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Diversity of G Proteins in Signal Transduction

MELVIN I. SIMON, MICHAEL P. STRATHMANN, NARASIMHAN GAUTAM

The heterotrimeric guanine nucleotide-binding proteins (G proteins) act as switches that regulate information processing circuits connecting cell surface receptors to a variety of effectors. The G proteins are present in all eukaryotic cells, and they control metabolic, humoral, neural, and developmental functions. More than a hundred different kinds of receptors and many different effectors have been described. The G proteins that coordinate receptor-effector activity are derived from a large gene family. At present, the family is known to contain at least sixteen different genes that encode the alpha subunit of the heterotrimer, four that encode beta subunits, and multiple genes encoding gamma subunits. Specific transient interactions between these components generate the pathways that modulate cellular responses to complex chemical signals.

ALL BIOLOGICAL SYSTEMS HAVE THE ABILITY TO PROCESS and respond to enormous amounts of information. Much of this information is provided to individual cells in the form of changes in concentration of hormones, growth factors, neuromodulators, or other molecules. These ligands interact with transmembrane receptors, and this binding event is transduced into an intracellular signal. Several families of cell surface receptors have been characterized that are coupled to different mechanisms of signal transduction. Here, we examine the signal processing mechanisms of receptors that are coupled to G proteins (*1*).

Signal transducing G proteins occur in two forms, the "small G proteins" that are generally found as single polypeptides composed of about 200 amino acids and the heterotrimeric G proteins that are made up of α, β, and γ subunits. The small G proteins function in regulating cell growth, protein secretion, and intracellular vesicle interaction (*3*). The heterotrimeric G proteins are associated with signal transduction from cell surface receptors (*4*) and are thought to act as switches that can exist in either of two states depending on bound nucleotide (Fig. 1). A large family of transmembrane receptor proteins that share a characteristic topological structure has been revealed by molecular cloning and biochemical or pharmacological studies. All of these proteins have seven membrane spanning domains and show considerable amino acid sequence similarity (*2*). Signal transduction by these receptors is initiated by ligand binding which stabilizes an alternate conformational form of the receptor and thus transmits information across the cell membrane. This leads to a complex series of events that we understand only in broad outline. The ligand-bound receptor initiates two processes; one leads to desensitization, and occurs through receptor modification (*5*), and the other is a signal generating process that begins with the activation of the heterotrimeric G protein (Fig. 1A). Interaction of the G protein with the activated receptor promotes the exchange of guanosine diphosphate (GDP), bound to the α subunit, for gua-

M. I. Simon and M. P. Strathmann are in the Division of Biology, California Institute of Technology, Pasadena, California 91125. N. Gautam is in the Department of Anesthesiology, Washington University School of Medicine, St. Louis, Missouri 63110.

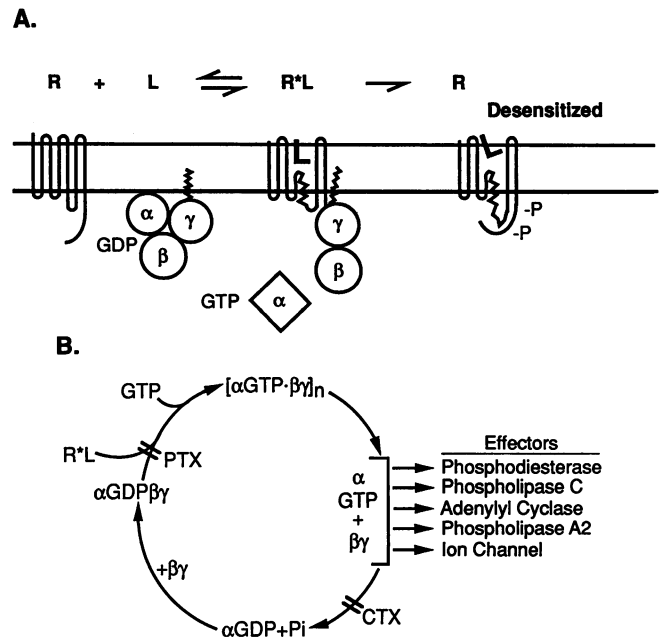

Fig. 1. Receptor G protein–mediated signal transduction. (**A**) Receptor (R) associates with a specific ligand (L), stabilizing an activated form of the receptor (R*), which can catalyze the exchange of GTP for GDP bound to the α subunit of a specific G protein. The βγ heterodimer may remain associated with the membrane through a 20-carbon isoprenyl modification (⧫) (*60*) of the γ subunit. The receptor is desensitized by specific phosphorylation (−P). (**B**) The G protein cycle. Pertussis toxin (PTX) blocks the catalysis of GTP exchange by the receptor. Activated α subunits (αGTP) and βγ heterodimers can interact with different effectors (E). Cholera toxin (CTX) blocks the GTPase activity of some α subunits fixing them in an activated form.

