundings, or to participate in a multicellular organism, it must communicate with that world. A major mechanism for information transfer across the lipid barrier is the G protein signal transduction system (Figure 1). The minimum components of this system are a receptor, a heterotrimeric G protein complex of G_α and $G_{\beta\gamma}$ subunits, and an effector. Roughly one thousand of the ~80,000 genes encoding specific proteins in humans are devoted to construction of specific receptors that thread themselves seven times across the lipid bilayer. We evolved from organisms swimming in the primal soup, in which various chemicals washed over the cell membranes, requiring the organisms to determine whether nearby objects were food, enemies, or potential mates. Thus, the largest group of these G protein–linked receptors are in the

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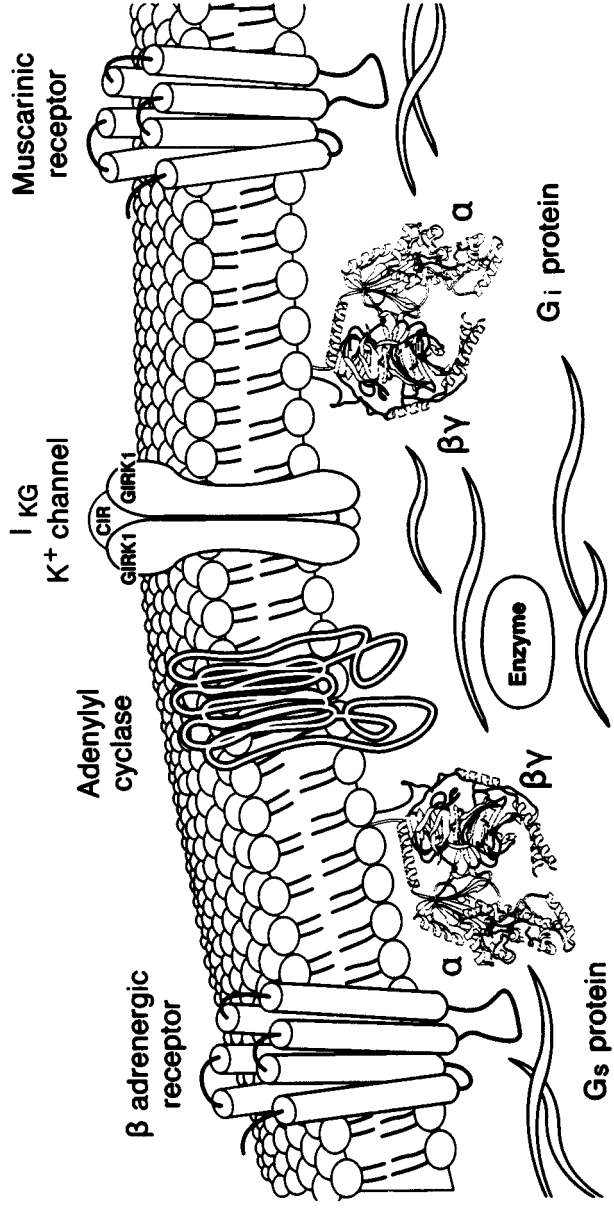

Figure 1 Components of the G protein–linked receptor signal transduction network for the β-adrenergic stimulation of adenylyl cyclase and the muscarinic-mediated activation of a K^+-selective ion channel.

olfactory system, although the mechanism by which the very large number of odorants are detected is only poorly understood. Probably the most remarkable receptor is the visual G protein–linked receptor rhodopsin, which can detect small numbers of photons. The other few hundred receptors specifically bind intercellular messenger molecules such as acetylcholine, glutamate, γ-aminobutyric acid, epinephrine, dopamine, histamine, opiates, and many others.

The G protein–linked receptor's extracellular face is adapted to recognize a very specific molecule. When bound to this specific ligand, it changes shape, and now its intracellular domain, which interacts with a specific heterotrimeric G protein complex, catalyzes the release of the guanine nucleotide diphosphate (GDP) from the G_α subunit. Unlike the G protein–linked receptors that are integral transmembrane proteins, the G proteins themselves are more loosely attached to the inner surface of the plasma membrane, partly through covalently attached lipids. Cytoplasmic GTP is abundant, and its concentration does not limit the exchange reaction. GTP replaces GDP in the guanine nucleotide binding cleft of G_α, initiating conformational changes in "switch" regions of the G_α subunit. These changes weaken the affinity of G_α and $G_{\beta\gamma}$ for each other so that GTP-bound G_α and $G_{\beta\gamma}$ subunits are freed to interact with other proteins.

