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# Heterotrimeric G Proteins: Organizers of Transmembrane Signals

Review

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Hundreds of chemical and physical signals constantly bombard the surface of all cells. Some of these do not enter the cell but, instead, bind to receptors at the cell surface and initiate a flow of information that moves to the cell interior. The receptors for many hormones (such as catecholamines, gonadotropins, parathyroid hormone, and glucagon), odorants, and light span the membrane seven times (reviewed by Dohlman et al., 1991). Stimulation of these receptors activates a group of coupling proteins (called G proteins because they bind GTP) that regulate a variety of enzymes and ion channels. The target enzymes or ion channels are called effectors because changes in their activity cause the changes in ionic composition or in second messenger levels (such as cAMP or inositol phosphate levels) that ultimately lead to the cellular response.

Every eukaryotic cell contains receptors for many kinds of chemical and/or physical signals, many different types of G proteins, and many effectors, each with multiple subtypes. A cell can only respond to those signals for which it has a receptor, but the specificity with which the receptor interacts with the coupling proteins (the G proteins) defines the range of responses that a cell is able to make. Receptors are highly selective for their ligands. If a receptor can interact with only one subtype of G protein that can, in turn, activate only one type of effector, the response will be very focused. In contrast, if a receptor can interact with several G proteins, each of which can interact with more than one effector, the response would be expected to be spread over several pathways. As will be discussed below, a cell may respond to some signals with a very defined set of actions, but may respond to others less specifically. Similarly, a ligand that gives a focused response in one cell may cause a pleiotypic response in another. Over the last decade, there has been enormous progress in defining the elements that are involved in transmembrane signaling. A very large number of receptors have been cloned, characterized, and subdivided into families. Four subfamilies of G protein α subunits have been defined, and multiple G protein β and γ subunits have been identified. We now know that effectors often come in several subtypes, each with different regulatory properties. What is still mysterious is exactly what determines specificity of the response of a cell to an extracellular stimulus. What is the grammar that controls the interpretation of signals? In this review, I will summarize some features of the structure and function of mammalian G protein subunits, then discuss how the elements of the cellular language may be ordered and weighted to allow the cell to respond properly to the message.

## The G Protein Cycle

G proteins are made up of three polypeptides: an α subunit that binds and hydrolyzes GTP, a β subunit, and a γ subunit. The β and γ subunits form a dimer that only dissociates when it is denatured and is, therefore, a functional monomer. Figure 1 illustrates the cycle of G protein activation and deactivation that transmits the signal from receptor to effector (reviewed by Gilman, 1987; Clapham and Neer, 1993; Neer, 1994). When GDP is bound, the α subunit associates with the βγ subunit to form an inactive heterotrimer that binds to the receptor. Both α and βγ subunits can bind to the receptor. Monomeric, GDP-liganded α subunits can interact with receptors, but the association is greatly enhanced by βγ. When a chemical or physical signal stimulates the receptor, the receptor becomes activated and changes its conformation. The GDP-liganded α subunit responds with a conformational change that decreases GDP affinity, so that GDP comes off the active site. Because the concentration of GTP in cells is much higher than that of GDP, the leaving GDP is replaced with GTP. Once GTP is bound, the α subunit assumes its activated conformation and dissociates both from the receptor and from βγ. The activated state lasts until the GTP is hydrolyzed to GDP by the intrinsic GTPase activity of the α subunit. 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). Once GTP is cleaved to GDP, the α and βγ subunits reassociate, become inactive, and return to the receptor. The free α and βγ subunits each activate target effectors. It is important to notice that the rate of GTP hydrolysis is a timing mechanism that controls the duration of both α and βγ subunit activation, but also of βγ. Reassociation turns off both subunits and primes the system to respond again. Thus, although the βγ subunit does not bind GTP, its active lifetime depends on the rate of GTP hydrolysis by an α subunit.

## Signal Transduction by G Protein Subunits

For a long time, the prevalent hypothesis for the mechanism of G protein–mediated signal transduction was that the GTP-liganded α subunit activated effectors, while the βγ subunit was only a negative regulator. Release of free βγ from an abundant G protein, such as $G_i$, was thought to deactivate other α subunits by forming inactive heterotrimers. Indeed, the βγ subunits can block activation of adenylyl cyclase by this mechanism (Gilman, 1987). Alternatively, the $G_{\beta\gamma}$ subunit was thought to decrease "noise" by blunting side reactions. This paradigm changed fundamentally with the discovery that the βγ subunit could activate the muscarinic $K^+$ channel and the realization that

