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COMMENTARY

Role of Subunit Diversity in Signaling by Heterotrimeric G Proteins

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ABSTRACT. The heterotrimeric G proteins are extensively involved in the regulation of cells by extracellular signals. The receptors that control them are often the targets of drugs. There are many isoforms of each of the three subunits that make up these proteins. Thus far, genes for at least sixteen α subunits, five β subunits, and eleven γ subunits have been identified. In addition, some of these proteins have splice variants or are differentially modified. Based upon what is already known, there are well over a thousand possible G protein heterotrimer combinations. The role of subunit diversity in heterotrimer formation and its effect on signaling by G proteins are still not well understood. However, many current lines of research are leading toward an understanding of these roles. The functional significance of subunit heterogeneity is related to the mechanisms used by G proteins to transmit and integrate the many signals coming into cells through this system. Described here are the basic mechanisms by which G proteins integrate cellular responses, the possible role of subunit heterogeneity in these mechanisms, and the evidence for and against their physiological significance. Recent studies suggest the likely possibility that subunit heterogeneity plays an important role in signaling by G proteins. This role has the potential to extend substantially the flexibility of G proteins in mediating cellular responses to extracellular signals. However, the details of this are yet to be worked out, and they are the subject of many different avenues of research. BIOCHEM PHARMACOL **54**;3:325–339, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. G protein; subunits; isoforms; receptor; cell signaling; physiological effects

G proteins are well understood as mediators of signaling by many extracellular compounds [1, 2]. By several estimates, G protein-coupled receptors are part of one of the most prevalent signaling systems used by mammalian cells [3–5]. The extensive role of these receptors in cell signaling suggests abundant mechanisms for controlling and integrating their function. For example, at the G protein level there are increasing numbers of accessory proteins modifying the incoming signal including GAP43 [6, 7], phosducin [8, 9], and, recently, RGS† proteins [10–15]. There are likely additional proteins as well [16, 17]. Importantly, however, the process of G protein activation and signaling leads directly to mechanisms for integrating signaling events within cells [18]. This article discusses current views of these mechanisms, both well established and yet to be proven, along with their physiological significance. Particular attention is paid to the emerging role of G protein β and γ subunit heterogeneity in these mechanisms.

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† *Abbreviations:* AC, adenylyl cyclase; βARK, β-adrenergic receptor kinase; MAP, mitogen-activated protein; PLC, phospholipase C; and RGS, regulator of G protein signaling.

CELLULAR SIGNALING THROUGH G PROTEINS

In the simplest possible case (Fig. 1A), a single receptor would couple to a single G protein, activating it, in turn, to regulate the function of a second messenger enzyme or ion channel. In fact, however, most cells probably contain many G protein-coupled receptors that choose between multiple G proteins to regulate a host of intracellular signaling processes. Even before the discovery of GTP-mediated signaling [19], it was clear that multiple receptors in a cell could converge on a single effector system, such as generation of cAMP by AC [20]. Such convergent receptor signaling (Fig. 1B), specifically through G proteins, was later revealed by the characterization of cyc$^-$ S49 cells that lack α_s along with AC responses to two different hormones [21]. Most recently, disruption of an α_i gene to produce an α_{i2} −/− mouse resulted in parallel decreases in receptor-mediated inhibition of AC for three different agonists in a single tissue [22]. Generally, most cells likely contain multiple receptors coupling to the same G protein subtype.

The isolation of G_s [23], G_i [24, 25], and G_t [26–29] established the G proteins as a family of signaling proteins regulating many different intracellular processes [30–32]. These same studies established that effectors such as AC can be regulated by more than one G protein and either positively or negatively. By 1991, sixteen α subunit genes

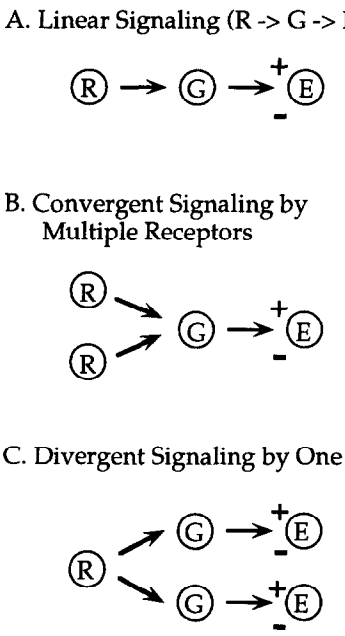

FIG. 1. Signaling mechanisms based upon receptor/G protein interactions.

