Protein Science (1994), *3*:3–14. Cambridge University Press. Printed in the USA.

REVIEW

G proteins: Critical control points for transmembrane signals

EVA J. NEER

Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts 02115

(RECEIVED July 20, 1993; ACCEPTED September 13, 1993)

Abstract

Heterotrimeric GTP-binding proteins (G proteins) that are made up of α and $\beta\gamma$ subunits couple many kinds of cell-surface receptors to intracellular effector enzymes or ion channels. Every cell contains several types of receptors, G proteins, and effectors. The specificity with which G protein subunits interact with receptors and effectors defines the range of responses a cell is able to make to an external signal. Thus, the G proteins act as a critical control point that determines whether a signal spreads through several pathways or is focused to a single pathway. In this review, I will summarize some features of the structure and function of mammalian G protein subunits, discuss the role of both α and $\beta\gamma$ subunits in regulation of effectors, the role of the $\beta\gamma$ subunit in macromolecular assembly, and the mechanisms that might make some responses extremely specific and others rather diffuse.

Keywords: G proteins; receptors; transmembrane signals

The surfaces of cells are constantly barraged by hundreds of chemical and physical signals that activate membrane-bound receptors. These activated receptors initiate a flow of information that passes through a set of coupling proteins (called G proteins because they bind GTP) to intracellular effector enzymes or ion channels. Changes in effector activity cause changes in second messenger levels (such as cAMP or inositol phosphates) or in ionic composition that ultimately lead to a cellular response. Most of the receptors that transmit signals through G proteins have a characteristic topology with seven membrane-spanning helices (Dohlman et al., 1991). Every cell contains receptors for many kinds of chemical signals, as well as many different types of G proteins. These proteins are heterotrimers made up of α, β, and γ subunits, each of which has several closely related isoforms. The specificity with which the G protein subunits interact with receptors and effectors defines the range of responses a cell is able to make to an external signal. Thus, the G proteins act as a critical control point that determines whether a signal spreads through several pathways leading to pleiotypic responses, or whether it is focused to a single pathway and a single response. Over the last decade, there has been enormous progress in understanding how signals are transmitted across the plasma membrane. However, it is still not understood exactly what determines the specificity of a cell's response to a hormone or other agonists. In this review, I will summarize some features of the structure and function of mammalian G protein subunits and then discuss the mechanisms that could make some responses extremely specific and others rather diffuse.

Recent detailed reviews covering various aspects of receptor structure and G protein action include: Gilman (1987), Ross (1989), Bourne et al. (1990, 1991), Kaziro et al. (1991), Simon et al. (1991), Kobilka (1992), Savarese and Frazier (1992), Spiegel et al. (1992), and Clapham and Neer (1993).

Reprint requests to: Eva J. Neer, Cardiovascular Division, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115.

Mechanism of action of G proteins

G proteins cycle between a GTP-liganded active form and a GDP-liganded inactive form (Fig. 1). The α subunits bind GTP and hydrolyze it to GDP. All isoforms of α subunits are GTPases, although the intrinsic rate of GTP hydrolysis varies greatly from one type of α subunit to another (Carty et al., 1990; Linder et al., 1990). Figure 1 illustrates the cycle of activation and inactivation. When GDP is bound to the α subunits, they associate with $\beta\gamma$ subunits to form the *inactive* heterotrimer. The *inactive* state of a G protein is the GDP-liganded heterotrimer. In this form, the G protein is able to associate with an inactive receptor. Although GDP-liganded α subunits are able to bind to receptor without $\beta\gamma$, the association is greatly enhanced by its presence (Fung, 1983; Florio & Sternweis, 1985; Hekman et al., 1987). Both the α and the $\beta\gamma$ subunits appear to bind to

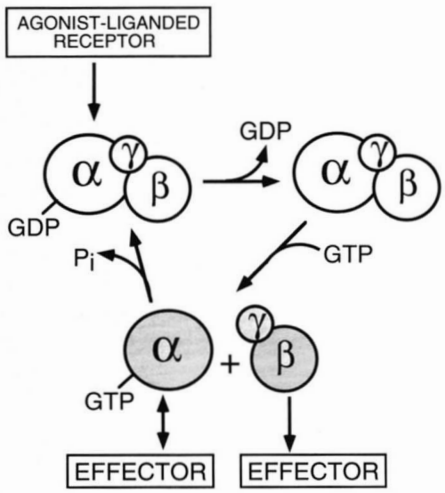

Fig. 1. The regulatory cycle of the heterotrimeric G proteins. See text for a description of the cycle. Open circles represent inactive states of the subunits; shaded circles represent active forms.

the receptor (Florio & Sternweis, 1985; Im et al., 1987; Kelleher & Johnson, 1988). Upon binding of agonist, the receptor becomes activated and undergoes a conformational change that is transmitted to the α subunit, causing the affinity of the α subunit for GDP to decrease. GDP comes off the active site, allowing GTP to bind. GTP binding is favored because in cells, the concentration of GTP is much higher than GDP, and because the affinity of the α subunit is greater for GTP than for GDP. Once GTP is bound, the α subunit assumes its activated conformation and dissociates both from the receptor and from $\beta\gamma$. The G proteins are functional dimers because β and γ do not dissociate unless they are denatured. When the α and $\beta\gamma$ subunits are dissociated from each other, each interacts with effectors (reviewed by Clapham & Neer, 1993). The patterns and mechanisms of effector regulation by the G protein subunits will be discussed below. The activated state lasts as long as GTP remains on the active site of the α subunit. Once GTP is cleaved to GDP, the subunits reassociate, become inactive, and return to the receptor. It is important to notice that the rate of GTP hydrolysis determines the timing of activation not only of the α subunit, but of $\beta\gamma$ as well. Reassociation turns off *both* subunits. Thus, although the $\beta\gamma$ subunit does not bind GTP, its activation depends on that of the α subunit.

In the last year, an important additional component has been added to our understanding of the regulation of G protein activation. Two effectors, phospholipase C (Berstein et al., 1992) and cGMP phosphodiesterase (Arshavzky & Bownds, 1992), have been shown to enhance the GTPase activity of the α subunit that regulates them. It had previously been thought that only the low molecular weight, monomeric GTP-binding proteins such as *ras* were regulated by GTPase-activating proteins (GAPs) (Trahey & McCormick, 1987; Gibbs et al., 1988). The finding that an effector can modulate the GTPase activity of the α subunit means that such an effector can influence the duration of its own activation. In principle, different effectors may be able to do so to different degrees.

Originally, it was thought that only the α subunit of G proteins regulated effectors, whereas the role of $\beta\gamma$ subunits was to inactivate α subunits, damp the signal, and prevent noise in the absence of hormonal stimulation (Birnbaumer, 1987; Birnbaumer et al., 1990; Gilman, 1987). There is ample evidence that activated α subunits do, indeed, directly activate effectors, but the paradigm changed with the discovery in 1987 that $\beta\gamma$ subunits could also regulate effectors (muscarinic K^+ channels in the heart) (Logothetis et al., 1987). It has now become apparent that many effectors are regulated both by α and by $\beta\gamma$ subunits in flexible and apparently complex ways. Table 1 lists the effectors that are regulated by α, $\beta\gamma$, or both subunits. When an effector is regulated by both subunits, the action of $\beta\gamma$ can be conditional upon activation by α, or regulation by each subunit can be independent of the other. One of the surprises in the last two years has been the extraordinary subtype specificity of the activation patterns. These different responses provide flexible systems to integrate or differentiate responses to external stimuli. Three examples will be discussed below.

Structure of α subunits

The α subunits are proteins with molecular weight from 39 to 52 kDa. There are over 20 G protein α subunits (16 gene products and several alternatively spliced isoforms; reviewed by Kaziro et al., 1991; Simon et al., 1991). There are four major classes of α subunits defined by amino acid sequence similarity. The α_s class was first recognized by its ability to activate adenylyl cyclase and includes the ubiquitous α_s and α_{olf} (an α subunit from olfactory neuroepithelium). The α_i class was named for the ability of some of its members to inhibit adenylyl cyclase and includes $\alpha_{i\text{-}1}$, $\alpha_{i\text{-}2}$, $\alpha_{i\text{-}3}$, α_o (a predominantly neural α subunit), α_t, α_{t2} (the retinal α subunits), and α_z. All

Table 1. *Effectors regulated by G protein subunits*

α Subunits	$\beta\gamma$ Subunits	References[a]
K^+ channel (I_{KAch})	K^+ channel	1
K^+ channel (I_{KATP})	–	2
Adenylyl cyclase I	Adenylyl cyclase I	3
Adenylyl cyclase II (IV)	Adenylyl cyclase II (IV)	
Adenylyl cyclase III	–	
Phospholipase Cβ1	Phospholipase Cβ1[b]	4
Phospholipase Cβ2	Phospholipase Cβ2	
Phospholipase Cβ3[b]	Phospholipase Cβ3	
–	Receptor kinases (β-adrenergic, muscarinic)	5
cGMP Phosphodiesterase	–	6
–	Phospholipase A_2	7
Calcium channel	Calcium channel (?)	8
–	Yeast pheromone response pathway	9

[a] References: **1.** Logothetis et al., 1987, 1988; Yatani et al., 1987, 1988; Kurachi et al., 1989a. **2.** Ito et al., 1992. **3.** Tang and Gilman, 1991, 1992. **4.** Blank et al., 1991, 1992; Taylor et al., 1991; Boyer et al., 1992; Camps et al., 1992; Conklin et al., 1992; Katz et al., 1992; Smrcka and Sternweis, 1993; Wu et al., 1993b. **5.** Haga and Haga, 1992; Pitcher et al., 1992. **6.** Stryer and Bourne, 1986. **7.** Jelsema and Axelrod, 1987. **8.** Kleuss et al., 1991, 1992, 1993. **9.** Whiteway et al., 1989.

[b] Forms of phospholipase Cβ that are weakly activated by the α or $\beta\gamma$ subunits.

the members of this class, except α_z, can be modified by pertussis toxin. The α_q class includes α_q, α_{11}, and α_{16}. Members of this class activate phospholipase C (see below). The fourth class includes α_{12} and α_{13}, whose function is not yet known. Some α subunits are very cell-specific (for example, α_t is found only in the retina and α_{olf} only in the olfactory neuroepithelium), but most are widely expressed, and individual cells contain many subtypes (Kim et al., 1988).

The α subunits of heterotrimeric G proteins make up one subfamily of a superfamily of guanine nucleotide-binding proteins that share considerable sequence similarity around their guanine nucleotide-binding sites. These proteins include elongation factors (EFTu), the oncogene *ras*, and the family of small GTP-binding proteins related to *ras*, such as *ral*, *rab*, *rac*, etc. (Bourne et al., 1990). The α subunit sequences that are similar to EFTu and *ras* are indicated in Figure 2. EFTu and *ras* have been crystallized (Jurnak, 1985; la Cour et al., 1985; Pai et al., 1989; Milburn et al., 1990). It seems reasonable, therefore, to predict the characteristics of the guanine nucleotide-binding site of the α subunit of heterotrimeric G proteins on the basis of the two known structures. For example, some mutations are known to inhibit the GTPase activity of *ras*. Mutations in the corresponding residues of the α subunit of heterotrimeric G proteins also inhibit GTPase activity. The characteristics of the guanine nucleotide-binding site of the heterotrimeric G proteins have been reviewed recently (Bourne et al., 1991). In this review, I will focus on the sites of protein–protein interaction that may be less obvious from comparisons with the available crystal structures. For another recent review on this subject, see Conklin and Bourne (1993).