The earliest functions established for individual subunits of G proteins were the activation of retinal cGMP phosphodiesterase by $G_{\alpha t}$ and of adenylyl cyclase by $G_{\alpha S}$ [reviewed by Gilman (1)]. Generalizing on these findings, G_α was postulated to be the effector regulatory subunit. $G_{\beta\gamma}$ was thought to turn off the activated G_α subunit and to enhance its membrane binding, but on the whole, its primary function was mysterious. The first clear evidence that $G_{\beta\gamma}$ could itself regulate effectors came to light when Logothetis et al (2) showed that $G_{\beta\gamma}$ activated a K^+-selective ion channel (I_{KACh}) in cardiac atrial cells. The following year, Whiteway et al (3) showed that $G_{\beta\gamma}$, not G_α, carried the signal from the yeast mating receptor to the response pathway. Although the hypothesis was heatedly opposed for several years by G_α advocates (4–6), $G_{\beta\gamma}$ has now been shown to directly bind and activate numerous effectors. Effectors that are regulated by one or both subunits are now known to be roughly equally represented, although identification of all potential effectors is still in its early stages. Twenty G_α, $6G\beta$, and $12G\gamma$ subunits nominally provide 1440 combinatorial signal transduction options. The bipartite signal of released active G_α and $G_{\beta\gamma}$ also enables control of two effectors simultaneously. The heterotrimeric nature of G proteins leads to amplification in the numbers of endpoints, control mechanisms, or turned-on effector molecules.

STRUCTURE OF $G_{\beta\gamma}$

Although the $G_{\beta\gamma}$ subunit is made up of two polypeptides, G_β and G_γ, it is functionally a monomer because the two subunits cannot be dissociated except

with denaturants. The G_β subunit is made up of two structurally distinct regions, an amino terminal segment, which is an α helix of approximately 20 amino acids, and the remainder of the molecule, which is made up of a sequence motif that is repeated seven times. This repeating sequence, called a WD repeat, is not unique to the G_β subunit but occurs in approximately 40 other proteins that make up the WD-repeat superfamily. Members of this family do not have an immediately obvious common function; they are involved in diverse cellular pathways such as signal transduction, pre-mRNA splicing, transcriptional regulation, assembly of the cytoskeleton, and vesicular traffic (7). The common thread seems to be that proteins with WD-repeats make up parts of large macromolecular assemblies. Therefore, the capacity to assemble multiple proteins may be an essential part of their function.

The regular expression that describes the WD-repeats predicts that the structure is made up of small antiparallel β strands (7). Recently, two groups have solved the crystal structure of the G protein heterotrimer. The crystal structure reveals that the core WD-repeat portion of G_β is indeed made up of β strands and that the β strands in the G_β subunit are arranged in a ring, forming a propeller structure [Figure 2; (8–10); see also Clapham (11) and Neer (12)]. Each blade of the propeller is made up of four twisted β strands. The circular structure is held closed by a molecular "velcro snap" in the seventh blade of the propeller. The outer strand of the seventh blade is made by a sequence arising from the N-terminal part of the protein, whereas the other three strands of the four-stranded blade come from the carboxyl terminus. The structure of $G_{\beta\gamma}$ helps explain the thermal stability of the dimer and the observation that tryptic cleavage at the one site accessible in the native molecule does not disrupt $G_{\beta\gamma}$ structure or function (13). The $G_{\beta\gamma}$ subunit also remains noncovalently associated after cleavage of $G_{\gamma 2}$ or $G_{\gamma 3}$ in purified brain $G_{\beta\gamma}$ by endopeptidase LysC (14). The WD-repeat can be divided into a highly conserved core at about 40 amino acids usually bounded by Gly-His (GH) and Trp-Asp (WD), a variable-length region between WD and the next GH (7). The variable region is variable only in the sense that no consensus can be found among all of the WD-repeat proteins analyzed. However, within a family, each of these regions is highly conserved. Thus, within a particular G_β subunit, the variable region between, for example, repeats two and three and repeats three and four are different from each other but very similar to the equivalent positions in G_β from evolutionarily very distant organisms. The variable regions of the G protein WD-repeats form the outer β strands of each blade. Together, they form a ring around the surface of the torus. The functions of these regions is not yet known, but it is very likely that they will be important for protein-protein contacts.