nosine triphosphate (GTP) and the subsequent dissociation of the α-GTP complex from the βγ heterodimer (Fig. 1B). A single receptor can activate multiple G protein molecules, thus amplifying the ligand binding event. The α subunit with GTP bound and the free βγ subunit may interact with effector proteins that further amplify the signal. Such effectors include ion channels and enzymes that generate regulatory molecules or second messengers. Low molecular weight second messengers such as cyclic adenosine-monophosphate (cAMP) or inositol triphosphate, in turn, generate dramatic intracellular changes including selective protein phosphorylation, gene transcription, cytoskeleton reorganization, secretion, and membrane depolarization. Termination of the signal occurs when GTP bound by the α subunit of the G protein is hydrolysed to GDP. The α subunit then reassociates with the βγ complex.

More than a hundred different G protein–coupled receptors have been found in mammals, including distinct receptors that bind the same ligand. At least five different muscarinic receptors, more than eight different adrenergic receptors, five different serotonin receptors, and four different opsins (*2*), have been identified. A growing family of receptors and receptor subtypes that respond to purines, bombesin, bradykinin, thrombin, histamine, dopamine, ecosinoids, vasopressin, growth hormone releasing hormone, and somatostatin are being cloned and characterized. Distinct forms, or subtypes, of receptors that respond to the same ligand can be differentiated by the intracellular responses that they elicit. These specific receptor subtypes are coupled to different second messenger pathways and to the regulation of different ion channels. Because a single receptor subtype can be coupled to multiple effectors and multiple receptor subtypes can activate a single effector (*2, 4, 6*), the G protein–coupled interactions form complicated networks. Furthermore, characterization of effectors has revealed that they too are specified by extensive gene families. Cloning studies have identified different types of adenylyl cyclases (*7*), five different phospholipase C isotypes (*8*), and multiple types of phospholipase A2 (*9*). There is also evidence for multiple calcium, potassium, and possibly sodium channels that are responsive to G proteins (*10*). This array of receptors and effectors raises questions about the nature of the information processing circuits that are formed. It remains unclear how many different G proteins are required to couple the receptor and effector subtypes, how specific receptors are linked through G proteins to form autonomous circuits, how circuits interact with each other, and how they are reshaped during cellular differentiation. Nor do we know what controls the specificity, the level of amplification, the timing, and the cross talk between signals in these circuits. To understand how the G proteins function we must identify the components of the G protein–mediated networks and the nature of their specific interactions.

Diversity of α Subunits of G Proteins

Cloning and sequencing techniques have been most productive in identifying and classifying new α subunits. Multiple G proteins have been identified in all of the eukaryotic organisms that have been examined. When the deduced amino acid sequences of all of the α subunits that have been cloned (more than 30 different cDNAs) are aligned, approximately 20% of the amino acids are found to be invariantly conserved (*11*). Amino acid sequence similarity provides a measure of the relatedness of different α subunits. A classification of the α subtypes of α subunits found in mammals based on amino acid sequence similarity is shown in Fig. 2. The family is made up of four classes (denoted G_x with x designating the specific class), and each class is composed of specific isotypes (denoted $G\alpha_x$, with x designating the specific isotype). Thus, the G_s class includes both the $G\alpha_s$ and $G\alpha_{olf}$ isotype (*12*). $G\alpha_{olf}$ shows 88% amino acid sequence identity with $G\alpha_s$. Both proteins are able to activate adenylyl cyclase to increase intracellular cAMP levels. Expression of $G\alpha_{olf}$ is restricted to specific neural tissues and is enriched in neurons in the olfactory epithelium. Individual isotypes are highly conserved between species. For example, there are no amino acid differences between $G\alpha_s$'s isolated from humans and from mice. It is therefore easy to identify the α subunit isotypes in a variety of mammals even though some of them such as $G\alpha_{i1}$ and $G\alpha_{i3}$ (*13*) differ in only 6% of their amino acid sequences. In addition to amino acid similarity there is conservation at the level of gene structure. The genes encoding $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_o$, and α subunits of the transducin rod and cone photoreceptors all conserve the positions of their introns and exons (*14*).