Interaction of G protein α subunits with receptors

Receptors bind to the carboxyl-terminal region of α subunits. Covalent modification of some classes of α subunits by a bacterial toxin from *Bordetella pertussis* gave the first clue to a potential site of receptor interaction and subunit. Pertussis toxin modifies a cysteine four amino acids from the carboxyl-terminus and prevents the G protein from communicating with the receptor (West et al., 1985). Mutagenesis of the carboxyl-terminus can block interaction of the G protein with receptor without affecting its ability to bind GTPγS or βγ (Sullivan et al., 1987). Some antibodies directed against peptides in the carboxyl-terminus can uncouple receptors from G proteins (Simonds et al., 1989b; Gutowski et al., 1991). Finally, Hamm et al. (1988) showed that a peptide representing the last 11 residues of the retinal G protein, α_{t1}, uncouples the G protein from the receptor, photorhodopsin. In addition, the peptide, itself, is able to induce a change in the absorption spectrum of photorhodopsin, suggesting that it is able to cause a conformational change in photorhodopsin. In an extension of these studies, Dratz et al. (1993) analyzed the interaction of the C-terminal 11 amino acid peptide of α_t by nuclear Overhauser effect spectroscopy (NOESY). Their results suggest that a glycine within the peptide forms part of a β turn that appears to bind directly to rhodopsin.

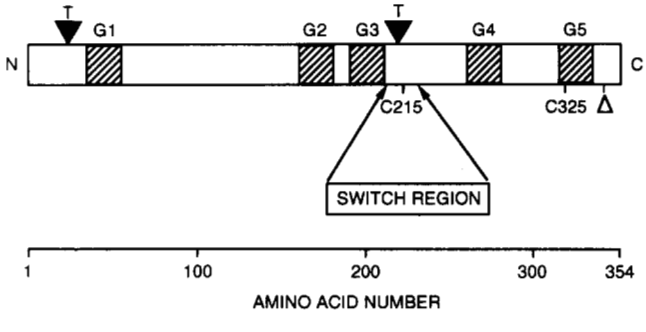

Fig. 2. Some functional regions of α_o. The five sequences that make up the guanine nucleotide-binding site are indicated by the shaded blocks marked G1–G5. The switch region is a region whose cognate in *ras* has the largest conformational difference between the GTP- and GDP-liganded forms (reviewed by Bourne et al., 1990). Solid triangles indicate the two sites accessible to cleavage by trypsin in the native molecule (Fung & Nash, 1983; Neer et al., 1988). The site in the switch region is only accessible in the GDP-liganded form of the α subunit. In the GTP- or GTPγS-liganded forms, trypsin only cleaves the site near the amino-terminus. C215 is the cysteine that can be cross-linked to βγ by bismaleimidehexane. Mutation of Cys 325 to alanine causes a 10-fold drop in affinity for GDP with no change in GTP affinity (Thomas et al., 1993a). The open triangle indicates the extent of the deletion in the carboxyl-terminus of α_o that led to a large decrease in GDP affinity (Denker et al., 1992b).

The essential function of the activated receptor is to induce a conformational change in α_o that decreases its affinity for GDP. It may do so by actually moving the carboxyl-terminus so as to relieve a constraint on GDP association. Deletion of 14 amino acids from the carboxyl-terminus of the G_o α subunit decreases its affinity for GDP without affecting the affinity for GTPγS. These results are consistent with the idea that the carboxyl-terminus may act as a lever whose position is changed or which is twisted by the receptor (Denker et al., 1992b). The extreme carboxyl-terminal of α subunits also has an important role in defining the specificity of G protein receptor interactions. Replacement of four amino acids at the carboxyl-terminus of α_q with the four amino acids normally found in $\alpha_{i\text{-}2}$ allowed α_q to couple to receptors different from those that normally activate it. Thus, the D_2-dopamine and A_1-adenosine receptors normally couple to $\alpha_{i\text{-}2}$ not to α_q and do not stimulate phospholipase C activity. However, they were able to activate a chimeric α_q molecule that had the four carboxyl-terminal amino acids of $\alpha_{i\text{-}2}$ and so stimulate phospholipase activity (Conklin et al., 1993). Several lines of evidence suggest that the amino- and carboxyl-termini of α subunits are on the same face of the molecule and, indeed, may be close to each other (Navon & Fung, 1988; Holbrook & Kim, 1989). However, it is not yet clear whether the N-terminus plays a role in binding to the receptor.

Another region important for receptor activation of α subunits is in the G5 region (see Fig. 2). The equivalent region in *ras* includes several amino acids whose side chains interact directly with the guanine ring of the nucleotide or stabilize interactions between the guanine ring and amino acid residues in the G4 region. Hamm et al. (1988) analyzed the effects of a peptide representing residues 311–328 of transducin α and found that this peptide also blocked activation of transducin by photorhodopsin and, like the C-terminal peptide, could itself induce changes in the spectrum of photorhodopsin. Furthermore, Hamm et al. (1988) found that the two peptides were able to act synergistically both in their effects on α subunit receptor interactions and on the induction of spectral changes in photorhodopsin. The importance of this region in regulating GDP affinity is further strengthened by the finding that mutation of a highly conserved cysteine (Cys 325) to alanine diminishes the affinity of α_o for GDP by approximately 10-fold without affecting

GTP affinity (Thomas et al., 1993a). It is likely that this region not only contains the site of interaction of α subunits with receptor but is a region of important conformational changes during G protein activation.

Interaction of G protein α subunits with effectors

Two different kinds of studies have defined the regions of α subunits that are likely to interact with effectors. In an extensive analysis of the regions of α_s responsible for activating adenylyl cyclase by systematic replacement of residues in α_s with alanine, Berlot and Bourne (1992) concluded that three regions in the carboxyl-terminal third of α_s determined the ability of the molecule to activate adenylyl cyclase. Substitution of α_s sequences from two of these regions into an $\alpha_{i\text{-}2}$ background was able to convert the $\alpha_{i\text{-}2}$, a molecule that normally inhibits adenylyl cyclase, into one that stimulates adenylyl cyclase. The importance of one of these regions was also highlighted by Artemyev et al. (1992) and Rarick et al. (1992) who found that a peptide corresponding to residues 293–314 of the retinal α subunit, α_t, was able to stimulate cGMP phosphodiesterase activity, suggesting that it was part of the effector surface of α_t. Both the receptor and effector binding regions of α subunits are found on the same plane of the molecule that has been termed the "membrane" face in contrast to the opposite side of the molecule that contains the GTP-binding pocket and is called the "cytosolic" face (Holbrook & Kim, 1989).

Interaction of α subunits with $\beta\gamma$

The association–dissociation of α and $\beta\gamma$ subunits is a hallmark of the mechanism of action of the heterotrimeric G proteins. Binding of the $\beta\gamma$ subunit to α increases the affinity of the α subunit for GDP 100-fold, stabilizing the heterotrimer in its associated, inactive form (Higashijima et al., 1987). What parts of the α subunit are important for association with $\beta\gamma$? One important region is the amino-terminus. Removal of 2 kDa from the amino-terminus, either by proteolysis or by mutation, blocks the formation of heterotrimers without affecting the GTPase activity of the remainder of the molecule (Fung & Nash, 1983; Neer et al., 1988; Graf et al., 1992).

The amino-terminus of some α subunits is myristoylated. This lipid modification enhances their ability to associate with $\beta\gamma$, although it is not absolutely essential (Buss et al., 1987; Linder et al., 1991; Denker et al., 1992a). The amino acids in the amino-terminal 2-kDa fragment are also important. Denker et al. (1992a) narrowed the important region by showing that amino acids 7–10 have an important role in α_o and $\beta\gamma$ interactions whether α_o is myristoylated or not. Deletion of these four amino acids (but not the four neighboring amino acids on the carboxyl-terminal side) diminishes the ability of α_o to interact with the $\beta\gamma$ subunit. However, other amino acids can be substituted at this position to restore the length, and the α_o subunit is again able to bind $\beta\gamma$. Analysis of chimeric molecules (Osawa et al., 1990) and chemical modifications (Dhanasekaran et al., 1988) have suggested that other regions of the α molecule may also be involved in $\beta\gamma$ interactions.

Although proteolysis, mutagenesis, and modification are useful for identifying regions that are potentially important for subunit interactions, they are not able to distinguish direct effects from overall changes in conformation. Analysis of chemical cross-linking can give more direct information about the orientation of protein surfaces. A homobifunctional cross-linking reagent, bismaleimidehexane, can cross-link five different α subunits to $\beta\gamma$, suggesting that a conserved cysteine is the site of cross-linking. Thomas et al. (1993a) showed that mutation of Cys 215 (a cysteine conserved in all five α subunits tested) to alanine had no effect on the ability of α_o subunits to form heterotrimers with $\beta\gamma$ subunits but completely blocked the cross-linking reaction. This cysteine is located on the membrane face of the molecule and is within the conformationally sensitive switch region (Fig. 2). For example, this region contains a tryptic cleavage site that is accessible in α-GDP but not in α-GTPγS. The equivalent region in *ras* shows the greatest guanine nucleotide-induced change in conformation (Pai et al., 1989; Milburn et al., 1990). It is in one of the most highly conserved regions of α subunits. Because β subunits are extremely similar to each other, it might be expected that the surface that binds β might be very similar among α subunits. Furthermore, because association of $\beta\gamma$ with α depends on the nucleotide bound to α, it is reasonable that $\beta\gamma$ should bind to a region that changes conformation. The putative $\beta\gamma$ binding surface potentially overlaps the effector binding site because the equivalent of Cys 215 in α_o is Cys 237 in α_s, a residue found in one of the three short clusters of amino acids that are essential for activation of adenylyl cyclase. The implication of partly overlapping sites is not clear. As will be discussed below, two subtypes of adenylyl cyclase (Type II and Type IV) can be activated synergistically by α and $\beta\gamma$ subunits (Tang & Gilman, 1991, 1992). These subtypes of adenylyl cyclase are not the ones that were present in the S49 lymphoma cells used to map the effector domains of α_s (Berlot & Bourne, 1992), but if all adenylyl cyclases bind to the same region on α_s, then it is unlikely that the α subunit can simultaneously bind effector and $\beta\gamma$. The inference is that α and $\beta\gamma$ will bind to separate sites on adenylyl cyclase and other effectors. In addition, the observation supports the idea that the subunits must dissociate to be active. Otherwise, the $\beta\gamma$ subunit might block the effector site on the α subunit.

Dissociation of α and $\beta\gamma$ in the membrane

In solution, binding of nonhydrolyzable GTP analogues (such as GTPγS) clearly causes α and $\beta\gamma$ subunits to dissociate (Huff & Neer, 1986; Gilman, 1987). GTP does not cause dissociation, although this is probably because it is rapidly cleaved to GDP. GTP also does not dissociate G_o that has been ADP-ribosylated by pertussis toxin, a modification that should block GTPase activity (Huff & Neer, 1986). An important question, therefore, is whether or not the α and $\beta\gamma$ subunits actually dissociate in the native membrane. This point is central if it is really true that α and $\beta\gamma$ subunits must dissociate to carry out their functions.