Figure 3 shows an $\alpha\beta\gamma$ heterotrimer (8, 9). From this diagram, one can see that the G_β subunit has several different surfaces: the α surface, the γ surface, the surface that makes a ring around the torus, the surface that lines the central

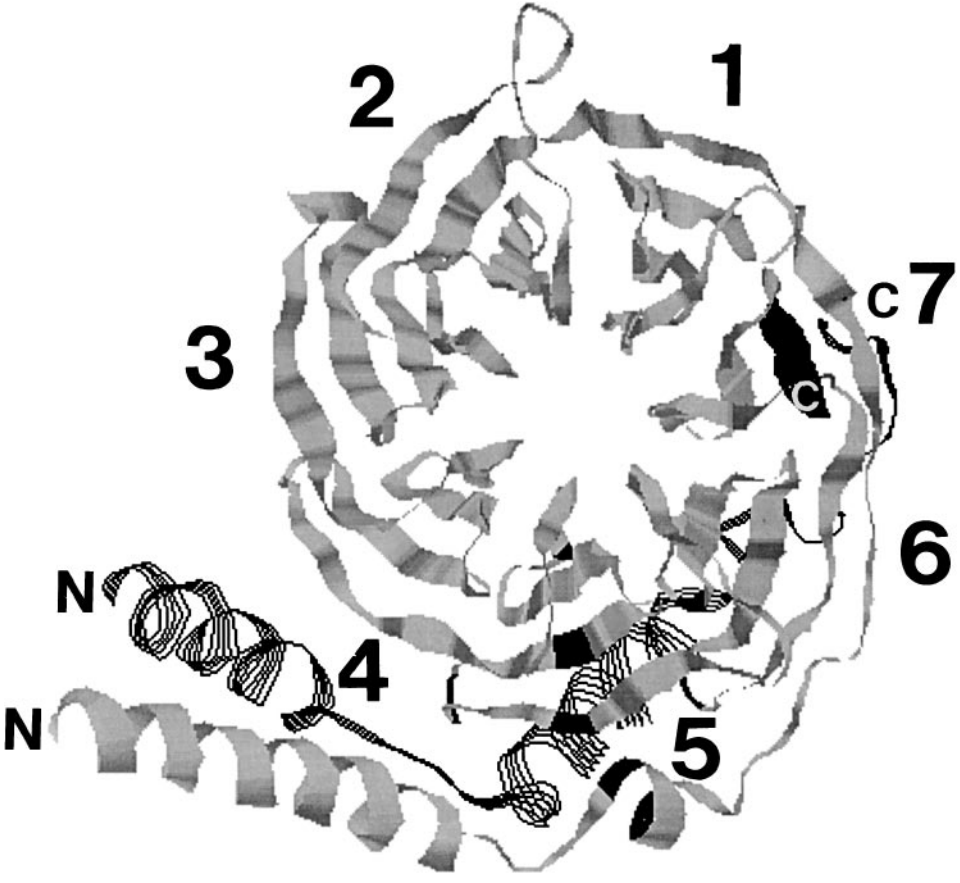

Figure 2 The $G_{\beta\gamma}$ subunit seen from the surface that faces G_α. The G_β subunit is shown in solid gray; the G_γ subunit is shown in black stripes. The C terminus of G_β is shown in black with a white C. It is located on the third β strand [counting from the inner (tunnel) surface] of blade 7. Note that the outer strand of blade 7 is formed from sequences near the N terminus of G_β. The association of the C terminus with the N-terminal area forms the "velcro" snap that holds the molecule together. The regions of G_β that contact the residues on $G_{\gamma 1}$ that determine the specificity of its interaction with $G_{\beta 1}$ or $G_{\beta 2}$ (20) are indicated in black on blade 5 and the adjacent small α helix. The blades are numbered so that the first core WD repeat (GH to WD) occurs in blade 1. This convention is different from that used by Sondek et al (10). The figure was created using coordinates kindly provided by Dr. S Sprang, University of Texas Southwestern, Dallas, Texas.