The functional role of specific α subunits is not obvious from their structural classification. One of the most effective tools for implicating G proteins in specific functions in intact cells has been the use of pertussis toxin (PTX) (*15*). The toxin uncouples the receptor from its G protein and thus blocks signal transduction by receptors that cause decreases in cAMP that regulate ion channels, and that activate phospholipases. Members of the Gi class of α subunits contain sites susceptible to modification by PTX and are therefore expected to mediate activation of the PTX-sensitive processes. Indeed, the $G\alpha_i$ and $G\alpha_o$ subtypes, have been shown to function in regulating ion channels. Activated $G\alpha_i$ subunits lower intracellular cAMP levels and $G\alpha_o$ has been implicated in increasing phosphoinositide release (*16*). However, the mechanism by which $G\alpha_i$ lowers cAMP is not clear (see below). Only in the case of $G\alpha_s$ and $G\alpha_{t1}$ (rod transducin) have preparations of the proteins been reconstituted with their purified effectors and receptors. Transducin couples rhodopsin to the activation of retinal phosphodiesterase (*17*) and the ubiquitous

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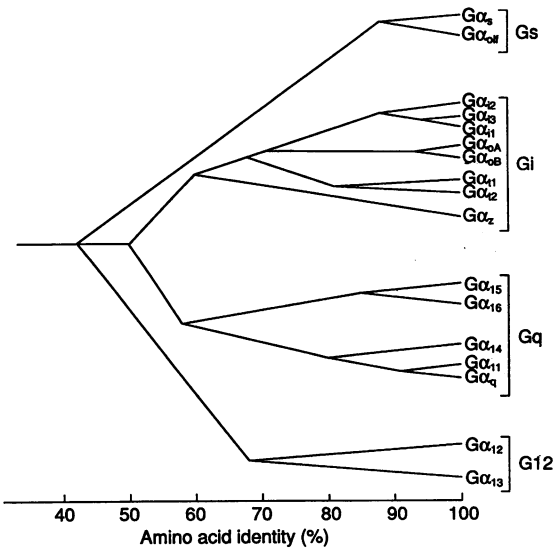

Fig. 2. Relationships among mammalian Gα subunits. The α subunits are grouped by amino acid sequence identity. Branch junctions approximate the values calculated for each pair of sequences. This figure extends the relationships shown by Kaziro *et al.* (*14*) and defines four distinct classes of Gα subunits. The splice variants of $G\alpha_s$ are not shown. Abbreviations for the amino acid residues are: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

$G\alpha_s$ and its splice variants activate purified reconstituted adenylyl cyclase (*18*).

GTP dependent signaling pathways that activate phospholipases but are resistant to PTX have also been described. Cloning experiments have revealed new G proteins that function in these pathways (*19*). Eight cDNA clones belonging to three different classes of Gα subunits have been characterized. They include two novel classes, G12 (*19*) and Gq (*20*) (Fig. 2); all of these lack the cysteine residue four amino acids from the COOH-terminal end that is the target for PTX-mediated adenosine diphosphate (ADP) ribosylation (Fig. 3). The $G\alpha_z$ (*21*) and $G\alpha_q$ (*22*) proteins have been isolated and shown to be refractory to PTX modification.

The $G\alpha_z$ subunit bears some resemblance to the G_i class (*23*), however, it differs in biochemical properties. The $G\alpha_z$ heterotrimer has been purified from bovine brain, and the α subunit has been expressed in *Escherichia coli* (*21*). The purified α subunit shows a very slow rate of guanine nucleotide exchange and an unusual Mg^{2+} ion dependence when compared to the $G\alpha_s$ and $G\alpha_i$ proteins. Furthermore, its intrinsic guanosine triphosphatase (GTPase) activity is extremely slow; approximately 100 times slower for $G\alpha_z$ than that determined for the other G protein α subunits. Even though the function of $G\alpha_z$ remains obscure, its kinetic properties and its distribution are of interest; it is found primarily in neurons, particularly cells with long axonal processes (*24*).

Both $G\alpha_q$ and $G\alpha_{11}$ are widely distributed and they lack a site for PTX modification (*20*). The amino acid sequences of the $G\alpha_{11}$ and $G\alpha_q$ isotypes differ from each other by less than 12% and almost all of these changes are confined to the NH_2-terminal region of the molecule. This region may be important in determining the specificity of interaction with the βγ subunit and the relative rate of nucleotide exchange and hydrolysis (see below). Thus, while $G\alpha_q$ and $G\alpha_{11}$ are often found in the same cell they may be responsible for generating signals with different time constants and they could interact with different subsets of receptors and effectors. Recent results point to the involvement of $G\alpha_q$ and $G\alpha_{11}$ in PTX-resistant coupling to phospholipase C activation. A novel 42-kD protein that activates phospholipase C in a PTX-resistant fashion has been partially purified (*22, 25*). The 42-kD G protein has amino acid sequence identity with the $G\alpha_q$ clone (*22*). In reconstitution experiments the 42-kD protein specifically activates the β isotype of phospholipase C (*26, 27*) and not the γ or δ form (*26*). Antisera to peptides with the specific sequences found at the COOH-terminus and in other regions of $G\alpha_q$ react with the 42-kD protein. Finally, in *Drosophila*, a cDNA was cloned encoding protein with a predicted amino acid sequence having 76% identity to mammalian $G\alpha_q$ (*20*). This gene was expressed in the eye (*28*) and may represent the G protein that couples *Drosophila* rhodopsin to the activation of the phospholipase C that is involved in the phototransduction cascade. Taken together, these experiments suggest that $G\alpha_q$ and $G\alpha_{11}$ are involved in coupling one type of phospholipase C to a specific set of receptor subtypes.