The best evidence for dissociation during the activation cycle is the observation that the subunits of the retinal G protein, G_t, dissociate from the membrane and each other upon activation by rhodopsin (Fung, 1983). The evidence for dissociation of other types of G proteins that remain membrane-bound throughout the activation cycle is still indirect but, on balance, suggests that they do dissociate. Exogenous subunits added to membranes can interact with endogenous G proteins, suggesting that exchange is possible (Gilman, 1987). A mutant α_s subunit (glycine 226 replaced by alanine) does not dissociate from $\beta\gamma$ (Miller et al., 1988). The isolated GTPγS-liganded mutant subunit is

able to activate adenylyl cyclase in vitro, but not when it is expressed in cells that also contain $\beta\gamma$. One explanation for the discrepancy between the observations in vitro and in vivo is that the mutant α subunit does not dissociate from $\beta\gamma$ in vivo but must do so to activate adenylyl cyclase (Lee et al., 1992a).

The subunits may dissociate and yet not be free to diffuse throughout the bilayer. Indeed, α subunits that have been cross-linked with bismaleimidehexane can bind GTPγS and assume an activated conformation (Yi et al., 1991). The subunits may pivot at the covalent cross-link and move apart, but they cannot dissociate completely. It is not known whether such a cross-linked G protein can activate effectors. Mattera et al. (1987) also identified an activated, pertussis toxin-resistant state of the α subunit that, on the basis of sedimentation properties, was apparently associated with $\beta\gamma$.

Interaction of α subunits with membranes

Myristoylation of some α subunits is essential for association with membranes. Mutation of the myristoylated glycine to alanine shifts the α subunit to the cytosol (Jones et al., 1990; Mumby et al., 1990b). This requirement does not seem linked to the ability to associate with $\beta\gamma$ because α subunits expressed in excess of endogenous $\beta\gamma$ associate with membranes (Bloch et al., 1989; Simonds et al., 1989a; Blumer & Thorner, 1990; Mumby et al., 1990b). Mumby et al. (1990b) calculated that membranes from COS cells expressing α_{12} contained about five times as much $\alpha_{i\text{-}2}$ as $\beta\gamma$. Thus, α associates with the membrane independently of its ability to form a heterotrimer.

It is not clear how myristoylated α subunits interact with the membrane. The hydrophobic myristate may intercalate into the lipid bilayer. Modification with a less hydrophobic fatty acid, 11 oxymyristate causes a significant fraction of α_o and α_i to be cytoplasmic (Mumby et al., 1990b). If some cells naturally incorporate a somewhat more hydrophilic fatty acid into α subunits than others, then the partitioning of the α subunit between membrane and cytosol may well vary. It remains to be seen how much heterogeneity there is in lipid modifications that occur in various cells (Kokame et al., 1992). At present, there is no evidence for a specific receptor for myristoylated α subunits. Some α subunits (α_s) are not myristoylated and these must be held in the membrane in other ways. The α_s subunit, for example, may be anchored through its carboxyl-terminus (Audigier et al., 1990), although there is conflicting evidence on this point (Juhnn et al., 1992). Myristoylation of α subunits affect two key functions: association with the membrane and with $\beta\gamma$. Alteration of either is likely to have major consequences for signal transduction.

In addition to myristoylation, several types of α subunits (α_o, $\alpha_{i1\text{-}3}$, α_z, α_q) are palmitoylated probably at a site within 30 amino acids of the amino-terminus (Linder et al., 1993). The function of the protein-bound palmitate is not yet known. However, palmitoylation is a reversible modification, and deacylation could cause release of G protein α subunits into the cytosol.

Structure of the $\beta\gamma$ subunit

Like the α subunit, the β subunit is a member of an extended family, but the functional consequences of family membership are much less clear with β than with α. The amino acid sequence of the β subunit suggests that it is made up of two different types of structures (Fig. 3). The 39-amino acid amino-terminal region is predicted to form an amphipathic α helix that might be involved in coiled-coil interactions (Lupas et al., 1992). Coiled-coil interactions have been proposed to hold β to γ, and a further triple coiled-coil was suggested to hold $\beta\gamma$ to α. It seems unlikely that an α helix at the amino-terminus of the α subunit is essential for heterotrimer formation because mutation of arginine residue 9 in the putative α helix to proline had no effect on association (Denker et al., 1992a). The remainder of the β protein is made up of seven repeating units of approximately 43 amino acids each (Fong et al., 1987). Similar repeating units are found in a large number of other proteins whose functions appear to have nothing to do with signal transduction (van der Voorn & Ploegh, 1992). It is not yet clear what functional features have conspired to conserve these repeating units in proteins that are found in all eukaryotic families.

The deduced amino acid sequences for the four known mammalian β subunits are between 83 and 90% identical to each other (Fong et al., 1987; Gao et al., 1987; Levine et al., 1990; von Weizsacker et al., 1992). The complete deduced amino acid sequences of seven mammalian γ subunits have been reported (Hurley et al., 1984; Yatsunami et al., 1985; Gautam et al., 1989; Cali et al., 1992). These molecules are much more different from each other than are the β subunits or α subunits. The retinal γ_1 subunit is only 38% identical to brain γ_2 and equally different from the other mammalian γs. The β subunit from squid is very similar to mammalian β_1, but the γ subunit from squid that was isolated in association with the β subunit is so dissimilar from mammalian γ that the relationship is only barely statistically significant compared to a random sequence (Lott et al., 1992). The existence of four different β subunits and at least seven γ subunits suggests that 28 different combinations are possible. In fact, not all the possible pairs can form. Analysis of the combinatorial possibilities by in vitro translation and by transient expression in COS cells showed that β_1 was able to interact with γ_1 and γ_2, but the very similar β_2 molecule was not able to form a dimer with γ_1, only with γ_2 (Schmidt et al., 1992; Pronin & Gautam, 1992). These results were confirmed by

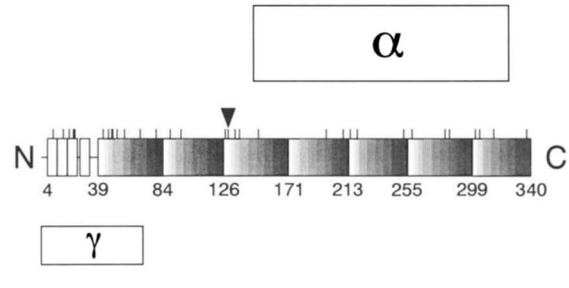

Fig. 3. Diagram of β subunit structure. The β subunit is represented as a linear sequence with its amino (N)- and carboxyl (C)-terminal ends indicated. The α and γ subunits have been placed relative to the β subunit in order to indicate the known regions of contact between these subunits (Bubis & Khorana, 1990; Yi et al., 1991; Thomas et al., 1993b). Two types of repetitive sequences have been identified within the primary sequence of β. These are putative α-helical heptad repeats (open bars) of the type observed in coiled-coil structures (Lupas et al., 1992), and WD-40 repeats (shaded bars) (Fong et al., 1987). The locations of 32 potential tryptic cleavage sites in β are indicated by vertical bars. The site at which the β subunit of native $\beta\gamma$ is cleaved by trypsin is indicated with an arrowhead. (Reprinted with permission from T.C. Thomas et al., 1993b, *Biochemistry 32*:8628–8635. Copyright 1993 American Chemical Society.)

synthesis and analysis of the proteins in insect Sf9 cells (e.g., Iniguez-Lluhi et al., 1992).

The different $\beta\gamma$ pairs differ in their ability to bind α subunits (Cerione et al., 1987; Casey et al., 1989; Fawzi et al., 1991) and to activate effectors. Retinal $\beta\gamma$ ($\beta_1\gamma_1$) is less effective than brain $\beta\gamma$ (predominantly $\beta_1\gamma_2$, $\beta_2\gamma_2$) in activating the cardiac atrial K^+ channel (Logothetis et al., 1988). The $\beta_1\gamma_2$ and $\beta_1\gamma_3$ dimers were 10–20 times more potent in stimulating Type II adenylyl cyclase than were $\beta_1\gamma_1$ dimers (Iniguez-Lluhi et al., 1992).

The γ subunits differ not only in their sequence but also in their lipid modification. Mammalian γ subunits undergo three modifications: first, prenylation on a cysteine four amino acids from the carboxyl-terminus, then cleavage of the last three amino acids followed by carboxyl methylation of the carboxyl-terminus (Backlund et al., 1990; Fung et al., 1990; Maltese & Robishaw, 1990; Mumby et al., 1990a; Yamane et al., 1990; Sanford et al., 1991). The 15-carbon farnesyl group on γ_1 (Lai et al., 1990) is less hydrophobic than the 20-carbon geranylgeranyl group found on γ_2 and presumably on other nonretinal γ subunits. Prenylation is not necessary for $\beta\gamma$ formation because mutation of the prenylated cysteine (Cys 68) to serine does not impair dimerization. However, $\beta\gamma$ dimers with unprenylated γ subunits cannot activate effectors and, in some cases, fail to form heterotrimers with α subunits (Iniguez-Lluhi et al., 1992; Katz et al., 1992; C.J. Schmidt & E.J. Neer, unpubl.). The importance of prenylation for $\alpha\beta\gamma$ association may depend critically on the α subunit. Wildman et al. (1993) found that transducin α subunits apparently do not discriminate between prenylated and nonprenylated forms of $\beta_1\gamma_2$. The $\beta\gamma$ subunit must be prenylated to associate with membranes. When normal β and γ subunits are transiently expressed in COS-M6 cells, the dimers are found in the membrane fraction. Mutation of Cys 68 of γ to serine prevents association of the $\beta\gamma$ dimer with the membrane (Simonds et al., 1991; Muntz et al., 1992).

The $\beta\gamma$ subunit forms a very stable structure whose function is not affected by cleavage of the molecule into two tryptic fragments. Despite the presence of 32 potential tryptic cleavage sites in the β_1 subunit, cleavage of purified native bovine brain $\beta\gamma$ with trypsin yields only two β-derived fragments. Trypsin-cleaved $\beta\gamma$ remains in a complex that has the same apparent sedimentation coefficient as intact $\beta\gamma$ and retains its ability to associate functionally with the α subunit (Fung & Nash, 1983; Thomas et al., 1993b). There are no disulfide bonds in the $\beta\gamma$ subunit, so that the stability of the cleaved molecule cannot be attributed to covalent linkages (Thomas et al., 1993b). The stability of the $\beta\gamma$ subunit may reflect strong noncovalent interactions among the repetitive structural units of β.

Although they apparently do not form separate functional domains, the two tryptic fragments are convenient for identifying portions of the molecule that interact with α and with γ. The 14-kDa amino-terminal fragment of β can be cross-linked to γ by bismaleimidehexane or copper phenanthroline, whereas the 26-kDa carboxyl-terminal portion can be cross-linked to α with bismaleimidehexane (Bubis & Khorana, 1990; Yi et al., 1991; Thomas et al., 1993b). The γ_2 subunit has only one cysteine at position 41 that is available for cross-linking (the other cysteine is prenylated). The γ_1 subunit does not contain this cysteine but has a pair of cysteines at positions 36 and 37. These cysteines were shown to cross-link to Cys 25 in β_1 (Bubis & Khorana, 1990). The precise site in β that is cross-linked to α is not yet known.

Regulation of effectors by G proteins

Table 1 lists the effectors that are regulated by G protein subunits. Three effectors that are regulated both by α and $\beta\gamma$ subunits are discussed below.