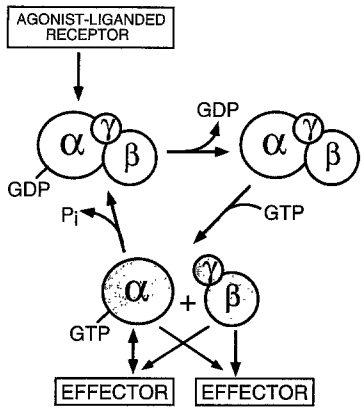


Figure 1. The Regulatory Cycle of Heterotrimeric G Proteins

See text for a description of the cycle. Open circles represent inactive states of the subunits; stippled circles represent active forms.

both α and βγ subunits positively regulate effectors (Logothetis et al., 1987). The initially surprising result with the $K^+$ channel was confirmed by other laboratories and, recently, in studies using recombinant βγ subunits and ion channels (reviewed by Clapham and Neer, 1993; Reuveny et al., 1994; Wickman et al., 1994). Subsequently, the βγ subunit was shown to be a positive regulator of a large number of effectors in addition to the $K^+$ channel, including adenylyl cyclase, phospholipase Cβ (PLCβ), phospholipase $A_2$ ($PLA_2$), phosphoinositide 3-kinase (PI3-kinase), and β-adrenergic receptor kinase (reviewed by Clapham and Neer, 1993). The βγ subunit may also act through *ras* to activate mitogen-activated protein (MAP) kinase pathways (Crespo et al., 1994; Faure et al., 1994). It is now clear that many effectors are regulated both by α and by βγ subunits, although as Tang and Gilman (1991) elegantly showed, the pattern of regulation is extraordinarily specific to the effector subtype: one subtype of adenylyl cyclase is activated by α and unaffected by βγ, a second subtype is activated by α and synergistically activated further by βγ, and a third type is activated by α but inhibited by βγ subunits. PLC has a different pattern of regulation. PLCγ is not activated by G protein subunits, while PLCβ can be independently activated either by α or by βγ subunits (Smrcka and Sternweis, 1993, and references therein).

## Structure of α Subunits

Mammals have over 20 different G protein α subunits (16 gene products, some with alternatively spliced isoforms; reviewed by Kaziro et al., 1991; Simon et al., 1991). As shown in Table 1, the proteins can be divided into four major classes according to the similarity of their amino acid sequences that ranges from 56%–95% identity. With the exception of G proteins that are found in sensory organs (such as $\alpha_t$, $\alpha_{gust}$, or $\alpha_{olf}$) and a few types of α subunits that are predominantly expressed in hematopoietic cells ($\alpha_{16}$) or in neural cells ($\alpha_o$), most α subunits are widely expressed. Individual cells usually contain at least four or five types of α subunits (reviewed by Neer, 1994).

A new era in understanding the structural basis for α subunit function opened when the crystal structure of GTP- and GDP-liganded transducin and $\alpha_{i1}$ was solved (Noel et al., 1993; Lambright et al., 1994; Coleman et al., 1994). We now know exactly which residues contact the guanine nucleotide and how the molecule changes as it goes from the inactive to the active form. The α subunit consists of two domains: one, a GTPase domain that contains the guanine nucleotide-binding pocket as well as sites for binding receptors, effectors, and βγ and a helical domain whose function is not clear. It may position a key residue (Arg-178 in $\alpha_t$) needed for GTP hydrolysis and so help to set the GTPase activity (Conklin and Bourne, 1993). The helical domain may also contribute to the effector-binding site (Coleman et al., 1994), along with other regions on the GTPase domain (see below). The βγ subunits, effectors, and receptors seem to bind to different surfaces of α subunits. Figure 2 shows a diagram of the α subunit as it might be viewed by the βγ subunit. The reader is looking at one face of the GTPase domain; the helical domain is behind the GTPase domain and hidden by it. The first 25 amino acids of the α subunit are essential for βγ binding (Fung and Nash, 1983; Denker et al., 1992a), but their position is unknown because they are mobile and do not show in the crystal (Coleman et al., 1994). The βγ-binding surface probably also includes the $\alpha_2$ helix because a cysteine on this helix (Cys-215 in $\alpha_o$) can be chemically cross-linked to βγ (Thomas et al., 1993a). Since binding to βγ depends critically on the nucleotide bound to the α subunit, it makes sense that the βγ contact surface would include a region such as the $\alpha_2$ helix that is different in the GDP- and GTP-liganded states (Lambright et al., 1994).