There are three other isotypes in the G_q class. They all show restricted patterns of tissue specific expression. $G\alpha_{14}$ is found primarily in stromal and epithelial cells (*29*), while $G\alpha_{15}$ and $G\alpha_{16}$ (*30*) are found in cells derived from the hematopoietic lineage. $G\alpha_{15}$ is found in murine B lymphocytes and $G\alpha_{16}$ in human T lymphocytes, and both are found in myeloid cells. Since there are multiple phospholipase C isotypes (*8*), it is possible that the other members of the G_q class interact with different members of the phospholipase family.

$G\alpha_{12}$ and $G\alpha_{13}$ (*31, 32*) represent yet another class of potential PTX-resistant α subunits (Figs. 2 and 3). Both $G\alpha_{12}$ and $G\alpha_{13}$ mRNA's are expressed ubiquitously. Again, we know little about

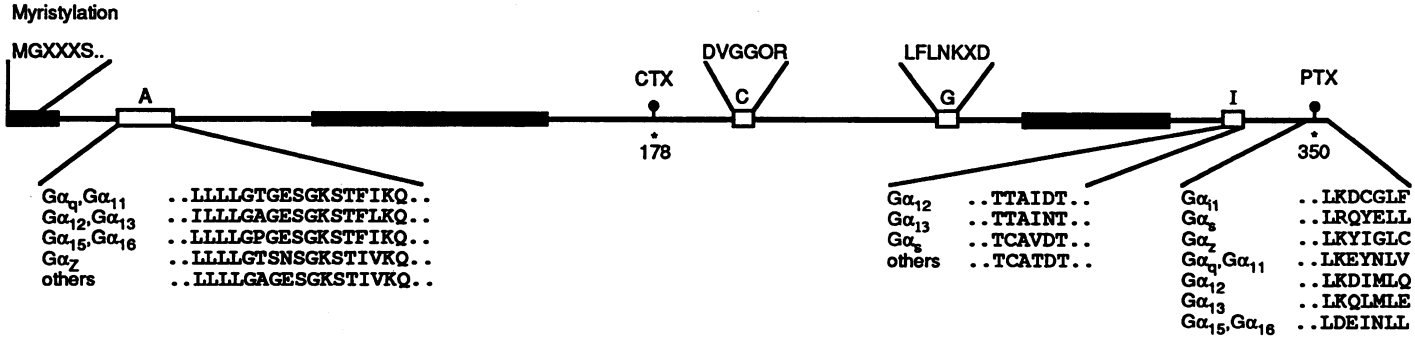

Fig. 3. The distribution of sequence specificity among the α subunits. The empty boxes represent the highly conserved domains found in all G proteins. These sequences are thought to be directly involved in interaction with the guanine nucleotide (see bold letters, A, C, G, and I). The single letter amino acid code is used to show the distinctive sequences for some of the Gα subunits. The full boxes indicate the regions that show the highest levels of amino acid sequence diversity. They are at the NH_2-terminus, between residues corresponding to amino acid 90 to 160 and amino acid 280 to 320 in the $G\alpha_{i1}$ sequence. The site for cholera toxin modification (Arg^{178}), pertussis toxin modification (Cys^{350}), and myristoylation (Gly^2, in certain α subunits) are shown.

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the function of these Gα proteins. There is some evidence indicating that a member of the $G\alpha_{12}$ family has been found in *Drosophila melanogaster*. A cDNA clone corresponding to the gene *concertina* (*cta*) in *Drosophila* has marked amino acid sequence homology with $G\alpha_{12}$ (*32*). It has been suggested that the *concertina* gene might play a role in regulating embryonic development in *Drosophila*.

The diversity of α subunit structure is further extended by differential splicing of complex genes. At least two variants can be generated by differential splicing of the G_o gene and most preparations of G_o contain both polypeptides (*33*). These variants differ in amino acid sequence at the COOH-terminal half of the protein and may therefore have different receptor and effector specificities (see below). It has been suggested that the G_o variants mediate PTX-sensitive activation of phospholipase C (*34*).