Independent activation of K^+ channels by α and $\beta\gamma$ subunits

Both the α and $\beta\gamma$ subunits can activate the muscarinic K^+ channel in cardiac atria (Logothetis et al., 1987, 1988; Yatani et al., 1987; Kurachi et al., 1989a). Another K^+ channel, the ATP-dependent K^+ channel, measured in the same excised membrane patches as the muscarinic K^+ channel, is activated only by α subunits, illustrating the effector subtype specificity of activation mechanisms (Ito et al., 1992). Several α subunits in the α_i family ($\alpha_{i\text{-}1}$, $\alpha_{i\text{-}2}$, $\alpha_{i\text{-}3}$, and α_o) can activate the muscarinic channel when applied to the cytoplasmic surface of an excised membrane patch, but the α_s and α_q types do not (Logothetis et al., 1988; Yatani et al., 1988). The channel can also be maximally activated by $\beta\gamma$ subunits from bovine brain (predominantly the $\beta_1\gamma_2$, $\beta_2\gamma_2$ type), whereas the $\beta\gamma$ subunits isolated from retina ($\beta_1\gamma_1$ type) are much less effective. Activation by α and $\beta\gamma$ subunits is not additive, so that the channel can be maximally activated by either one or the other (Logothetis et al., 1988). The precise mechanism of activation by either α or $\beta\gamma$ subunits is not known. The $\beta\gamma$ subunit probably has at least two pathways for activating the channel—one indirect, through activation of phospholipase A_2 and subsequent formation of arachidonic acid metabolites (Kim et al., 1989; Kurachi et al., 1989b), and the other possibly direct. The mechanisms for activating the channel cannot be precisely defined until channels have been isolated, cloned, and reconstituted in purified systems.

Synergistic regulation of adenylyl cyclase by α and $\beta\gamma$ subunits

Adenylyl cyclase is the best understood example of interactive regulation of an effector by α and $\beta\gamma$ subunits (Tang & Gilman, 1991, 1992). All subtypes of adenylyl cyclase can be activated by the α_s class of α subunits but not by other types of α subunits. However, the response to $\beta\gamma$ is very specific to subtypes. Thus, Type I adenylyl cyclase, the calmodulin-sensitive adenylate cyclase predominantly found in the nervous system, is inhibited by $\beta\gamma$. Type III adenylyl cyclase is neither inhibited nor further activated by $\beta\gamma$. In contrast, Types II and IV adenylyl cyclases are activated by α_s and are further synergistically activated five- to sixfold by $\beta\gamma$. The activation seems to be direct because both α and $\beta\gamma$ subunits are able to activate purified enzyme (Taussig et al., 1993). Regulation of Type II adenylyl cyclase by α and $\beta\gamma$ subunits can occur in cells, not only in reconstituted systems. Federman et al. (1992) created COS cells expressing a variety of transfected receptors, G protein subunits, and Type II adenylyl cyclase. Receptors acting through G proteins whose α subunit normally *inhibits* adenylyl cyclase were able to *stimulate* Type II adenylyl cyclase provided the $\beta\gamma$ subunit and some activated α_s were also present. Because the α subunits with which these receptors interact do not stimulate adenylyl cyclase, the activation by these receptors was presumably through the $\beta\gamma$ subunits of their G proteins.

What is the usefulness of such dual regulation? Bourne and Nicoll (1993) suggested that such systems could work as coinci-

dence detectors that allow a powerful response to paired signals, but only a weak or insignificant response to unpaired signals. The presence of a particular adenylyl cyclase might then determine the pattern of response to activation of cell-surface receptors. Indeed, some subtypes of adenylyl cyclase are expressed in very specific subsets of neurons (Glatt & Snyder, 1993). Such mechanisms might be important at synapses to allow neurons to coordinate their responses to incoming stimuli.

Regulation of phospholipase C by α and βγ subunits

Phospholipase C exists in several versions, some of which (such as PLCβ1–4) are regulated by G protein subunits, whereas others (such as PLCγ) are not (Blank et al., 1991, 1992; Taylor et al., 1991; Boyer et al., 1992; Camps et al., 1992; Conklin et al., 1992; Katz et al., 1992; Rhee & Choi, 1992; Wu et al., 1993a, 1993b). As with adenylyl cyclase, the pattern of regulation of PLCβ by α and βγ subunits is characteristic for each isoform. The G protein α subunits of the α_q class that includes α_q, α_{11}, and α_{16} activate PLCβ1 = PLCβ2 > PLCβ3. All of these α subunits lack the site for covalent modification by pertussis toxin so they are resistant to inhibition by the toxin. The βγ subunits stimulate PLCβ in a different rank order: PLCβ3 > PLCβ2 > PLCβ1 (Smrcka & Sternweis, 1993). In contrast to adenylyl cyclase, the activation of PLCβ isoforms by either subunit is independent and does not require priming of the enzyme by α or βγ (Smrcka & Sternweis, 1993). When both activated subunits are present simultaneously, the resultant activity is sometimes additive and sometimes not (Smrcka & Sternweis, 1993). The independent actions of the subunits on PLCβ activity reflects their binding to different regions of the enzyme: βγ binds in the amino-terminal two-thirds of the molecule, whereas α binds in the carboxyl-terminal region (Park et al., 1993; Wu et al., 1993a, 1993b; P. Gierschik & J. Exton, pers. comm.).

Muscarinic cholinergic receptors transiently expressed in COS cells can activate PLCβ2, provided that βγ is also transfected (Katz et al., 1992). Pertussis toxin blocks the activation of PLCβ2 through the muscarinic receptor. Stimulation of PLCβ isoforms by βγ may explain the sensitivity of PLC in some cells to inhibition by pertussis toxin. Pertussis toxin uncouples the G protein from the receptor, thus preventing exchange of GTP for GDP on the α subunit and blocking dissociation and activation of both α and βγ. It is a reasonable hypothesis that sensitivity to pertussis toxin indicates activation of PLC by βγ.

Role of the βγ subunit in macromolecular assembly

Another way that G protein subunits can regulate protein function is by promoting the assembly of active complexes. The βγ subunit plays such a role at two stages of receptor function: activation and down-regulation. G protein-coupled receptors exist in two forms, free receptor (low-affinity receptor) and receptor in a ternary complex with inactive αβγ heterotrimers (Kent et al., 1980). The latter has a high affinity for agonists and is poised to activate the G protein. Although α subunits can bind to receptors, the formation of the complete ternary complex is enhanced by βγ subunits (Fung, 1983; Florio & Sternweis, 1985; Phillips et al., 1992).

Once ligand activates the receptor, α and βγ are released and βγ takes on its second function with respect to receptors: to protect the cell from overstimulation by promoting feedback inactivation of the receptor. This function has been best studied for the β-adrenergic and muscarinic receptors. These receptors are regulated by phosphorylation at serine and threonine residues on their cytoplasmic carboxyl-terminal tails by similar or identical receptor-specific kinases. The phosphorylated receptor is less susceptible to subsequent activation. Thus, a feedback loop is created that controls the duration of receptor activation (Sibley et al., 1987). Recently, Haga and Haga (1992) and Pitcher et al. (1992) have found that the βγ subunit stimulates phosphorylation of the muscarinic and β-adrenergic receptors by their respective kinases. Phosphorylation of the β_2-adrenergic receptor by β-adrenergic receptor kinase (βARK) is increased approximately 10-fold by βγ when all the components were reconstituted in phospholipid vesicles. In contrast, the βγ subunit had no effect on the ability of βARK to phosphorylate synthetic peptide substrates. Pitcher et al. (1992) concluded that the activating effect of the subunits was not on the intrinsic catalytic activity of βARK but was caused by assembling βARK with its receptor substrate, thus effectively increasing the substrate concentration. The same laboratory had earlier shown that activation of the β-adrenergic receptor causes translocation of βARK from the cytosol to the membrane (Strasser et al., 1986). Thus, it appears that the βγ subunit plays two roles in receptor regulation, each of them involving macromolecular assembly. It facilitates the association of α subunits and βARK with receptors. It is tempting to consider that this assembly function is not unique to receptor regulation, but is a common property of the βγ subunit, and that this mechanism may be involved in some of the other instances of activation by βγ that are less clearly understood.

The specificity question

Although clearly a great deal is known about the structure, organization, and function of the transmembrane signaling system, a key question remains mysterious. Given the exuberant abundance of receptors, G proteins and effectors, all with similar structures, how is the specificity of cellular responses to hormone maintained? The nature of the question can be defined by considering two examples of cellular specificity, one natural and one experimentally created.

The heart is an example of a tissue that keeps opposing signals quite distinct: it responds to stimulation of β-adrenergic receptors with an increase in the rate and force of contraction and to stimulation of muscarinic cholinergic receptors with a decrease in the rate and the force of contraction. The β-adrenergic receptor is coupled to G_s, the G protein that causes stimulation of adenylyl cyclase, whereas the muscarinic receptor is coupled through the G_i class of G proteins to a variety of functions including activation of phospholipase C and of an inward-rectifying K^+ channel (Neer & Clapham, 1988). Cardiac cells are able to keep the pathways quite distinct. Even when the β-adrenergic receptor is maximally activated, it is not able to activate the K^+ channel (D. Clapham, unpubl.). Similarly, the muscarinic receptor does not cause any increase in adenylyl cyclase. One straightforward idea for the biochemical basis of this specificity would be that the β-adrenergic receptor is able to interact with G_s but not with G_i. This idea could be tested by reconstituting purified β-adrenergic receptors with G_s and G_i and asking whether a productive interaction between the two can take place. Such experiments were first carried out by Asano et al. (1984) and by Cerione et al. (1985), and the results revealed a surprising degree of cross-talk. In fact, in a reconstituted system, the pure

β-adrenergic receptor could couple both to G_s and to G_i, although it interacted with G_s two- to threefold better than with G_i. However, in the cardiac cell, the concentration of G_i is substantially greater than G_s. Why then does the β-adrenergic receptor not activate the K^+ channel?

The relative promiscuity of receptor G protein interactions has been borne out in many other subsequent reconstitution studies (e.g., Cerione et al., 1986; Senogles et al., 1990; Munshi et al., 1991). In some cases, differences in affinity have been found, but the rule seems to be that receptors discriminate rather poorly among α subunits when pure components are reassembled in phospholipid vesicles. Receptors that are able to discriminate one family of G proteins from another (e.g., G_s from G_i) discriminate much less effectively among the isoforms of G_i. As discussed above, there seems to be greater specificity in the ability of G protein subunits to activate effectors. Nevertheless, specificity at the G protein–effector interface cannot overcome any diffusion of the signal that originates at the receptor–G protein interface. If a receptor activates multiple G proteins and all of them very specifically activate their effectors, the signal initiated by that receptor will still spread through multiple second messenger systems.

A second striking example of specificity in transmembrane signaling comes from an elegant series of experiments by Kleuss et al. (1991, 1992, 1993). These investigators inhibited the synthesis of two alternatively spliced forms of G_o, β, and γ subunits in GH3 cells by injection of antisense oligonucleotides. The results revealed an extraordinary specificity of action. Elimination of one isoform of G_o blocked inhibition of a calcium channel by the somatostatin receptor, whereas elimination of the other alternatively spliced isoform eliminated inhibition of the same calcium channel by the muscarinic receptor. Because the two alternatively spliced forms of α_o differ in their carboxyl-termini (Hsu et al., 1990; Strathmann et al., 1990), a region thought to define the receptor interaction, it makes sense that the alternatively spliced G protein might couple to different receptors.