Table 1. Classes of $G_\alpha$ Subunits[a]

| Class | Members | Modifying Toxin | Some Functions |
|---|---|---|---|
| $\alpha_s$ | $\alpha_s$, $\alpha_{olf}$ | Cholera | Stimulate adenylyl cyclase, regulate $Ca^{2+}$ channels |
| $\alpha_i$ | $\alpha_{i-1}$, $\alpha_{i-2}$, $\alpha_{i-3}$, $\alpha_o$, $\alpha_{t-1}$, $\alpha_{t-2}$, $\alpha_{gust}$, $\alpha_z$ | Pertussis (except $\alpha_z$) | Inhibit adenylyl cyclase, regulate $K^+$ and $Ca^{2+}$ channels, activate cGMP phosphodiesterase |
| $\alpha_q$ | $\alpha_q$, $\alpha_{11}$, $\alpha_{14}$, $\alpha_{15}$, $\alpha_{16}$ | — | Activate PLC |
| $\alpha_{12}$ | $\alpha_{12}$, $\alpha_{13}$ | — | Regulate $Na^+/K^+$ exchange[b] |

[a] See Gilman, 1987; Simon et al., 1991; Clapham and Neer, 1993; Neer, 1994 for references.
[b] Voyno-Yasenetskaya et al., 1994.

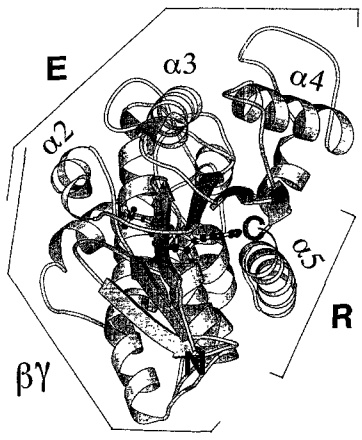


Figure 2. Structure of the α Subunit of Transducin

A diagram of the α subunit of transducin drawn from coordinates of Noel et al. (1993). The first 25 residues at the N-terminus (N) and the last 6 residues at the C-terminus (C) are not shown. The faces of the molecule that interact with receptors (R), effectors (E), and βγ subunits and the α helices discussed in the text are indicated.

The effector-binding region has been mapped only for the pairs $\alpha_s$/adenylyl cyclase and $\alpha_i$/cGMP phosphodiesterase (reviewed by Conklin and Bourne, 1993). The effector-binding region of $\alpha_s$ includes the $\alpha_2$ helix (see Figure 2) and partially overlaps the putative βγ-binding surface. Therefore, it is unlikely that the α subunit can simultaneously bind effector and βγ. Further, when an effector, such as type II adenylyl cyclase, is activated both by α and by βγ, it is likely that α and βγ bind to distinct sites on the enzyme.

The extreme C-terminus and parts of the $\alpha_5$ helix are important sites of interaction with receptors (reviewed by Conklin and Bourne, 1993; Neer, 1994). An activated receptor triggers the intracellular responses by dramatically decreasing the affinity of the α subunit for GDP, perhaps by moving or twisting the C-terminal α helix to loosen the grip of the α subunit on GDP. This effect is mimicked by deletion of 14 amino acids from the C-terminus of $\alpha_o$ (Denker et al., 1992b). Motion of the $\alpha_5$ helix would be transmitted to the loop at its N-terminal end. Mutations of amino acids in this loop also decrease GDP affinity (Thomas et al., 1993a; Iiri et al., 1994).

The C-terminus has an important role in defining the specificity of G protein receptor interactions, at least for some G proteins. Conklin et al. (1993) replaced three amino acids at the C-terminus of $\alpha_q$ with four amino acids normally found in $\alpha_{i\text{-}2}$, allowing $\alpha_q$ to couple to receptors that normally interact only with $\alpha_{i\text{-}2}$. While these residues are important, they are clearly not the only determinants of specificity. For example, several naturally occurring α subunits are identical at the extreme C-terminus but, nevertheless, interact with different receptors (Cerione et al., 1986; Kleuss et al., 1991). The $\alpha_{16}$ subunit interacts with the C5a receptor, while the $\alpha_{11}$ subunit does not. Chimeras of $\alpha_{11}/\alpha_{16}$ with a large portion of the $\alpha_{16}$ C-terminus did not function like $\alpha_{11}$ unless an additional region of $\alpha_{16}$ (residues 220–240) was present (Lee et al., 1995). Thus, the relative importance of the C-terminus of α subunits for receptor specificity seems to differ from one α subtype to another. Equivalent mutations of the GTP-binding site in different α subunits cause very similar phenotypes because the GTP-binding site is their most conserved part. However, as shown in the above example, the uniformity may not extend to changes that affect protein–protein interactions or overall conformation. Comparing the consequences of equivalent changes in different α subunits may reveal regions that specify their unique functions.