The use of the polymerase chain reaction has revealed new Gα subunits (*19*). However, it is not clear whether all members of the family have been identified. It is possible that some α subunits are expressed only in small subsets of cells, or that the sequences of novel subunits could diverge in conserved regions such that the probing techniques would not detect them. Splice variants of α subunit cDNAs may have been overlooked during the search. However, there has not been a great deal of pressure to search for new G proteins, since in terms of assigning functions to known G proteins, we already face an embarrassment of riches.

Structure Function Relations Among the Gα Subunits

The crystal structure is not yet known for any α subunit of the heterotrimeric G proteins. An approximation of the three-dimensional structure of the Gα subunit has been developed on the basis of the crystal structure of the small, G protein Ras and of elongation factor TU, another GTP binding protein (*35*). The disposition of functional domains in a "normalized" Gα subunit based on site-specific mutagenesis and studies with chimeric genes is shown in Fig. 3. This work has been reviewed recently (*36*) and we briefly summarize some of the results. Mutations in the Gα subunit that are analogous to those that have been studied in Ras exemplify both the differences and the similarities between the two systems. Ras ordinarily hydrolyzes GTP very slowly (approximately 100 times slower than $G\alpha_s$). The rate of hydrolysis is accelerated by interaction with another protein called the GTPase activating protein (GAP). The substitution of valine for glycine in the A box (Fig. 3) in ras lowers its GTPase activity and also results in its inability to be activated by the GAP protein. The homologous change introduced in $G\alpha_s$ results in a two- to fourfold change in GTPase activity (*37*). However, changes of the amino acid at the arginine residue that is modified by ADP ribosylation with cholera toxin (*38*), or in other nearby residues (for example, Glu^{227}) leads to large (30 to 100 times) decreases in intrinsic GTPase activity. It has been suggested that this portion of the molecule (from approximately residue 100 to 230) (Fig. 3) has a critical role in regulating the GTPase activity. Similar mutations have been found in $G\alpha_{i2}$ and $G\alpha_s$ when tumors were screened for amino acid changes in Gα proteins (*39*). These results suggest that mutations that lock the G protein in the GTP-bound form are dominant and in some tissues may lead to changes in growth control and oncogenesis.

The NH_2-terminal region of the Gα subunit is thought to be involved in interaction with the βγ subunit. This notion is supported by the finding that proteolysis of the NH_2-terminus prevents the Gα subunit from binding to the βγ subunit (*40*). The NH_2-terminal region is also the site of myristoylation on some of the G proteins ($G\alpha_o$, $G\alpha_{i1}$, $G\alpha_{i2}$, and $G\alpha_{i3}$). Myristoylation increases the affinity of the α subunit for the βγ subunit and facilitates heterotrimer formation (*41*). It has been suggested that the COOH-terminal region of the G protein is involved in receptor interactions. This suggestion is supported by the observation that modification of the Gα subunit by pertussis toxin blocks its interaction with receptor. Antibodies or peptides that specifically interact with COOH-terminal regions of some of the Gα proteins also block interaction with receptors (*42*). G protein–effector interactions have been examined by constructing chimeric α subunits. The results suggest that sequences in the COOH-terminal half of the Gα subunit can determine effector specificity (*43*). However, this domain is not well defined. Reconstituted systems containing purified components may eventually allow mapping of the amino acid residues required for specific protein binding and thus pinpoint the nature of the interactions.

Experiments demonstrating a direct role of Gα subunits in the activation of ion channels have increased our understanding of G protein function. In addition to activating adenylyl cyclase, $G\alpha_s$ subunits have been shown to regulate calcium channels (*44*). $G\alpha_o$ regulates a variety of neuronal and atrial potassium channels as well as calcium channels in dorsal root ganglia (*45*). The $G\alpha_{i3}$ and $G\alpha_{i1}$ proteins appear to activate potassium channels (*10, 16*). The use of patch-clamp techniques, with highly purified or recombinant G proteins and the observation of rapid channel activation suggest that the effect of the activated α subunit is directly on the channel. Channels are also known to be regulated by the activities of some second messengers. Thus, in addition to a direct effect on ion channel function, channels can be regulated indirectly through G protein interaction with specific effectors that in turn activate second messengers and kinases that can modify channel activity.