The tight specificity extends also to β and γ subunits. Antisense oligonucleotides against β_1 block the somatostatin response, whereas antisense oligonucleotides aimed at β_3 block the muscarinic response. The GH3 cells used in these experiments also contain β_2, but that seemed not to be involved either with the muscarinic or the somatostatin receptor. Antisense oligonucleotide experiments that eliminate γ subunit isoforms showed that γ_2 has the same response pattern as β_1, and γ_4 correlates with β_3. Thus, the conclusion would be that the somatostatin response is mediated through $\alpha_{o1}\beta_1\gamma_2$, whereas the muscarinic response is mediated by $\alpha_{o2}\beta_3\gamma_4$. Eliminating the $\beta\gamma$ subunits completely blocks the response to its corresponding receptor. One interpretation of this is that particular pairs of $\beta\gamma$ subunits are required to bring the α subunits back to their correct receptors, but that the effector regulation is carried out entirely by the α subunit. An alternative explanation is that $\beta\gamma$ subunits might also be able to inhibit the channel, and either α or $\beta\gamma$ subunits can produce the full effect. This would be a pattern similar to that seen for α and $\beta\gamma$ subunits with respect to the K^+ channel (Logothetis et al., 1988).

Although these examples are certainly striking, the specificity of cellular responses should not be overstated and is not universal. For example, Mortensen et al. (1991) eliminated all expression of $\alpha_{i\text{-}2}$ from mouse embryonic stem cells. In these cells, $\alpha_{i\text{-}2}$ is the predominant α subunit of the α_i class. Yet, the cells grew normally and differentiated into myocytes, neurites, and other cell types, as readily as control cells. Either $\alpha_{i\text{-}2}$ has no function in these cells or, more likely, the function can be taken over by other α_i isoforms. There are examples of receptors that appear, even in the context of intact cells, to be able to interact with more than one G protein and thus are able to initiate more than one signaling pathway (e.g., Abou-Samra et al., 1992; Eason et al., 1992; Gudermann et al., 1992; Dell'Acqua et al., 1993). The problem to resolve is how the cell defines which pathways must proceed through a single second messenger system and which can be allowed to spread through more than one.

Part of the explanation is likely to lie in the kinetics of the reaction and in the particular set of receptor α, β, γ, and effector subtypes expressed in a given cell. This precise fingerprint is part of what distinguishes one cell from another. The reciprocal regulation of α subunits and effectors may turn out to be an important element in specificity. An effector may be a good GTPase-activating protein (GAP) for one G protein but not for another. If the effector has a large effect on the GTPase activity, activation will be short lived, whereas if it has a small effect, the activation will last longer. Modulating the ability of an effector to exert its GAP function is one way to increase the discrimination among subunits. It is even possible that the $\beta\gamma$ subunit affects the GAP activity of effectors.

One obvious difference between reconstitution systems (including even transfection experiments in COS cells) and an intact, undisturbed cell is that the physical organization of the transmembrane signaling system is likely to be quite different. Simply making membranes from cells increases the mobility of membrane proteins by more than an order of magnitude (Beth et al., 1986). Thus, in an intact cell, receptors, G proteins, and effectors may be arranged and organized in microdomains and not have free access to all other components of the system. There is good evidence that pools of second messengers do exist in cells, perhaps reflecting the spatial organizations of the enzymes and channels that generate them (Dufau et al., 1978; Buxton & Brunton, 1983; Lechleiter et al., 1991). Such a possibility reframes the question, so that to understand specificity, we must understand what determines the assembly of the complete signaling system. There is some evidence that such geographical arrangements do, in fact, exist, although the evidence for functional importance is far from conclusive because localization is to cellular regions that are very large (e.g., apical or basolateral surfaces of polarized cells [Peraldi et al., 1989; Ercolani et al., 1990] or growth cones of neurites [Strittmatter et al., 1990; Zubiaur & Neer, 1993]) compared to the microdomains that might affect the specificity of transmembrane signaling.

Covalent modification of G proteins may affect the specificity of their interactions. Several types of G protein subunits ($\alpha_{i\text{-}2}$, α_z) can be phosphorylated on serine or threonine residues, but the phosphorylation has been difficult to correlate exactly with changes in activity (Katada et al., 1985; Daniel-Issakani et al., 1989; Bushfield et al., 1990; Lounsbury et al., 1991). Recombinant α subunits of various types can be phosphorylated in vitro on tyrosine residues by $pp60^{c\text{-}src}$ or by insulin receptors, but changes in activity were modest (Hausdorff et al., 1992) or nonexistent (Krupinski et al., 1988). None of the studies of phosphorylation of G proteins have tested the idea that phosphorylation of a G protein affects the *specificity* of its interaction with receptors or effectors. There is now increasing evidence from other phosphorylation systems, in particular the

role of SH2 domains in recognizing tyrosine phosphorylated proteins, suggesting that the role of phosphorylation may not be to change the function of the protein, but rather to change its localization or to specify the other proteins with which it may interact (Koch et al., 1991).

Another way to enhance specificity is through intracellular molecules whose role is specifically to enhance or blunt the function of certain activated G protein subunits. There are examples both for α and for $\beta\gamma$ of such intracellular regulatory proteins. For $\beta\gamma$, the best characterized example is phosphoducin, a phosphoprotein first identified in the retina that binds to $\beta\gamma$. Phosducin is able to inhibit $\beta\gamma$ function in vitro and may serve a similar role in vivo (Lee et al., 1992b). Calmodulin is another candidate $\beta\gamma$ regulator (Katada et al., 1987). An example of a regulatory protein for an α subunit is GAP43 or neuromodulin, a growth cone-associated protein. Despite its name, it is unrelated to GTPase activating proteins also called GAP. The neuromodulin (GAP43) protein is able to enhance GTPγS binding to the G_o subunit by a mechanism that appears to be different from hormone receptors because the activation is not blocked by a pertussis toxin (Strittmatter et al., 1991). Although the interaction of GAP43 with G_o suggests that the transmembrane signaling system could be modulated by intracellular proteins, the physiological significance of the interaction is, as yet, not understood.

There is considerable evidence that the specificity of cellular responses is not reflected in the selectivity of receptor interactions with G proteins. It is still an open question under what circumstances and to what extent similar G proteins can substitute for each other in the cell. It is also not clear to what degree any answer can be generalized from one cell type to another. I have outlined above a variety of mechanisms that might be superimposed on the minimal degree of selectivity seen in reconstitution experiments to restrict the potential for cross-talk among different signaling pathways. The weight given to each of these potential mechanisms might vary greatly from cell type to cell type. Although there is no doubt that the minimal number of proteins needed to transmit a signal across the plasma membrane of a cell is a receptor, G protein, and effector (Cerione et al., 1984; May et al., 1985), the challenge now is to define the further extent of the system. The signal-transducing proteins are not isolated in the cell membrane but interact with the cytoskeleton, with intracellular enzymes, and with several classes of receptors. Interaction with any one of these is very likely to alter both the possibility and the consequences of interactions with other parts of the macromolecular array. Unraveling how it all really works in the cell is sure to produce many surprises, but also new insights into the mechanisms that control these complex information networks.

Acknowledgments

This work was supported by NIH grants GM36259 and GM46370. I thank Mrs. Paula McColgan for expertly and patiently typing this manuscript, and the members of my laboratory for critical reading of the work. I also thank Drs. T. Thomas, D. Spring, and T. Michel for help with the figures.

References

Abou-Samra AB, Juppner H, Force T, Freeman MW, Kong XF, Schipani E, Urena P, Richards J, Bonventre JV, Potts JT Jr, Kronenberg HM, Segre GV. 1992. Expression cloning of a common receptor for parathyroid hormone and parathyroid hormone-related peptide from rat osteoblast-like cells: A single receptor stimulates intracellular accumulation of both cAMP and inositol trisphosphates and increases intracellular free calcium. *Proc Natl Acad Sci USA 89*:2732–2736.

Arshavsky VY, Bownds MD. 1992. Regulation of deactivation of photoreceptor G protein by its target enzyme and cGMP. *Nature 357*:416–417.

Artemyev NO, Rarick HM, Mills JS, Skiba NP, Hamm HE. 1992. Sites of interaction between rod G-protein α-subunit and cGMP-phosphodiesterase γ-subunit. Implications for the phosphodiesterase activation mechanism. *J Biol Chem 267*:25067–25072.

Asano T, Katada T, Gilman AG, Ross EM. 1984. Activation of the inhibitory GTP-binding protein of adenylate cyclase, G_i, by β-adrenergic receptors in reconstituted phospholipid vesicles. *J Biol Chem 259*:9351–9354.

Audigier Y, Journot L, Pantaloni C, Bockaert J. 1990. The carboxy-terminal domain of Gsα is necessary for anchorage of the activated form in the plasma membrane. *J Cell Biol 111*:1427–1435.

Backlund PS Jr, Simonds WF, Spiegel AM. 1990. Carboxyl methylation and COOH-terminal processing of the brain G-protein γ-subunit. *J Biol Chem 265*:15572–15576.

Berlot CH, Bourne HR. 1992. Identification of effector-activating residues of $G_{s\alpha}$. *Cell 68*:911–922.

Berstein G, Blank JL, Jhon DY, Exton JH, Rhee SG, Ross EM. 1992. Phospholipase C-β1 is a GTPase activating protein (GAP) for $G_{q/11}$, its physiologic regulator. *Cell 70*:411–418.

Beth AH, Conturo TE, Venkataramu SD, Staros JV. 1986. Dynamics and interactions of the anion channel in intact human erythrocytes: An electron paramagnetic resonance spectroscopic study employing a new membrane-impermeant bifunctional spin-label. *Biochemistry 25*:3824–3832.

Birnbaumer L. 1987. Which G protein subunits are the active mediators in signal transduction? *Trends Pharmacol Sci 8*:209–211.

Birnbaumer L, Abramowitz J, Brown AM. 1990. Receptor-effector coupling by G proteins. *Biochim Biophys Acta 1031*:163–224.

Blank JL, Brattain KA, Exton JH. 1992. Activation of cytosolic phosphoinositide phospholipase C by G-protein $\beta\gamma$ subunits. *J Biol Chem 267*:23069–23075.

Blank JL, Ross AH, Exton JH. 1991. Purification and characterization of two G-proteins that activate the β-1 isozyme of phosphoinositide-specific phospholipase C. Identification as members of the G_q class. *J Biol Chem 266*:18206–18216.

Bloch DB, Bonventre JV, Neer EJ, Seidman JG. 1989. The G protein alpha O subunit alters morphology, growth kinetics, and phospholipid metabolism of somatic cells. *Mol Cell Biol 9*:5434–5439.

Blumer KJ, Thorner J. 1990. β and γ subunits of a yeast guanine nucleotide-binding protein are not essential for membrane association of the α subunit but are required for receptor coupling. *Proc Natl Acad Sci USA 87*:4363–4367.

Bourne HR, Nicoll R. 1993. Molecular machines integrate coincident synaptic signals. *Cell 72*(10):65–75.

Bourne HR, Sanders DA, McCormick F. 1990. The GTPase superfamily: A conserved switch for diverse cell functions. *Nature 348*:125–132.

Bourne HR, Sanders DA, McCormick F. 1991. The GTPase superfamily: Conserved structure and molecular mechanism. *Nature 349*:117–127.

Boyer JL, Waldo GL, Harden TK. 1992. $\beta\gamma$-Subunit activation of G-protein-regulated phospholipase C. *J Biol Chem 267*:25451–25456.

Bubis J, Khorana HC. 1990. Sites of interaction in the complex between β- and γ-subunits of transducin. *J Biol Chem 265*:12995–12999.