### Structure of βγ Subunits

The five known mammalian β subunits are between 53% and 90% identical to each other (reviewed by Simon et al., 1991; Watson et al., 1994). In contrast, the six γ subunits are much more different from each other than are the β subunits or the α subunits (Cali et al., 1992). Five different β subunits and at least six γ subunits could produce 30 different combinations. In fact, not all the possible pairs can form. For example, the $\beta_1$ subunit is able to interact with $\gamma_1$ and $\gamma_2$, but the very similar $\beta_2$ molecule is able to form a dimer only with $\gamma_2$ and not with $\gamma_1$ (Schmidt et al., 1992; Pronin and Gautam, 1992). So far, there has been no difference in the ability of reconstituted βγ pairs to activate effectors or interact with α subunits, except for $\beta_1\gamma_1$, which is sometimes much less effective (for example, see Clapham and Neer, 1993; Wickman et al., 1994; Smrcka and Sternweis, 1993; Cerione et al., 1987). However, $\beta_1\gamma_1$ is only found in the retina, so this selectivity does not help answer questions about specific βγ function in other cells.

The β subunit is predicted to contain two types of structures: an N-terminal region thought to form an amphipathic α helix such as might form coiled-coils (Lupas et al., 1992), followed by seven repeating units of approximately 43 amino acids each (Simon et al., 1991). The repeating units in β are examples of a class of repeating sequences (WD repeats) found in a family of proteins engaged not only in signal transduction, but also in control of cell division, transcription, processing of pre-mRNA, cytoskeletal assembly, and vesicle fusion. The WD repeat, diagrammed below, consists of a conserved core of 23–41 residues usually bounded by Gly–His and Trp–Asp (Neer et al., 1994). The conserved cores are separated by variable regions that are rather short in the β subunits (7–11 amino acids) and probably form loops. There is no consensus in the variable region that fits most WD repeat proteins, but some of these regions are highly conserved within the β subunit subfamily.

$$\{X_{6\text{-}94}\text{----------}[GH\text{----}X_{23\text{-}41}\text{--}WD]\}^{N_{4\text{-}8}}$$

Variable length loop — Constant length core

A repeating structure can be made up of units that are functionally interchangeable or specialized. If each repeating unit has a specialized function and if that specialized function is conserved over long evolutionary periods, its structure might also be conserved. The repeating units of β subunits can be identified by their position in the sequence (unit 1, unit 2, etc.). If repeating units are differenti-

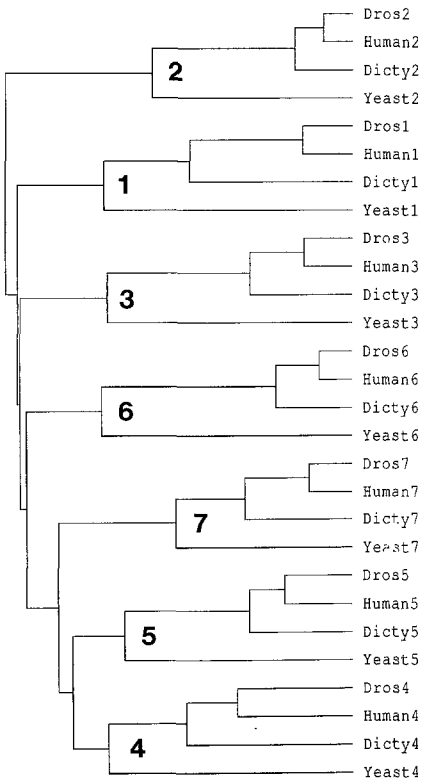


Figure 3. Relationship of WD Repeats in β Subunits from Widely Separated Species

The WD repeats from β subunits from human, Drosophila melanogaster, Dictyostelium discoideum, and Saccharomyces cerevesiae were numbered one to seven from N-terminus to C-terminus, divided into separate files, and compared using the Pileup program of the University of Wisconsin GCG Software package. In a more stringent analysis, we showed the cores of repeating units at equivalent positions in β subunits clustered together even when compared with the approximately 300 other WD repeat sequences (Neer et al., 1994).

ated, then a unit at any position in β might be more like a repeating unit at the same position in a β from a very distant organism than it is like neighboring units in the same polypeptide. We tested this hypothesis by analysis of β sequences from eight species, including Dictyostelium and human, that diverged 1000–1200 million years ago (Neer et al., 1994). Figure 3 shows that repeating units at equivalent positions cluster together, even though they come from widely separated organisms. For example, the first repeating unit from human β is more like the first repeating unit from Dictyostelium β than it is like any other repeating unit in the human β protein (see also Figure 1, Neer et al., 1994). The βγ subunit is known to interact directly with at least seven kinds of proteins (α subunits, receptors, adenylyl cyclase, PLCβ, βARK, calmodulin, and phosducin) and probably also interacts directly with $PLA_2$, $K^+$ channels, and PI3-kinase (Clapham and Neer, 1993). These proteins have no obvious common βγ-binding sequence motif. The number of different interacting proteins suggests that no single repeating unit specifies a partner and that each partner protein interacts with more than one repeating unit. Specific subsets of repeating units may define the binding surface for different effectors.