Gα Subunit Evolution

There is no evidence for a cell surface receptor-coupled G protein system in bacteria. In fungi, homologs of the subunits of heterotrimeric G proteins do exist. Two genes encoding Gα subunits have been found in yeast. One of the heterotrimeric G proteins in yeast is coupled to the mating type receptor. The mechanism of action of G proteins is different in yeast (*Saccharomyces cerevisiae*) than in multicellular organisms; it appears that the βγ heterodimer rather than the α subunit interacts with effector (*46*). Nonetheless, the overall features of the system are conserved, since mammalian Gα subunits can restore partial function to yeast mutants. Mammalian $G\alpha_s$ and $G\alpha_i$ subunits are apparently able to interact well with the yeast βγ subunit and thus inhibit the mating type pathway, but the mammalian proteins respond poorly to the yeast mating type receptor because they lack the appropriate receptor specificity (*47*). However, if the gene for the mating type receptor is replaced by the gene for the mammalian β-adrenergic receptor, catecholamines will trigger the yeast mating response (*47*). This kind of hybrid system promises to be very useful in screening for effective receptor agonists and antagonists.

In *Dictyostelium discoideum*, the slime mold, an extensive family of genes encoding Gα subunits has been described; these participate in regulating cellular aggregation and development (*48*). The amino acid sequences of the Gα subunits maintain the conserved GTP binding and hydrolysis motifs (Fig. 3) but do not bear a direct relation to the G protein classes found in mammalian cells. However, several of the G proteins from simple multicellular organisms do show a clear relation to the classes found in mammals. G proteins similar to $G\alpha_s$ and $G\alpha_o$ have been identified in *Caenorhabditis elegans* (*49*). In *Drosophila* (*50*) sequences similar to Go, Gi, Gs, Gq, and G12 α subunit classes have all been found. These sequences gener-

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ally bear from 60 to 75% amino acid identity with the mammalian proteins. If the amino acid sequence identity among members of classes of Gα subunits reflect their interaction with similar subsets of effectors or receptors, we can expect insights into mammalian G protein function from studies on these simpler systems.

Diversity and Function of βγ Subunits

In mammals four distinct β subunit isotypes have been found (*51, 52*). They share more than 80% amino acid sequence identity. However, an individual isotype, β_1, cloned from different mammalian sources has identical amino acid sequence. The same is true for β_2, suggesting that each isotype has a conserved sequence and may also have conserved function. β_1, β_2, and β_3 are ubiquitously expressed while β_4 is abundant in brain and lung tissue but is found at low levels in other tissues (*52*). All of the β subunits are made up of eight segments of amino acid sequence. Each segment shares a repetitive 40–amino acid sequence motif that is characterized by certain amino acids, including a tryptophan-aspartic acid pair. This motif has been referred to as the WD-40 repeat (*51*). The repeat structure is common to β subunits found in all organisms. Recently, this same motif has been found in a large number of other proteins (*53*). The function of the WD-40 repeat is not known and its distribution in other genes has not as yet provided a useful clue. The first 30 to 40 amino acids on the NH_2-terminal end of the β subunit do not contain the repeated sequence. Cross-linking studies with transducin suggest that this is the region where β and γ subunits may interact (*54*).

Diversity among the γ subunits has been demonstrated by electrophoretic, immunological, and protein sequencing techniques (*55*). However, these data do not allow us to distinguish between heterogeneity due to primary structure or to post-translational modification. Four distinct isotypes have been isolated as cDNA clones (*56*). Peptide sequences obtained from purified γ subunit proteins suggest the existence of at least two more isotypes. One γ isotype, $G\gamma_1$ is expressed only in photoreceptors, while another $G\gamma_2$ is expressed at different levels in all tissues that were examined; $G\gamma_3$ is expressed primarily in brain and in testis. The proteins are most divergent at their NH_2-terminal sequence and they share considerable sequence homology at their COOH-terminus. The amino acid sequence near the COOH-terminus of the γ subunits (as deduced from the cDNA), resembles the Ras oncogene sequence with a characteristic cysteine four residues from the end of the protein. The γ subunits are all modified (*57*) by the removal of the three COOH-terminal amino acids adjacent to the cysteine, and by the carboxymethylation, and isoprenylation of the terminal cysteine residue. The modification of $G\gamma_1$ most closely resembles the modification of Ras, a 15 carbon farnesyl group is found on the carboxymethylated cysteine (*58*). Without modification, $G\gamma_1$ complexed to β is inactive (*59*). Thus, isoprenylation could participate in the regulation of signal transduction. The γ subunits extracted from brain were also found to be polyisoprenylated. However, they are modified by the addition of a 20-carbon all trans-geranylgeranyl moiety (*60*). This addition may be required to anchor the γ subunit in the membrane.

There are a few examples of direct effects of βγ subunits on purified components of mammalian signaling systems. Addition of βγ subunits to rod photoreceptor outer segments apparently activates phospholipase A2 (*61*). The activation of phospholipase A2 with subsequent release of arachadonic acid could account for some of the observations that addition of βγ subunits to membrane patches activates potassium channels. However, inhibitors of phospholipase A2 do not block acetylcholine activation of K^+ channels and recent reports suggest that βγ subunits can inhibit K^+ channel activation by interaction with α subunits (*62*). Part of the confusion in these and other experiments designed to look for an effect of βγ subunits may result from the complex nature of the βγ subunit mixtures that are added to membrane preparations and from the complexity of the membrane patch system.