Bushfield M, Lavan BE, Houslay MD. 1991. Okadaic acid influences a phosphorylation/dephosphorylation cycle controlling the inhibitory guanine-nucleotide-binding regulatory protein G_{i2}. *Biochem J 274*:317–321.

Buss JE, Mumby SM, Casey PJ, Gilman AG, Sefton BM. 1987. Myristoylated α subunits of guanine nucleotide-binding regulatory proteins. *Proc Natl Acad Sci USA 84*:7493–7497.

Buxton IL, Brunton LL. 1983. Compartments of cyclic AMP and protein kinase in mammalian cardiomyocytes. *J Biol Chem 258*:10233–10239.

Cali JJ, Balcueva EA, Rybalkin I, Robishaw JD. 1992. Selective tissue distribution of G protein γ subunits, including a new form of the γ subunits identified by cDNA cloning. *J Biol Chem 267*:24023–24027.

Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P. 1992. Isozyme-selective stimulation of phospholipase C-β2 by G protein $\beta\gamma$ subunits. *Nature 360*:684–686.

Carty DJ, Padrell E, Codina J, Birnbaumer L, Hildebrandt JD, Iyengar RJ. 1990. Distinct guanine nucleotide binding and release properties of the three G_i proteins. *J Biol Chem 265*:6268–6273.

Casey PJ, Graziano MP, Gilman AG. 1989. G protein $\beta\gamma$ subunits from bovine brain and retina: Equivalent catalytic support of ADP-ribosylation of α subunits by pertussis toxin but differential interactions with $G_s\alpha$. *Biochemistry 28*:611–616.

Cerione RA, Gierschik P, Staniszewski C, Benovic JL, Codina J, Somers R, Birnbaumer L, Spiegel AM, Lefkowitz RJ, Caron MG. 1987. Func-

tional differences in the $\beta\gamma$ complexes of transducin and the inhibitory guanine nucleotide regulatory protein. *Biochemistry 26*:1485-1491.
Cerione RA, Regan JW, Nakata H, Codina J, Benovic JL, Gierschik P, Somers RL, Spiegel AM, Birnbaumer L, Lefkowitz RJ, Caron MG. 1986. Functional reconstitution of the α_2-adrenergic receptor with guanine nucleotide regulatory proteins in phospholipid vesicles. *J Biol Chem 261*: 3901-3909.
Cerione RA, Sibley DR, Codina J, Benovic JL, Winslow J, Neer EJ, Birnbaumer L, Caron MG, Lefkowitz RJ. 1984. Reconstitution of a hormone-sensitive adenylate cyclase. *J Biol Chem 259*:9979-9982.
Cerione RA, Staniszewski C, Benovic JL, Lefkowitz RJ, Caron MG. 1985. Specificity of the functional interactions of the β-adrenergic receptor and rhodopsin with guanine nucleotide regulatory proteins reconstituted in phospholipid vesicles. *J Biol Chem 260*:1493-1500.
Clapham DE, Neer EJ. 1993. New roles for G protein $\beta\gamma$ dimers in transmembrane signalling. *Nature 365*:403-406.
Conklin BR, Bourne HR. 1993. Structural elements of $G\alpha$ subunits that interact with $G\beta\gamma$, receptors and effectors. *Cell*. Forthcoming.
Conklin BR, Chabre O, Wong YH, Federman AD, Bourne HR. 1992. Recombinant $G_q\alpha$. Mutational activation and coupling to receptors and phospholipase C. *J Biol Chem 267*:31-34.
Conklin RB, Farfel Z, Lustig KD, Julius D, Bourne HR. 1993. Substitution of three amino acids switches receptor specificity of $G_q\alpha$ to that of $G_i\alpha$. *Nature 363*:274-276.
Daniel-Issakani S, Spiegel AM, Strulovici B. 1989. Lipopolysaccharide response is linked to the GTP binding protein, G_{i-2}, in the promonocytic cell line U937. *J Biol Chem 264*:20240-20247.
Dell'Acqua ML, Carroll RC, Peralta EG. 1993. Transfected m2 muscarinic acetylcholine receptors couple to $G\alpha_{i-2}$ and $G\alpha_{i-3}$ in Chinese hamster ovary cells. Activation and desensitization of the phospholipase C signaling pathway. *J Biol Chem 268*:5676-5685.
Denker BM, Neer EJ, Schmidt CJ. 1992a. Mutagenesis of the amino terminus of the α subunit of the G protein, G_o. in vitro characterization of $\alpha_o\beta\gamma$ interactions. *J Biol Chem 267*:6272-6277.
Denker BM, Schmidt CJ, Neer EJ. 1992b. Promotion of the GTP-liganded state of the $G_{\alpha o}$ protein by deletion of the C terminus. *J Biol Chem 267*:9998-10002.
Dhanasekaran N, Weslling-Resnick M, Kelleher DJ, Johnson GL, Ruoho AE. 1988. Mapping of the carboxyl terminus within the tertiary structure of transducin's α subunit using the heterobifunctional cross-linking reagent, ^{125}I-N-(3-iodo-4-azidophenylpropio-namido-*S*-(2-thiopyridyl)-cysteine). *J Biol Chem 263*:17942-17950.
Dohlman HG, Thorner J, Caron MG, Lefkowitz RJ. 1991. Model systems for the study of seven-transmembrane-segment receptors. *Annu Rev Biochem 60*:653-688.
Dratz EA, Furstenau JE, Lambert CG, Thireault DL, Rarick H, Schepers T, Pakhlevaniants S, Hamm HE. 1993. NMR structure of a receptor-bound G-protein peptide. *Nature 363*:276-281.
Dufau ML, Horner KA, Hayashi K, Tsuruhara T, Conn PM, Catt KJ. 1978. Actions of choleragen and gonadotropin in isolated Leydig cells. Functional compartmentalization of the hormone-activated cyclic AMP response. *J Biol Chem 253*:3721-3729.
Eason MG, Kurose H, Holt BD, Raymond JR, Liggett SB. 1992. Simultaneous coupling of α_2-adrenergic receptors to two G-proteins with opposing effects. Subtype-selective coupling of α_2C10, α_2C4, and α_2C2 adrenergic receptors to G_i and G_s. *J Biol Chem 267*:15795-15801.
Ercolani L, Stow JL, Boyle JF, Holtzman EJ, Lin H, Grove JR, Ausiello DA. 1990. Membrane localization of the pertussis toxin-sensitive G-protein subunits α_{i-2} and α_{i-3} and expression of a metallothionein-α_{i-2} fusion gene in LLC-PK$_1$ cells. *Proc Natl Acad Sci USA 87*:4635-4639.
Fawzi AB, Fay DS, Murphy EA, Tamir H, Erdos JJ, Northup JK. 1991. Rhodopsin and the retinal G-protein distinguish among G protein $\beta\gamma$ subunit forms. *J Biol Chem 266*:12194-12200.
Federman AD, Conklin BR, Schrader KA, Reed RR, Bourne HR. 1992. Hormonal stimulation of adenylyl cyclase through G_i-protein $\beta\gamma$ subunits. *Nature 356*:159-161.
Florio VA, Sternweis PC. 1985. Reconstitution of resolved muscarinic cholinergic receptors with purified GTP-binding proteins. *J Biol Chem 260*: 3477-3483.
Fong HKW, Amatruda TT, Birren BW, Simon MI. 1987. Distinct forms of the β subunit of GTP-binding regulatory proteins identified by molecular cloning. *Proc Natl Acad Sci USA 84*:3792-3796.
Fung BKK. 1983. Characterization of transducin from bovine retinal rod outer segments. I. Separation and reconstitution of the subunits. *J Biol Chem 258*:10495-10502.
Fung BKK, Nash CR. 1983. Characterization of transducin from bovine retinal rod outer segments. II. Evidence for distinct binding sites and conformational changes revealed by limited proteolysis with trypsin. *J Biol Chem 258*:10503-10510.
Fung BKK, Tamane HK, Ota IM, Clarke S. 1990. The γ subunit of brain G proteins is methyl esterified at a C-terminal cysteine. *FEBS Lett 260*: 313-317.
Gao B, Gilman AG, Robishaw JD. 1987. A second form of the β subunit of signal-transducing G proteins. *Proc Natl Acad Sci USA 84*:6122-6125.
Gautam N, Baetscher M, Aebersold R, Simon MI. 1989. A G protein γ subunit shares homology with *ras* proteins. *Science 244*:971-974.
Gibbs JB, Schaber MD, Allard WJ, Sigal IS, Scolnick EM. 1988. Purification of *ras* ATPase activating protein from bovine brain. *Proc Natl Acad Sci USA 85*:5026-5030.
Gilman AG. 1987. G proteins: Transducers of receptor-generated signals. *Annu Rev Biochem 56*:615-649.
Glatt CE, Snyder SH. 1993. Cloning and expression of an adenylyl cyclase localized to the corpus striatum. *Nature 361*:536-538.
Graf R, Mattera R, Codina J, Estes MK, Birnbaumer L. 1992. A truncated recombinant α subunit of G_{i3} with a reduced affinity for $\beta\gamma$ dimers and altered guanosine 5'-3-O'(thio)triphosphate binding. *J Biol Chem 267*: 24307-24314.
Gudermann T, Birnbaumer M, Birnbaumer L. 1992. Evidence for dual coupling of murine luteinizing hormone receptor to adenylyl cyclase and phosphoinositide breakdown and Ca^{2+} mobilization. Studies with the cloned murine luteinizing hormone receptor expressed in L cells. *J Biol Chem 267*:4479-4488.
Gutowski S, Smrcka A, Nowak L, Wu D, Simon M, Sternweis PC. 1991. Antibodies to the α_q subfamily guanine nucleotide-binding regulatory protein α subunits attenuate activation of phosphatidylinositol 4,5-bisphosphate hydrolysis by hormones. *J Biol Chem 266*:20519-20524.
Haga K, Haga T. 1992. Activation by G protein $\beta\gamma$ subunits of agonist- or light-dependent phosphorylation of muscarinic acetylcholine receptors and rhodopsin. *J Biol Chem 267*:2222-2227.
Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, Hofmann KP. 1988. Site of G protein binding to rhodopsin mapped with synthetic peptides from the α subunit. *Science 241*:832-835.
Hausdorff WP, Pitcher JA, Luttrell DK, Linder ME, Kurose H, Parsons SJ, Caron MG, Lefkowitz RJ. 1992. Tyrosine phosphorylation of G protein α subunits by pp60$^{c\text{-}src}$. *Proc Natl Acad Sci USA 89*:5720-5724.
Hekman M, Holzhofer A, Gierschik P, Im MJ, Jakobs KH, Pfeuffer T, Helmreich EJ. 1987. Regulation of signal transfer from β1-adrenoceptor to adenylate cyclase by $\beta\gamma$ subunits in a reconstituted system. *Eur J Biochem 169*:431-439.
Higashijima T, Ferguson KM, Sternweis PC, Smigel MD, Gilman AG. 1987. Effects of Mg^{2+} and the $\beta\gamma$ subunit complex on the interactions of guanine nucleotides with G proteins. *J Biol Chem 262*:762-766.
Holbrook SR, Kim SH. 1989. Molecular model of the G protein α subunit based on the crystal structure of the HRAS protein. *Proc Natl Acad Sci USA 86*:1751-1755.
Hsu WH, Rudolph U, Sanford J, Beertrand P, Olate J, Nelson C, Moss LG, Boyd AE, Codina J, Birnbaumer L. 1990. Molecular cloning of a novel splice variant of the α subunit of the mammalian G_o protein. *J Biol Chem 265*:11220-11226.
Huff RM, Neer EJ. 1986. Subunit interactions of native and ADP-ribosylated α_{39} and α_{41}, two guanine nucleotide-binding proteins from bovine cerebral cortex. *J Biol Chem 261*:1105-1110.
Hurley JB, Fong HK, Teplow DB, Dreyer WJ, Simon MI. 1984. Isolation and characterization of a cDNA clone for the γ subunit of bovine retinal transducin. *Proc Natl Acad Sci USA 81*:6948-6952.
Im MJ, Holzhofer A, Keenan AK, Gierschik P, Hekman M, Helmreich EJM, Pfeuffer T. 1987. The role of β,γ-subunits of guanine nucleotide binding proteins in control of a reconstituted signal transmission chain containing purified components of the adenylate cyclase system. *J Receptor Res 7*:17-42.
Iniguez-Lluhi JA, Simon MI, Robishaw JD, Gilman AG. 1992. G protein $\beta\gamma$ subunits synthesized in Sf9 cells. *J Biol Chem 267*:23409-23417.
Ito H, Tung RT, Sugimoto T, Kobayashi I, Takahashi K, Katada T, Ui M, Kurachi Y. 1992. On the mechanism of G protein $\beta\gamma$ subunit activation of the muscarinic K^+ channel in guinea pig atria cell membrane. Comparison with the ATP-sensitive K^+ channel. *J Gen Physiol 99*: 961-983.
Jelsema CL, Axelrod J. 1987. Stimulation of phospholipase A_2 activity in bovine rod outer segments by the $\beta\gamma$ subunits of transducin and its inhibition by the α subunit. *Proc Natl Acad Sci USA 84*: 3623-3627.
Jones TLZ, Simonds WF, Merendino JJ Jr, Brann MR, Spiegel AM. 1990. Myristoylation of an inhibitory GTP-binding protein α subunit is essential for its membrane attachment. *Proc Natl Acad Sci USA 87*:568-572.
Juhnn YS, Jones TLZ, Spiegel AM. 1992. Amino- and carboxyl-terminal deletion mutants for $Gs\alpha$ are localized to the particulate fraction of transfected COS cells. *J Cell Biol 119*:523-530.
Jurnak F. 1985. Structure of the GDP domain of EF-Tu and location of the amino acids homologous to *ras* oncogene proteins. *Science 230*:32-36.
Katada T, Gilman AG, Watanabe Y, Bauer S, Jakobs KH. 1985. Protein ki-