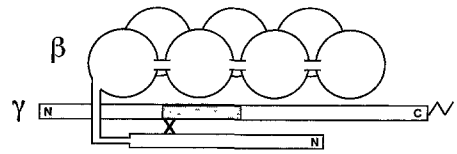


Figure 4. Model of the βγ Subunit

The cores of the WD repeats are represented by circles connected by the variable regions. Each core is predicted to be a structure made up of a β strand–turn–β strand–turn–β strand. The putative α-helical region in the N-terminus of β is shown as a rectangle. The area of γ that determines the specificity of interaction with $\beta_1$ or $\beta_2$ is stippled (Spring and Neer, 1994). The X represents the site of the cross-link introduced by Bubis and Khorana (1990). The C-terminal prenyl group is indicated by a zigzag line.

Figure 4 presents a diagram of the βγ subunit intended only to serve as a framework to organize what is known about βγ structure and to suggest testable hypotheses. The cores of the repeating units are represented by circles connected by the variable regions. The variable regions are long enough that the cores could pack in many ways other than what is shown in the diagram. At present, nothing is known about their packing. Each core is predicted to be a structure made up of a β strand–turn–β strand–turn–β strand (Neer et al., 1994). By hydrodynamic and chemical analysis, the βγ subunit is a globular structure with very tight interactions among its parts. It stays together after cleavage with trypsin between repeats 2 and 3, although it has no disulfides (Huff et al., 1985; Thomas et al., 1993b). This observation is consistent with the predicted structure of the conserved cores, whose small β strands may be stabilized by interactions with β strands in other cores.

The β and γ subunits bind very tightly to each other and can only be separated by denaturants. The putative α helix at the N-terminus of β makes up part, but not all, of the βγ interaction site (Lupas et al., 1992; Garritsen and Simonds, 1994). The N-terminal portion of β must lie close to γ because it contains a cysteine that can be cross-linked to a cysteine in γ (Bubis and Khorana, 1990). However, selectivity (the ability of $\beta_1$ to dimerize with $\gamma_1$ but not $\gamma_2$) is determined by multiple sites in the WD repeat region, especially residues 215–255 in repeat 5 (Pronin and Gautam, 1992; Garritsen and Simonds, 1994; Katz and Simon, 1995).

The γ subunit is predicted to be largely α helical (Lupas et al., 1992). In Figure 4, γ is shown extended along the repeating units of β and held in place by N-terminal α helix of the β subunit. The prenyl group at the C-terminus of γ is likely to be on the outer surface of βγ because it is essential for membrane attachment (reviewed by Casey, 1994). Selectivity of the γ subunits for different β subunits is determined by a stretch of 14 amino acids in the middle of the γ subunit (Spring and Neer, 1994). This 14 amino acid region contains the cysteine that was cross-linked to β (Bubis and Khorana, 1990). Because the specificity region of γ is in the middle of the γ molecule, βγ could be oriented C-terminus to N-terminus (as shown) or N-terminus to N-terminus.

The α subunit seems to be able to interact with γ as well

as β (Rahmatullah and Robishaw, 1994), so perhaps the surface of βγ that binds α is at the bottom of the diagram. Binding of a GDP-liganded α subunit to βγ blocks the ability of βγ to stimulate effectors (for example, Logothetis et al., 1987), either because $\alpha_{GDP}$ induces a conformational change in βγ, or because it covers the site of βγ–effector interaction. The relationship of the $\alpha_{GDP}$-binding site on βγ to the effector-binding site must be defined before this issue can be resolved.

In what way are the functions of βγ like those of other WD repeat proteins? Many WD repeat proteins help to assemble macromolecular complexes (Neer et al., 1994). There are two cases in which βγ performs such a role. First, it facilitates association of α with membrane receptors to form the ternary complex of receptor–αβγ that is poised to bind ligands with high affinity (reviewed by Neer, 1994). Second, it facilitates the formation of a complex that includes a receptor and a specific receptor kinase that phosphorylates the liganded receptor (Pitcher et al., 1992). The βγ subunit binds to such a kinase, the β-adrenergic receptor kinase (βARK), through a region that contains sequences homologous to the platelet protein pleckstrin (Touhara et al., 1994). Pleckstrin homology (PH) domains in other proteins, including the GTPase-activating proteins for *ras*, spectrin, and PLCγ, also bind βγ, albeit much more weakly than βARK (Touhara et al., 1994). The great variation in βγ binding affinity among different PH domains suggests that not all of them are designed to bind βγ. A plausible hypothesis is that different PH domains interact with different members of the WD repeat protein family to form multiprotein assemblies.