The suppressive effect of added βγ subunits on the activity of GTP activated α subunits has been demonstrated in a number of contexts. In fact, this is the basis for one explanation of the mechanism of Gi-mediated inhibition of adenylyl cyclase (*63*). Indirect evidence has implicated activation of $G\alpha_i$ in lowering intracellular cAMP levels. However, the purified α subunit shows only mild activity in reconstituted systems. This led to the hypothesis that activation of $G\alpha_i$ frees βγ subunits to interact with endogenous $G\alpha_s$, thus inactivating $G\alpha_s$. The scheme has been criticized on kinetic and other grounds (*64, 16*), but it remains a consistent explanation of the data. Another hypothesis is that βγ subunits act directly. This notion is based on the observation that βγ subunits added to adenylyl cyclase inhibit its activity (*65*). Again, some of these effects might be indirect; they may result from the interaction of βγ with calmodulin, thereby inhibiting the Ca^{2+} calmodulin-mediated stimulation of adenylyl cyclase activity (*66*).

During the signaling process, βγ subunits act to coordinate cellular responses. They have several functions: (i) stabilizing the interaction of α subunits with receptors and perhaps inducing formation of appropriate receptor complexes exhibiting specific activation kinetics, (ii) modulating the effects of activated α subunits, and (iii) regulating, at least indirectly, channel and phospholipase activity. The extensive modification of the γ subunits, the diversity of β and γ subunits, as well as the cross talk between βγ subunits that are associated with different α subunits, all point to an important function for βγ subunits in establishing specific receptor G protein associations and in integrating the effects and timing of the various G protein–mediated circuits.

G Protein Networks

In exploratory experiments with cloned cell lines appropriate probes have been used to detect $G\alpha_s$, $G\alpha_{i1}$, $G\alpha_{i3}$, $G\alpha_o$, $G\alpha_z$, $G\alpha_q$, and $G\alpha_{11}$ mRNA in the same cell. There are also detectable amounts of four β and at least three γ subunit gene products expressed in some cloned cell lines. If all of these subunits associated combinatorially and at random there would be almost one hundred different kinds of heterotrimers. Different combinations could have different affinities for individual receptors. These combinatorial relations could regulate the association between G protein and receptor and the kinetics of the activation response, because each receptor, when transiently activated, would interact with the subset of combinations of G protein subunits for which it had the highest affinity. Alternatively, there may be a mechanism that assembles the heterotrimer in a specific manner and transports specific assemblies to intracellular compartments that are enriched for the presence of appropriate receptors or effectors. There is evidence for compartmentalization (*67*) and for the preferential association of subsets of βγ heterodimers with specific α subunits. Reconstitution experiments have shown that $G\alpha_s$, $G\alpha_i$, and transducin have different affinities for different βγ heterodimers (*68*) and the differential elution of α subunits from βγ columns further attests to differences in their relative affinities (*69*). A βγ complex isolated from placenta has been shown to have distinctive properties (*70*) and different γ subunits were found to be associated in heterotrimers with the same α subunit when isolated from different tissues (*71*). A precise function of βγ subunits in determining receptor or effector specificity in vivo has not been described.

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Network specificity can be controlled by feedback processes. Activation of a particular G protein–coupled pathway can generate second messengers that regulate protein kinases. The kinases, in turn, can influence the information processing system. There are a number of examples where the addition of ligand leads to rapid phosphorylation of the Gα subunit. In *Dictyostelium* the $G\alpha_2$ protein is required for transduction of signals from cyclic AMP receptors. The addition of ligand to the cells results in rapid phosphorylation of $G\alpha_2$ (*72*). Phosphorylation is transient and it is not clear how this modification affects the activity of $G\alpha_2$. It could lead to inactivation and thus reflect a desensitization or adaptation process. In the yeast mating type system where mutants in the α subunit lead to constitutively active βγ subunits, a process that desensitizes and reverses the long-term effects of free βγ subunits results in adaptation (*73*). There is also evidence for the rapid phosphorylation of activated $G\alpha_z$ and of $G\alpha_{i2}$ (*74*). Again, it is not clear how these modifications affect function. There are a variety of other modifications including myrisoylation, isoprenylation, carboxymethylation and ADP ribosylation that could also be regulated to modulate the activity of different G proteins.