nase C phosphorylates the inhibitory guanine-nucleotide-binding regulatory component and apparently suppresses its function in hormonal inhibition of adenylate cyclase. *Eur J Biochem 151*:431–437.

Katada T, Kusakabe K, Oinuma M, Ui M. 1987. A novel mechanism for the inhibition of adenylate cyclase via inhibitory GTP-binding proteins. Calmodulin-dependent inhibition of the cyclase catalyst by the $\beta\gamma$ subunits of GTP-binding proteins. *J Biol Chem 262*:11897–11900.

Katz A, Wu D, Simon MI. 1992. Subunits $\beta\gamma$ of heterotrimeric G protein activate $\beta 2$ isoform of phospholipase C. *Nature 360*:686–689.

Kaziro Y, Itoh H, Kozasa T, Nakafuku M, Satoh T. 1991. Structure and function of signal-transducing GTP-binding proteins. *Annu Rev Biochem 60*: 349–400.

Kelleher DJ, Johnson GL. 1988. Transducin inhibition of light-dependent rhodopsin phosphorylation. Evidence for $\beta\gamma$ subunit interaction with rhodopsin. *Mol Pharmacol 34*:452–460.

Kent RS, DeLean A, Lefkowitz RJ. 1980. A quantitative analysis of beta-adrenergic receptor interactions: Resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol Pharmacol 17*:14–23.

Kim S, Ang SL, Bloch DB, Bloch KD, Kawahara Y, Tolman C, Lee R, Seidman JG, Neer EJ. 1988. Identification of cDNA encoding an additional α subunit of a human GTP-binding protein. Expression of three α_i subtypes in human tissues and cell lines. *Proc Natl Acad Sci USA 85*: 4153–4157.

Kim D, Lewis DL, Graziadei L, Neer EJ, Bar-Sagi D, Clapham DE. 1989. G protein $\beta\gamma$ subunits activate the cardiac muscarinic K^+ channel via phospholipase A_2. *Nature 337*:557–560.

Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G, Wittig B. 1991. Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature 353*:43–48.

Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B. 1992. Different β-subunits determine G-protein interaction with transmembrane receptors. *Nature 358*:424–426.

Kleuss C, Scherubl H, Hescheler J, Schultz G, Wittig B. 1993. Selectivity in signal transduction determined by γ subunits of heterotrimeric G proteins. *Science 259*:832–834.

Kobilka B. 1992. Adrenergic receptors as models for G protein-coupled receptors. *Annu Rev Neurosci 15*:87–114.

Koch CA, Anderson D, Moran MF, Ellis C, Pawson T. 1991. SH2 and SH3 domains: Elements that control interactions of cytoplasmic signaling proteins. *Science 252*:668–674.

Kokame K, Fukada Y, Yoshizawa T, Takao T, Shimonishi Y. 1992. Lipid modification at the N terminus of photoreceptor G-protein α-subunit. *Nature 359*:749–752.

Krupinski J, Rajaram R, Lakonishok M, Benovic JL, Cerione RA. 1988. Insulin-dependent phosphorylation of GTP-binding proteins in phospholipid vesicles. *J Biol Chem 263*:12333–12341.

Kurachi Y, Ito H, Sugimoto T, Katada T, Ui M. 1989a. Activation of atrial muscarinic K^+ channels by low concentrations of $\beta\gamma$ subunits of rat brain G proteins. *Pfluger's Arch 413*:325–327.

Kurachi Y, Ito H, Sugimoto T, Shimizu T, Miki I, Ui M. 1989b. Arachidonic acid metabolites as intracellular modulators of the G protein-gated cardiac K^+ channel. *Nature 337*:555–557.

Kurose H, Regan JW, Caron MG, Lefkowitz RJ. 1991. Functional interactions of recombinant α_2-adrenergic receptor subtypes and G proteins in reconstituted phospholipid vesicles. *Biochemistry 30*:3335–3341.

la Cour TF, Nyborg J, Thirup S, Clark BF. 1985. Structural details of the binding of guanosine diphosphate to elongation factor Tu from *E. coli* as studied by X-ray crystallography. *EMBO J 4*:2385–2388.

Lai RK, Perez-Sala D, Canada RJ, Rando RR. 1990. The γ subunit of transducin is farnesylated. *Proc Natl Acad Sci USA 87*:7673–7677.

Lechleiter J, Girard S, Clapham D, Peralta E. 1991. Subcellular patterns of calcium release determined by G protein-specific residues of muscarinic receptors. *Nature 350*:505–508.

Lee E, Taussig R, Gilman AC. 1992a. The G226A mutant of $G_{s\alpha}$ highlights the requirement for dissociation of G protein subunits. *J Biol Chem 267*:1212–1218.

Lee RH, Ting TD, Lieberman BS, Tobias DE, Lolley RN, Ho YK. 1992b. Regulation of retinal cGMP cascade by phosducin in bovine rod photoreceptor cells. Interaction of phosducin and transducin. *J Biol Chem 267*:25104–25112.

Levine MA, Smallwood PM, Moen PT Jr, Helman LJ, Ahn TG. 1990. Molecular cloning of $\beta 3$ subunit, a third form of the G protein β-subunit polypeptide. *Proc Natl Acad Sci USA 87*:2329–2333.

Linder ME, Ewald DA, Miller RJ, Gilman AG. 1990. Purification and characterization of $G_{o\alpha}$ and three types of $G_{i\alpha}$ after expression in *Escherichia coli*. *J Biol Chem 265*:8243–8251.

Linder ME, Middleton P, Hepler JR, Taussig R, Gilman AG, Mumby SM. 1993. Lipid modifications of G proteins: α subunits are palmitoylated. *Proc Natl Acad Sci USA 90*:3675–3679.

Linder ME, Pang IH, Duronio RJ, Gordon JI, Sternweis PC, Gilman AG. 1991. Lipid modifications of G protein subunits. Myristoylation of $G_{o\alpha}$ increases its affinity for $\beta\gamma$. *J Biol Chem 266*:4654–4659.

Logothetis DE, Kim D, Northup JK, Neer EJ, Clapham DE. 1988. Specificity of action of guanine nucleotide-binding regulatory protein subunits on the cardiac muscarinic K^+ channel. *Proc Natl Acad Sci USA 85*: 5815–5818.

Logothetis DE, Kurachi Y, Galper J, Neer EJ, Clapham DE. 1987. The $\beta\gamma$ subunits of GTP-binding proteins activate the muscarinic K^+ channel in heart. *Nature 325*:321–326.

Lounsbury KM, Casey PJ, Brass LF, Manning DR. 1991. Phosphorylation of G_z in human platelets. Selectivity and site of modification. *J Biol Chem 266*:22051–22056.

Lott JS, Ryba NJP, Pottinger JDD, Keen JN, Carne A, Findlay JBC. 1992. The γ-subunit of the principal G-protein from squid (*Loligo forbesi*) photoreceptors contains a novel N-terminal sequence. *FEBS Lett 312*:241–244.

Lupas AN, Lupas JM, Stock JB. 1992. Do G protein subunits associate via a three-stranded coiled coil? *FEBS Lett 314*:105–108.

Maltese WA, Robishaw JD. 1990. Isoprenylation of C-terminal cysteine in a G-protein γ subunit. *J Biol Chem 265*:18071–18074.

Mattera R, Codina J, Sekura RD, Birnbaumer L. 1987. Guanosine 5'-O-(3-thiotriphosphate) reduces ADP-ribosylation of the inhibitory guanine nucleotide-binding regulatory protein of adenylyl cyclase (N_i) by pertussis toxin without causing dissociation of the subunits of N_i. Evidence of existence of heterotrimeric pt_+ and pt_- conformations of N_i. *J Biol Chem 262*:11247–11251.

May DC, Ross EM, Gilman AG, Smigel MD. 1985. Reconstitution of catecholamine stimulated adenylate cyclase activity using three purified proteins. *J Biol Chem 260*:15829–15833.

Mazzoni MR, Hamm HE. 1989. Effect of monoclonal antibody binding on α-$\beta\gamma$ subunit interactions in the rod outer segment G protein, G_i. *Biochemistry 28*:9873–9880.

Milburn MV, Tong L, DeVos AM, Brunger A, Yamaizumi Z, Nishimura S, Kim SH. 1990. Molecular switch for signal transduction: Structural differences between active and inactive forms of protooncogenic *ras* proteins. *Science 247*:939–945.

Miller RT, Masters SB, Sullivan KA, Beiderman B, Bourne HR. 1988. A mutation that prevents GTP-dependent activation of the α chain of G_s. *Nature 334*:712–715.

Mortensen RM, Zubiaur M, Neer EJ, Seidman JG. 1991. Embryonic stem cells lacking a functional inhibitory G-protein subunit ($\alpha_{i\text{-}2}$) produced by gene targeting of both alleles. *Proc Natl Acad Sci USA 88*:7036–7040.

Mumby SM, Casey PJ, Gilman AG, Gutowski S, Sternweis PC. 1990a. G protein γ subunits contain a 20-carbon isoprenoid. *Proc Natl Acad Sci USA 87*:5873–5877.