## Control Points for Transmembrane Signals

### Receptor–G Protein–Effector Interfaces

Cellular responses to external stimuli are sometimes very selective. One example is the heart, which responds accurately to opposing signals. Stimulation of its β-adrenergic receptor increases the rate and force of contraction, while stimulation of muscarinic cholinergic receptors decreases the rate and force of contraction. Each of these receptors is coupled to a different G protein: the β-adrenergic receptor interacts with $G_s$, while the muscarinic receptor interacts with the $G_i$ class of G proteins ($G_i$ and $G_o$) and with $G_q$. In the intact cell, there seems to be no cross–talk between these pathways. Another example is the pituitary-derived cell line $GH_3$, in which somatostatin and muscarinic receptors both regulate $Ca^{2+}$ channels, but each uses a different alternatively spliced form of $\alpha_o$ and different βγ subunits (Kleuss et al., 1991, 1992, 1993).

The simplest way to encode specificity would be for each kind of receptor to interact with a single kind of G protein. However, there are more kinds of receptors than G proteins. $G_s$, for example, can be activated by 30 or more receptors. Conversely, individual receptors can activate more than one G protein (see below). The intrinsic ability of receptors to activate G proteins can be tested with purified receptors and G proteins reconstituted in lipid vesicles. When receptors, G proteins, and effectors were first purified, the optimistic hope was that reconstitution experiments would answer all questions about the specificity of the signal transduction mechanisms. Reality proved to be more complicated because the experiments revealed an unexpected lack of specificity (Asano et al., 1984; Cerione et al., 1985). In a reconstituted phospholipid vesicle, the β-adrenergic receptor could activate both $G_s$ and $G_i$, although it activated $G_s$ 2- or 3-fold better than $G_i$. But in a cardiac cell, the concentration of $G_i$ is greater than $G_s$. Furthermore, βγ liberated from either $G_s$ or $G_i$ should activate the $K^+$ channel. It is still not clear why the β-adrenergic receptor only activates adenylyl cyclase and not the $K^+$ channel.

Other reconstituted receptors may be able to discriminate one class of α subunits from another (for example, $\alpha_s$ from $\alpha_i$) but select much less well among isoforms within a class (Senogles et al., 1990; Cerione et al., 1986; Munshi et al., 1991, and others). The cytoplasmic regions of G protein–coupled receptors determine G protein selectivity. Specificity for G proteins depends not only on the presence of a correct G protein recognition sequence, but also on its proper control by other cytoplasmic regions. The selectivity of a receptor can be greatly diminished by altering cytoplasmic regions outside of the G protein recognition sequence (Wong and Ross, 1994).

The specificity of some cellular responses to hormones is striking but is not universal. There are several examples of receptors that interact with more than one G protein even in intact cells, and thus initiate more than one signaling pathway (for example, Abou-Samra et al., 1992; Gudermann et al., 1992; Allgeier et al., 1994; Chabre et al., 1994). For example, the parathyroid hormone receptor transfected into COS7 cells couples to two G proteins of different classes to activate adenylyl cyclase and PLC, while the $\beta_2$-adrenergic receptor transfected into the same cell type only activates adenylyl cyclase (Abou-Samra et al., 1992).

The pattern of cellular responses can be only partly predicted from the properties of receptors in phospholipid vesicles. Effectors discriminate better among G protein α subunits than do receptors: only $\alpha_s$ activates adenylyl cyclase and only $\alpha_q/\alpha_{11}$ activate PLCβ. In contrast, many kinds of βγ subunits activate effectors equally. But, if a liganded receptor can activate several kinds of G proteins, the response would spread over several effectors, even if G proteins were entirely specific for effectors. Thus, strict specificity at the G protein–effector interface cannot undo the spread of the signal that begins at the receptor–G protein interface. Clearly, other factors, besides the "lock and key" fit of receptors and G proteins, must play an important role in shaping the final response of the cell to any external stimulus. A few of these are discussed below.

### Kinetics

Different α subunits have different intrinsic rates of GTP hydrolysis (Carty et al., 1990; Linder et al., 1990). If a receptor activates two α subunits that have very different activated half-lives, one signal will be quickly extinguished while another could be sustained and predominant. These intrinsic differences may be modulated by intracellular proteins including some effectors. The reciprocal regulation

of α subunits and effectors is potentially an important element in specificity. Two effectors (PLC and retinal cGMP phosphodiesterase) have been shown to increase the GTPase activity of their activating G proteins (Arshavsky and Bownds, 1992; Berstein et al., 1992). Thus, they hasten the deactivation of the α subunit and limit the time of their own active state. An effector may strongly affect the rate of GTP hydrolysis by one G protein, but not by another. The lifetime of effector activation by the former would be shortened more than the lifetime of the latter. Interaction with other proteins might modulate the ability of a particular effector to act as a GTPase-activating protein. Possibly, interaction of the effector with the βγ subunit might have this effect.