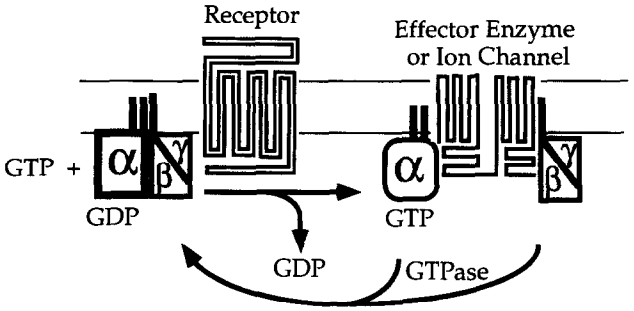

FIG. 2. Mechanism of G protein activation and signaling.

had been identified [33, 34]. It was also clear that receptors could activate more than one G protein subtype, leading to divergent signaling pathways in cells (Fig. 1C). This was first shown for the β-adrenergic receptor, which activated its suspected target, G_s, along with transducin, now called G_t, and even the apparently contradictory protein G_i [35]. The converse example has also been demonstrated whereby the α_2-adrenergic receptor activates both its suspected target, G_i, and G_s [36].

Offermanns *et al.* [37] have described a general assay for G protein activation based upon receptor-stimulated binding of [α-^{32}P]GTP azidoanilide and subsequent immunoprecipitation of labeled α subunits with isoform-specific antibodies. With this assay many receptors have a wide range of G protein specificity [38]. For example, the TSH receptor in human thyroid membranes activates G proteins containing some eleven different α subunits [39]. Other receptors, such as the thromboxane A_2, thrombin, TRH, and α_2-adrenergic receptors, may be nearly as promiscuous (reviewed in Ref. 38). Significantly, however, there are cases, using this assay, indicating receptors with more restricted G protein preferences, such as the LH [40] and histamine H_1 [41] receptors.

A challenging problem is to determine the physiological significance of promiscuity of receptor activation of G proteins. At first thought, this would seem inconsistent with regulation of specific cellular events. However, most signaling processes in cells are complexly regulated. Perhaps these processes respond to subtle integrated changes in several signaling systems simultaneously, leading in a complex way to specific cellular responses. As noted by others [38], for endogenous receptors it is difficult to rule out the presence of receptor isoforms as an explanation of receptor promiscuity. Conversely, expressed, recombinant receptors may behave differently in an artificial environment unlike that of their physiological site or level of expression. This is also true of reconstituted systems where receptors, purified or expressed, are recombined with purified G proteins. For example, muscarinic receptors in brain membranes couple poorly to G_o, but if the membranes are transiently treated with detergent, they interact strongly with G_o [42]. A major problem is to determine why some receptors are very promiscuous, while others are very specific in their interactions with G proteins. Both cases may be physiologically significant, but some of these observations may reflect the biases of the experimental approaches used to define them. Ultimately the issue of receptor isoforms in biological membranes must be dealt with and the repertoire of G proteins responding to receptors determined in a native, physiological environment by more than one experimental approach.

G PROTEIN ACTIVATION BY SUBUNIT DISSOCIATION

G protein activation has been studied extensively [43, 44] and requires a dual mechanism involving nucleotide exchange (GTP for GDP) and subunit dissociation (Fig. 2). This led to the realization (Fig. 3) that βγ from G_i could inhibit adenylyl cyclase by suppressing the activation of G_s [45, 46]. Inhibition of adenylyl cyclase turns out to be very complex. There are multiple mechanisms involved [47–49], and there are many AC isoforms with varying properties [50–53]. Even when proposed, βγ suppression of α_s was realized not to be the only mechanism of G_i action since inhibition of AC does not obligatorily require G_s [46, 54, 55]. Nevertheless, the importance of this idea was the realization that both α and βγ can convey information during signaling through the interaction of G proteins with one another. Furthermore, this mechanism remains a viable explanation for some instances of inhibition of AC, particularly for effects on basal activity [56], as opposed to hormone-stimulated activity [57]. Related phenomena have been observed for G_i activation of potassium channels [58] and for α_q regulation of PLCβ [59]. Thus, G protein interactions through exchange of their βγ dimers may have general significance. In particular, this mechanism allows