Mumby SM, Heukeroth RO, Gordon JI, Gilman AG. 1990b. G-protein α-subunit expression, myristoylation, and membrane association in COS cells. *Proc Natl Acad Sci USA 87*:728–732.

Munshi R, Pang IH, Sternweis PC, Linden J. 1991. A_1 adenosine receptors of bovine brain couple to guanine nucleotide-binding proteins G_{i1}, G_{i2}, and G_o. *J Biol Chem 266*:22285–22289.

Muntz KH, Sternweis PC, Gilman AG, Mumby SM. 1992. Influence of γ subunit prenylation on association of guanine nucleotide-binding regulatory proteins with membranes. *Mol Biol Cell 3*:49–61.

Navon SE, Fung BKK. 1988. Characterization of transducin from bovine retinal rod outer segments. *J Biol Chem 263*:489–496.

Neer EJ, Clapham DE. 1988. Roles of G protein subunits in transmembrane signalling. *Nature 333*:129–134.

Neer EJ, Pulsifer L, Wolf LG. 1988. The amino terminus of G protein α subunits is required for interaction with $\beta\gamma$. *J Biol Chem 263*:8996–9000.

Osawa S, Dhanasekaran N, Woon CW, Johnson GL. 1990. $G\alpha_i$–$G\alpha_s$ chimeras define the function of α chain domains in control of G protein activation and $\beta\gamma$ subunit complex interactions. *Cell 63*:697–706.

Pai EJ, Kabsch W, Krengel U, Holmes KC, John J, Wittinghofer A. 1989. Structure of the guanine-nucleotide-binding domain of the Ha-ras oncogene product p21 in the triphosphate conformation. *Nature 341*:209–214.

Park D, Jhon DY, Lee CW, Ryu SH, Rhee SG. 1993. Removal of the carboxyl-terminal region of phospholipase C-$\beta 1$ by calpain abolishes activation by $G\alpha_q$. *J Biol Chem 268*:3710–3714.

Peraldi S, Nguyen Than Dao B, Brabet P, Homburger V, Rouot B, Toutant M, Bouille C, Assenmacher I, Bockaert J. 1989. Apical localization of the alpha subunit of GTP-binding protein G_o in choroidal and ciliated ependymocytes. *J Neurosci 9*:806–814.

Phillips WJ, Wong SC, Cerione RA. 1992. Rhodopsin/transducin interactions. II. Influence of the transducin-$\beta\gamma$ subunit complex on the coupling of the transducin-α subunit to rhodopsin. *J Biol Chem 267*:17040–17046.

Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C, Benovic JL, Kwatra MM, Caron MG, Lefkowitz RJ. 1992. Role of $\beta\gamma$ subunits of

G proteins in targeting the β-adrenergic receptor kinase to membrane-bound receptors. *Science 257*:1264-1267.
Pronin AN, Gautam N. 1992. Interaction between G-protein β and γ subunit types is selective. *Proc Natl Acad Sci USA 89*:6220-6224.
Rarick HM, Artemyev NO, Hamm HE. 1992. A site on rod G protein α subunit that mediates effector activation. *Science 256*:1031-1033.
Rhee SG, Choi KD. 1992. Regulation of inositol phospholipid-specific phospholipase C isozymes. *J Biol Chem 267*:12393-12396.
Ross EM. 1989. Signal sorting and amplification through G protein-coupled receptors. *Neuron 3*:141-152.
Sanford J, Codina J, Birnbaumer L. 1991. γ-Subunits of G proteins, but not their α- or β-subunits, are polyisoprenylated. Studies on post-translational modifications using in vitro translation with rabbit reticulocyte lysates. *J Biol Chem 266*:9570-9579.
Savarese TM, Fraser CM. 1992. In vitro mutagenesis and the search for structure-function relationships among G protein-coupled receptors. *Biochem J 283*:1-19.
Schlichting I, Almo SC, Rapp G, Wilson K, Petratos K, Lentfer A, Wittinghofer A, Kabsch W, Pai EF, Petsko GA, Goody RS. 1990. Time-resolved X-ray crystallographic study of the conformational change in Ha-Ras p21 protein on GTP hydrolysis. *Nature 345*:309-315.
Schmidt CJ, Neer EJ. 1991. In vitro synthesis of G protein βγ dimers. *J Biol Chem 266*:4538-4544.
Schmidt CJ, Thomas TC, Levine MA, Neer EJ. 1992. Specificity of G protein β and γ subunit interactions. *J Biol Chem 267*:13807-13810.
Senogles SE, Spiegel AM, Padrell E, Iyengar R, Caron MG. 1990. Specificity of receptor-G protein interactions. *J Biol Chem 265*:4507-4514.
Sibley DR, Benovic JL, Caron MG, Lefkowitz RJ. 1987. Regulation of transmembrane signaling by receptor phosphorylation. *Cell 48*:913-922.
Simon MI, Strathmann MP, Gautam N. 1991. Diversity of G proteins in signal transduction. *Science 252*:802-808.
Simonds WF, Butrynski JE, Gautam N, Unson CG, Spiegel AM. 1991. G-protein βγ dimers. *J Biol Chem 266*:5363-5366.
Simonds WF, Collins RM, Spiegel AM, Brann MR. 1989a. Membrane attachment of recombinant G-protein α-subunits in excess of βγ subunits in a eukaryotic expression system. *Biochem Biophys Res Commun 164*:46-53.
Simonds WF, Goldsmith PK, Codina J, Unson CG, Spiegel AM. 1989b. G_{i2} mediates α_2-adrenergic inhibition of adenylyl cyclase in platelet membranes. In situ identification with G_α C-terminal antibodies. *Proc Natl Acad Sci USA 86*:7809-7813.
Smrcka AV, Sternweis PC. 1993. Regulation of purified subtypes of phosphatidylinositol-specific phospholipase Cβ by G protein α and βγ subunits. *J Biol Chem 268*:9667-9674.
Spiegel AM, Shenker A, Weinstein LS. 1992. Receptor-effector coupling by G proteins: Implications for normal and abnormal signal transduction. *Endocrine Rev 13*:536-565.
Strasser RH, Benovic JL, Caron MG, Lefkowitz RJ. 1986. β-Agonist- and prostaglandin E_1-induced translocation of the β-adrenergic receptor kinase: Evidence that the kinase may act on multiple adenylate cyclase-coupled receptors. *Proc Natl Acad Sci USA 83*:6362-6366.
Strathmann M, Wilkie TM, Simon MI. 1990. Alternative splicing produces transcripts encoding two forms of the α subunit of GTP-binding protein G_o. *Proc Natl Acad Sci USA 87*:6477-6481.
Strittmatter SM, Valenzuela D, Kennedy TE, Neer EJ, Fishman MC. 1990. G_o is a major growth cone protein subject to regulation by GAP-43. *Nature 344*:836-841.
Strittmatter SM, Valenzuela D, Sudo Y, Linder ME, Fishman MC. 1991. An intracellular guanine nucleotide release protein for G_o. GAP-43 stimulates isolated α subunits by a novel mechanism. *J Biol Chem 266*:22465-22471.
Stryer L, Bourne HR. 1986. G proteins: A family of signal transducers. *Annu Rev Cell Biol 2*:391-419.
Sullivan KA, Miller RT, Masters SB, Beiderman B, Heideman W, Bourne HR. 1987. Identification of receptor contact site involved in receptor-G protein coupling. *Nature 330*:758-760.
Tang WJ, Gilman AG. 1991. Type-specific regulation of adenylyl cyclase by G protein βγ subunits. *Science 254*:1500-1503.
Tang WJ, Gilman AG. 1992. Adenylyl cyclases. *Cell 70*:869-872.
Taussig R, Quarmby LM, Gilman AG. 1993. Regulation of purified Type I and Type II adenylylcylases by G protein βγ subunits. *J Biol Chem 268*:9-12.
Taylor SJ, Chae HZ, Rhee SG, Exton JH. 1991. Activation of the β1 isozyme of phospholipase C by α subunits of the α_q class of G proteins. *Nature 350*:516-518.
Thomas TC, Schmidt CJ, Neer EJ. 1993a. G protein α_o subunit: Mutation of conserved cysteines identifies a subunit contact surface and alters GDP affinity. *Proc Natl Acad Sci USA*. Forthcoming.
Thomas TC, Sladek T, Yi F, Smith T, Neer EJ. 1993b. The G protein βγ subunit: Physical and chemical characterization. *Biochemistry 32*:8628-8635.
Trahey M, McCormick F. 1987. A cytoplasmic protein stimulates normal N-*ras* p21 GTPase, but does not affect oncogenic mutants. *Science 238*:542-545.
van der Voorn L, Ploegh HL. 1992. The WD-40 repeat. *FEBS Lett 307*:131-134.
von Weizsacker E, Strathmann MP, Simon MI. 1992. Diversity among the beta subunits of heterotrimeric GTP-binding proteins: Characterization of a novel beta-subunit cDNA. *Biochem Biophys Res Commun 183*:350-356.
West RE Jr, Moss J, Vaughan M, Liu T, Liu TY. 1985. Pertussis toxin-catalyzed ADP-ribosylation of transducin. *J Biol Chem 260*:14428-14430.
Whiteway M, Hougan L, Dignard D, Thomas DY, Bell L, Saari GC, Grant FJ, O'Hara P, MacKay VL. 1989. The STE4 and STE18 genes of yeast encode potential β and γ subunits of the mating factor receptor-coupled G protein. *Cell 56*:467-477.
Wildman DE, Tamir H, Leberer E, Northup JK, Dennis M. 1993. Prenyl modification of guanine nucleotide regulatory protein γ2 subunits is not required for interaction with the transducin α subunit or rhodopsin. *Proc Natl Acad Sci USA 90*:794-798.
Wu D, Jiang H, Katz A, Simon MI. 1993a. Identification of critical regions on phospholipase C-β1 required for activation by G-proteins. *J Biol Chem 268*:3704-3709.
Wu D, Katz A, Simon MI. 1993b. Activation of phospholipase C β2 by the α and βγ subunits of trimeric GTP-binding protein. *Proc Natl Acad Sci USA 90*:5297-5301.
Yamane HK, Farnsworth CC, Xie H, Howald W, Fung BKK, Clarke S, Gelb MH, Glomset JA. 1990. Brain G protein γ subunits contain an all-trans-geranylgeranyl-cysteine methyl ester at their carboxyl termini. *Proc Natl Acad Sci USA 87*:5868-5872.
Yatani A, Codina J, Brown AM, Birnbaumer L. 1987. Direct activation of mammalian atrial muscarinic potassium channels by GTP regulatory protein G_k. *Science 235*:207-211.
Yatani A, Mattera R, Codina J, Graf R, Okabe K, Padrell E, Iyengar R, Brown AM, Birnbaumer L. 1988. The G protein-gated atrial K^+ channel is stimulated by three distinct $G_i\alpha$ subunits. *Nature 336*:680-682.
Yatsunami K, Pandya BV, Oprian DD, Khorana HG. 1985. cDNA-derived amino acid sequence of the γ subunit of GTPase from bovine rod outer segments. *Proc Natl Acad Sci USA 82*:1936-1940.
Yi F, Denker BM, Neer EJ. 1991. Structural and functional studies of cross-linked G_o protein subunits. *J Biol Chem 266*:3900-3906.
Zubiaur M, Neer EJ. 1993. Nerve growth factor changes G protein levels and localization in PC12 cells. *J Neurosci Res 35*:207-217.