### Stoichiometry

One cell is distinguished from another by the particular complement of receptors, α, β, and γ subunits, and effectors that it expresses, although the complement may change substantially with developmental or metabolic state. The precise stoichiometry among the signaling components can determine the predominant response pathway. When the porcine $M_2$ muscarinic receptor was transfected into CHO cells, the stimulation of phosphoinositol turnover was very dependent on receptor number, while inhibition of adenylyl cyclase was similar regardless of receptor number (Ashkenazi et al., 1987). The results suggest that in these cells, adenylyl cyclase inhibition is limited by enzyme or G protein, while PLC activation is limited by receptor number. Naturally occurring cells with few $M_2$ receptors might respond to acetylcholine with only a decrease in cAMP, whereas cells with more receptors would show both responses.

### Covalent Modification

#### *Phosphorylation*

Several types of α subunits ($\alpha_{i\text{-}2}$, $\alpha_z$) can be phosphorylated on serine or threonine residues in vivo and in vitro, and several types of α subunits can be phosphorylated in vitro on tyrosine residues by $pp60^{c\text{-}src}$ or by insulin receptor (for example, Daniel-Issakani et al., 1989; Lounsbury et al., 1991; Hausdorff et al., 1992). Changes in activity upon phosphorylation have been modest at best. None of the studies of phosphorylated G proteins have so far tested the idea that phosphorylation affects the specificity of its interaction with receptors or effectors. In many cases, phosphorylation does not change the activity of the protein, but rather changes its localization or association with other proteins (Koch et al., 1991). Phosphorylation may have an important role in assembling macromolecular regulatory complexes in this system, as it does in others.

The βγ subunit can act as a direct activator of effectors, but the tantalizing possibility of an additional mode of regulation was raised by the finding that βγ can be phosphorylated on histidine residues in membranes from human leukemia cells (Wieland et al., 1993). The phosphate comes from GTP and can be transferred back to GDP, perhaps locally regenerating GTP. Recently, Maeda et al. (1994) showed that phosphate transferred from histidine in a yeast osmolarity sensor to a regulatory protein inhibits the ability of the regulator to activate enzymes in the MAP kinase pathway. Is there any link between this regulatory mechanism and the regulation of other MAP kinase pathways by βγ? The analogy cannot be exact because β does not have the characteristic sequence of bacterial and yeast histidine kinases, nor has the phosphate from β been shown to transfer to a protein aspartate carboxyl group. Nevertheless, the histidine phosphorylation of β may be the first inkling of a new regulatory role for βγ.

#### *Modification by Lipids*

Some α subunits are myristoylated at the N-terminal glycine ($\alpha_o$, $\alpha_i$, $\alpha_z$); others are not ($\alpha_s$, $\alpha_q$). Myristoylation is necessary for membrane attachment and facilitates binding of βγ (reviewed by Casey, 1994). However, myristoylation is an irreversible covalent modification and apparently does not serve a regulatory role. In addition, some α subunits are palmitoylated at Cys-3. In contrast with myristoylation, palmitoylation is reversible. Activation of the β-adrenergic receptor leads to rapid depalmitoylation of $\alpha_s$, and depalmitoylated $\alpha_s$ does not activate adenylyl cyclase. Depalmitoylation might be a mechanism to turn off $\alpha_s$ and so to desensitize the cell to β-adrenergic stimulation. Control of palmitoylation might also be a mechanism to control the pathway that is activated. A receptor that interacts with two G proteins might cause depalmitoylation of one α subunit with different kinetics or potency than another, thus tilting the response toward the latter (reviewed by Casey, 1994).

The γ subunits differ from each other in the prenyl group modifying the C-terminal cysteine (reviewed by Casey, 1994) and perhaps in other, as yet unidentified lipids (Wilcox et al., 1994). Prenylation of γ is not necessary for βγ formation, but is necessary for membrane attachment of the βγ dimer and, in some cases, for association with the α subunit (reviewed by Casey, 1994). It may influence the specificity of βγ–receptor interactions (Kisselev et al., 1994).

### Accessory Proteins

A number of intracellular proteins modulate the function of G proteins. For example, GAP43 or neuromodulin is a growth cone–associated protein that enhances GTPγS binding to the $G_o$ subunit by a mechanism that appears to be different from hormone receptors (Strittmatter et al., 1990). The interaction of GAP43 with $G_o$ suggests that the transmembrane signaling system in the growth cone could be modulated by an intracellular protein, but the physiological significance of the interaction is, as yet, not understood. Phosducin is a retinal protein that binds βγ. It is able to inhibit βγ function in vitro and may serve a similar role in vivo (Lee et al., 1992). Calmodulin is another protein that binds βγ and may affect βγ regulation of adenylyl cyclase (Katada et al., 1987).