Some of the circuitry mediated by G proteins is presumably "hardwired" and can serve the function of signal distribution. For example, $G\alpha_s$ when activated, is capable of both opening Ca^{2+} channels and increasing the intracellular concentration of cAMP. Thus, these responses are presumably coordinated by the activation of a single $G\alpha_s$ protein. G protein similarities can generate cross talk between circuits, resulting in signal integration. If two G proteins such as $G\alpha_{i2}$ and $G\alpha_o$ are activated by different receptors and characteristically deliver signals to distinct effectors but are capable of interacting at low efficiency with other effectors, we would expect that the activation of either one of these pathways could elicit activation of the other. Thus, cross activation can be an essential part of the information transducing circuit. On the other hand, parts of the intracellular system may be built to shield against cross talk. For example, it has been found that in polarized renal epithelium cells $G\alpha_{i2}$ is localized to the basal lateral membrane of the cell while $G\alpha_{i3}$ is found in the Golgi and in apical membranes (*75*). By confining specific G protein and perhaps their effectors to local regions of the cell their ability to cross talk or interact could be effectively stymied.

There are other proteins that appear to augment the function of G proteins. Nucleoside diphosphate kinase (NDPK) has been reported to form complexes with the various GTP-binding proteins (*76*). The enzyme is a source of GTP and its association with the α subunits may reflect functional interactions. There is also indirect evidence suggesting that the small GTP-binding proteins such as Ras bound to GAP (77) might interact with the Gα subunits.

The properties of G protein networks are well suited to the needs of the complex processing that takes place in the nervous system. Many neuromodulators and neurotransmitters operate through G protein–coupled pathways, and G proteins can effect specific ion channel function. It is therefore not surprising that G proteins are thought to function in generating long-term potentiation (LTP) in the mammalian hippocampus (*78*). In aplysia, associative learning is known to be mediated by multiple G protein pathways (*79*). We are beginning to get indications of a most exciting aspect of the complexity of G protein networks—their role in coordination and integration of information during neural function.

Function of G Proteins in Differentiation

As cells differentiate, their signaling characteristics change, and new pathways are expressed and integrated into the function of the cell. Perhaps the clearest example of expression of new G proteins as a function of differentiation is found in *Dictyostelium*, where the transitions from amoeba to aggregate and from slug to stalk and spore are all accompanied by the appearance of new Gα subunits (*80*). Presumably, the new transducers can establish and generate circuits that produce a pattern of second messengers that help stabilize the differentiated state of these cells. Studies of the effects of one G protein–coupled pathway on another suggest that there are feedback mechanisms that regulate gene expression of the components of the pathway (*81*).

There are a variety of regulatory peptide factors and hormones that can influence cell growth. Many of these agents, such as thrombin, bombesin, serotonin, and angiotensin, bind to specific receptors that are coupled to G proteins. In some cases, PTX has been reported to block the proliferative response. The toxin has also been used to show that growth factor receptors may be coupled to G proteins (*82*). Marked effects on the signaling mechanisms of insulin-like growth factor (IGF), have been described and peptides that contain a sequence derived from the IGF reeptor (*83*) have been shown to carry the specificity to activate certain G proteins. This implies that apart from the seven-pass membrane receptors a whole new class of receptors with single transmembrane segments could be coupled to G proteins.

G proteins may also play a role in modulating cell movement, cytoskeletal structure, and chemotaxis. G proteins have been reported to interact with tubulin (*84*), collagen (*85*), and actin (*86*). Furthermore, PTX blocks some of these interactions. One hope is that genetic systems can be used to analyze complex functions of G protein pathways. In *Drosophila*, a maternal effects gene called *concertina* (*cta*) that is required for appropriate morphological development has been cloned and shown to be a Gα subunit (*32*). G proteins may influence the effects of growth factors to direct a cell along a particular developmental pathway. For example, ligands that activate G protein–coupled receptors that increase the concentration of cAMP in cells enhance the differentiation of PC12 cells initiated by nerve growth factor. Tyrosine kinase–coupled receptors and G protein–coupled receptors activate different phospholipase C isotypes. These two pathways could act synergistically. Thus, the activation of G protein–mediated pathways in the appropriate context might initiate, facilitate, or amplify changes required for cellular differentiation.

Cloning and sequencing have been used to establish the nature of the G proteins, and experiments with purified protein to reconstitute steps of signaling pathways in vitro have helped to define the mechanism of action of G protein–coupled systems. However, these approaches give us little insight into the integrative properties of the system or into its function in vivo. New microscopes and specific fluorescent markers may allow us to visualize the effects of different circuits simultaneously. Applications of homologous recombination and antisense oligonucleotides should provide ways to inactivate individual genes and allow assessments of their function. Extensive studies with dominant mutants in mammalian systems and in transgenic mice have proven to be useful and may be extended to define the function of individual components and provide the means to analyze the complex interactions within the signal transduction network.

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