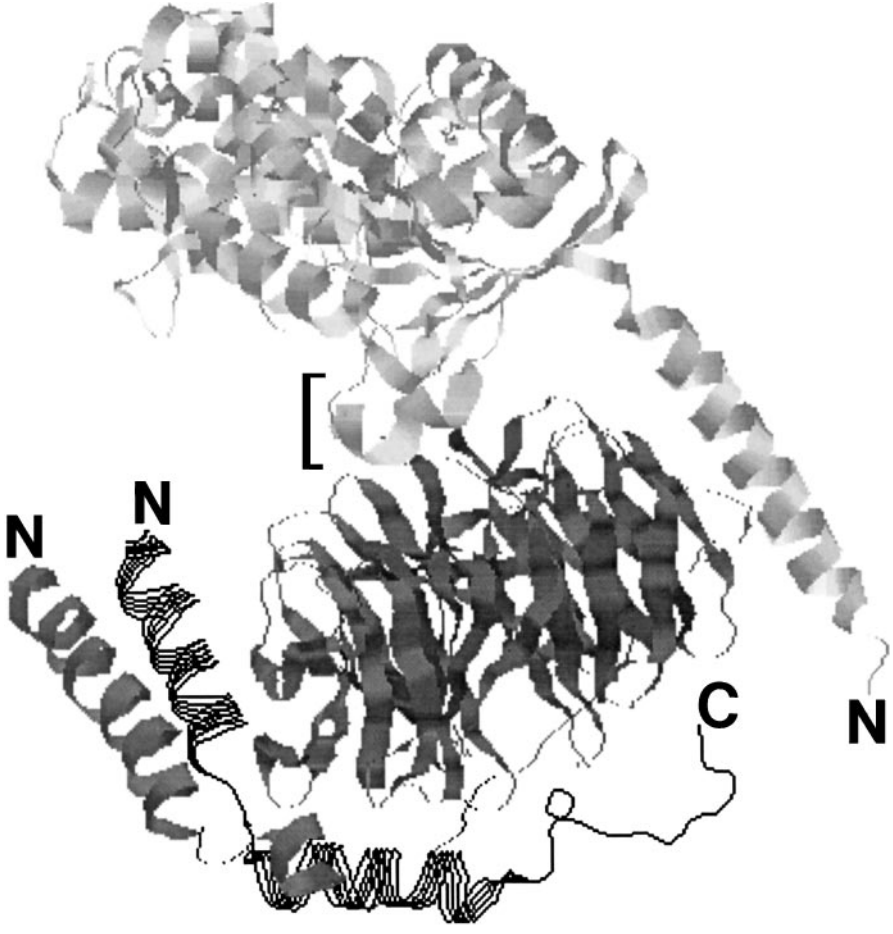

Figure 3 The $G_{\alpha\beta\gamma}$ heterotrimer. The G_α subunit is shown in light gray. The bracket marks the switch-II region, one of the regions that has a different conformation in the GDP and GTP bound state of G_α. The G_β subunit is shown in dark gray. The G_γ subunit is shown in black stripes. The N and C termini are marked except for the C termini of G_β and G_α that are not visible. The figure was created using coordinates kindly provided by Dr. S Sprang, University of Texas Southwestern, Dallas, Texas.

tunnel, and the surface of the N-terminal α helix. G_α binds asymmetrically over the narrow end of $G_{\beta\gamma}$, making contacts principally with residues in blades 1, 2, and 3. The switch-II region of G_α, a region that changes conformation between the GDP- and GTP-liganded forms (see below) is positioned over the central tunnel of G_β. The γ subunit is extended across the wider surface (see also Figure 2). Its amino terminus forms a coiled-coil with the amino terminal non-WD-repeat region of the β subunit [as predicted by Lupas et al (15)], and the

remainder of the molecule extends across the bottom face contacting residues in blades 5, 6, and 7. The structure explains why the association of G_β and G_γ is so tight. The G_γ subunit makes virtually no contacts with itself but makes all of its contacts with the G_β subunit.

Although the structure of G_β is a repeating one, the repeats are not identical. Analysis of the repeating sequences of G_β subunits taken from organisms widely separated by evolutionary time suggests that each of the repeating units in G_β acquired a specialized function very early and that this specialization was then conserved over at least the last 1.2 billion years (7). The crystal structure now allows us to understand what the function of some of these specializations might be. Some blades, for example, are specialized to interact with G_γ. Others have specializations on the opposite surface necessary to interact specifically with G_α. Still other sequences that give each blade of the propeller its individual character may have been conserved to interact with receptors or effectors.