### Coincidence Detection

A coincidence detector is a device that responds briskly to the simultaneous presence of two signals, but poorly or not at all to either alone. When a single effector molecule is regulated by more than one G protein subunit, the effector can act as a coincidence detector to integrate and to

modulate the relative impact of different receptors (Bourne and Nicoll, 1993). Type II adenylyl cyclase is activated a little by $\alpha_s$ but synergistically by $\beta\gamma$. Full activation depends on the coincident presence of activated $\alpha_s$ and excess $\beta\gamma$ (Tang and Gilman, 1991). In brain slices, stimulation of the β-adrenergic receptor by isoproterenol increases cAMP. The response is greatly enhanced by agents (such as α-adrenergic agonists) that, by themselves, do not affect the synthesis or breakdown of cAMP but that do release $\beta\gamma$. The $\beta\gamma$ may activate adenylate cyclase primed by $\alpha_s$ activated through the β-adrenergic receptor. Federman et al. (1992) showed that such a mechanism could operate in transfected cells. In principle, a neuron might contain both type II adenylyl cyclase that is regulated by $\alpha_s$ and $\beta\gamma$ and another effector also stimulated by the β-adrenergic receptor but regulated by one subunit only. The latter might give rise to the predominant response without coincident input from a second receptor type, but be relatively less important when multiple signals converge.

### Compartmentation of Signaling Proteins

In intact cells, some sets of receptors, G proteins, and effectors may be organized into microdomains and not have access to other sets. This organization is clearly missing from phospholipid vesicles and even from isolated membranes. Making membranes from cells increases the mobility of membrane proteins by more than an order of magnitude (Beth et al., 1986) and may disrupt microdomains. Even transfected cells may not faithfully mirror the organization of wild-type cells because overexpression may saturate normal compartments and put signaling components into abnormal places.

Functional studies have revealed intracellular pools of second messengers and have long argued for compartmentation of signaling proteins. Separate pools of cAMP seem to exist in testicular Leydig cells (Dufau et al., 1978) and in cardiac myocytes (Buxton and Brunton, 1983; Xiao et al., 1994). Different receptors generate different spatial redistribution of $Ca^{2+}$ in the same cell (Lechleiter et al., 1991). Pools of second messengers probably reflect the spatial organization of the enzymes and channels that generated them. Lack of competition for G proteins by different receptors, even when the amount of G protein was made limiting, also argues for compartmentation. Such competition might be expected if all the components were freely mobile (Graeser and Neubig, 1993).

There are several examples of cells, especially polarized epithelial cells, with asymmetrically distributed receptors or G proteins (most recently, von Zastrow et al., 1993; Keefer et al., 1994; see also review by Neer, 1994). In some cells, G proteins are found in specialized membrane regions called caveoli that mediate transcytosis and uptake of small molecules (Sargiacomo et al., 1993). In neurites and PC12 cells, the $G_o$ protein is highly concentrated in the growth cones (Strittmatter et al., 1990).

The $\beta\gamma$ subunits have been shown to fractionate with the cytoskeleton (Carlson et al., 1986). Recently, Hansen et al. (1994) showed that $\gamma_5$ is localized in focal adhesions of neonatal cardiac fibroblasts and other cells and colocalized with vinculin, extending a short way out along the vinculin-associated stress fiber. It is not known yet whether a subset of β subunits are also present in the focal adhesions, whether the $\gamma_5$ subunits are specific for a signaling pathway, nor whether the association with cytoskeletal elements is through γ or its associated β.

The localization of signaling proteins to certain cellular surfaces or regions supports the idea that stable assemblies of signaling molecules may exist and be important—but these areas are very large and still allow a large range of motion. There is little direct evidence for microdomains or multiprotein assemblies, although in some cases, neutron target size analysis has suggested that the basal state of a signal transduction complex may be an entity with a mass of millions of daltons (Schlegel et al., 1979). Further, Jahangeer and Rodbell (1993) solubilized large complexes containing G proteins and suggested these represented multimeric G protein structures that might disassemble upon activation.

### Conclusion

For a cell to understand external messages requires a grammar that orders the incessant flow of signals. The words of the cellular language (the receptors, the G proteins, and the effectors) each have a structure and an allowed usage that varies according to the context. The challenge is to define the rules of this grammar: how much is innate, common to all cells, and encoded in the structures of the elements, and how much is acquired and modified as cells develop and specialize. Understanding the language is an essential first step toward being able to correct the inappropriate responses to external signals that contribute to abnormal cell function.

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