Specificity of β and γ Interactions

At present, there are 6 different G_βs and 12 different G_γs known (15a–c). If all of these could combine to form $\beta\gamma$ dimers, there would be 72 potential combinations. Five of the G_β subunits share 80% identity over their ~340 amino acid length. $G_{\beta5}$ has only 53% identity to other known G_β subunits and has 13 additional amino acid residues, but it is able to associate functionally with numerous G_γ subunits. $G_{\beta5}$ is least like other G_β subunits at its amino terminus, the domain involved in the coiled-coil interaction with the amino terminus of the G_γ subunit. Predicted molecular weights of all known G_β subunits vary between 35 and 39 kDa. If all G_β and G_γ pairs could form, the number of potential $G_{\beta\gamma}$ subunit pairs would exceed the known number of G_αs by a factor of three. While it appears that most pairs can indeed form, there are exceptions (16–19). For example, the $G_{\beta1}$ subunit can combine with both $G_{\gamma1}$ and $G_{\gamma2}$ (and all other known $G\gamma$s), while $G_{\beta2}$ can combine with $G_{\gamma2}$ but not $G_{\gamma1}$. The region of G_γ that defines the specificity of its interaction with $G_{\beta1}$ or $G_{\beta2}$ is located in a 14–amino acid segment close to the middle of the molecule (20). Further studies have shown that 5 amino acids within the 14–amino acid stretch of $G_{\gamma1}$ are particularly important, including the triplets Glu38-Glu39-Phe40 (21) and Cys36-Cys37-Glu38 (22). Figure 2 shows the partial footprint of G_γ on G_β. The residues in G_β (9) that contact the region of G_γ necessary to define specificity are highlighted. They are principally clustered on blade 5 and a small section of the N-terminal region. Thus, one of the specialized functions of blade 5 is to define the specificity of $G_{\beta\gamma}$ interactions.

Assembly of β and γ

Whereas G_α subunits can be synthesized in almost any expression system including the bacterial, the $G_{\beta\gamma}$ subunit is much more finicky. For example, native

$G_{\beta\gamma}$ subunits can be synthesized in vitro in a rabbit reticulocyte lysate (23). The two subunits do not need to be cotranslated in order to assemble. However, either by cotranslation or by subsequent assembly, the formation of $G_{\beta\gamma}$ is not complete. Only about 30–50% of the G_{β} synthesized seems to be competent to form $G_{\beta\gamma}$. Furthermore, $G_{\beta\gamma}$ needs to be made in a rabbit reticulocyte lysate and cannot assemble when synthesized from a wheat germ extract. The wheat germ extract is as efficient as the rabbit reticulocyte lysate in synthesizing G_{β}, but the G_{β} synthesized in the wheat germ system is not competent to dimerize with G_{γ}. In contrast, G_{γ} can be made either in the wheat germ system, the rabbit reticulocyte lysate, or in bacteria and will effectively assemble with G_{β} made in the reticulocyte lysate. This specificity suggests that chaperones may be important for allowing G_{β} to fold into a native structure (24). Inanobe et al (25) reported that $G_{\beta\gamma}$ can associate with hsp90 (90-kDa heat shock protein), although the functional consequence of the association is not known. Unpublished studies from the laboratory of EJN show that antibodies to hsp90 will immunoprecipitate the aggregated nondimerized forms of G_{β} but not native $G_{\beta\gamma}$, a finding consistent with the idea that chaperones may be necessary for proper assembly. Although native $G_{\beta\gamma}$ cannot be made in bacteria, insect cells have been successfully used to produce large amounts of native $G_{\beta\gamma}$ (16, 26).

Given the highly integrated structure of $G_{\beta\gamma}$ it is not surprising that truncations at almost any point prevent correct assembly. For example, truncation of 19 amino acids from the amino terminus that removes only the α helix and does not include the WD-repeat portion of the molecule prevents $G_{\beta\gamma}$ assembly (27). In contrast, not all of G_{γ} is essential. Removal of 15 amino acids from the amino terminus markedly diminishes but does not entirely block formation of $G_{\beta\gamma}$ dimers, while removal of 10 amino acids from the carboxyl terminus has no effect on assembly (24).

COVALENT MODIFICATION OF $G_{\beta\gamma}$

Lipid Modification

The carboxyl terminus of the γ subunit contains a CAAX motif that directs prenylation of the molecule. γ subunits differ in their prenylation; $G_{\gamma 1}$ is farnesylated, while $G_{\gamma 2}$ is geranylgeranylated. Farnesyl or geranylgeranyl moieties are attached to γ via a stable thioesther bond to the cysteine located in the C-terminal CAAX box. Prenylation is followed by proteolytic removal of the C-terminal three amino acids and subsequent carboxylmethylation at the new C terminus [see Casey (28) for review]. The function of the carboxy methyl group is unknown (29–31) and may contribute to signaling (32,) although recent studies have not found a role for carboxymethylation in the ability of $G_{\beta\gamma}$ to interact with G_{α} or to activate PLC or PIP3 kinase (33). Farnesylation or geranylgeranylation of the appropriate γ subunit is not required for $G_{\beta\gamma}$

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