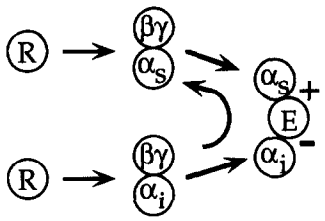

FIG. 3. Signaling mechanisms based upon subunit dissociation as a mechanism of G protein activation. This specific case uses the action of G_s and G_i to regulate the effector AC as an example.

one G protein to conditionally suppress spontaneous activation of another, depending upon its specific activation by a receptor [47].

DUAL SIGNALING BY α SUBUNITS AND βγ Dimers

Direct inhibition of calmodulin-stimulated AC by βγ dimers [60], βγ stimulation of phospholipase A_2 [61], and, significantly, βγ stimulation of potassium channels [62] demonstrated that both activated α-GTP and βγ dimers directly mediate downstream effects of G proteins (Fig. 4A). This is now extensively recognized [63–67]. In fact, the list of βγ targets continues to grow. For example, it is likely that βγ activation of the MAP kinase pathway [68–70] accounts for the observation of pertussis toxin-sensitive pathways regulating cell growth [71–73]. Such studies presaged the involvement of a host of G protein-related mechanisms in the positive regulation of cell growth [74–76], leading to the realization that G protein and tyrosine kinase pathways are intertwined in the combined regulation of many phenomena [77, 78]. The mechanism of this effect is still not clear [76, 78]. It could involve βγ regulation of serine/threonine (Raf) kinases [79], tyrosine kinases such as Tsk/Itk and Btk [80], or some unidentified tyrosine kinase [81, 82], perhaps a member of the Src family of nonreceptor kinases [83]. Recently, it was suggested that such a kinase could be downstream of βγ activation of PI3 kinase-γ [84]. Likely, the complexity of cell growth control allows for multiple roles of βγ.

Recently, several types of calcium channels, potentially targets of Gα subunits, were shown to be regulated by βγ dimers [85]. These include N-type [86, 87] and P/Q-type [86] channels involved in neurotransmitter release. Direct interaction of several different calcium channel α subunits with G protein βγ dimers mediates these effects [88, 89]. This is significant both for this new role of βγ and because, once again, α_o seems to be left without a definitive downstream target. Calcium channels are quite diverse [90], and so some may yet turn out to be direct targets of α_o. For example, α_o copurifies with ω-conotoxin-sensitive calcium channels [91]. Nevertheless, regulation by βγ explains why many receptors, through multiple G protein pathways, regulate several different calcium currents (reviewed in Ref. 92). Some other potential roles of α_o may also be mediated

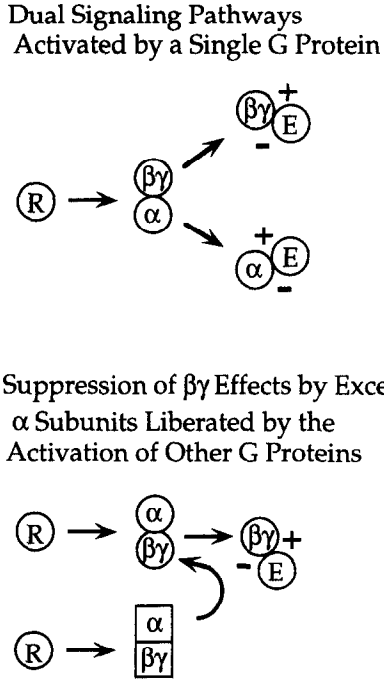

FIG. 4. Signaling mechanisms based upon effects of both activated α subunits and free βγ dimers.

by βγ [93]. $G_o\alpha$ can weakly inhibit AC1 [94], but it is difficult to believe that this accounts for its extremely high level in brain, 1–2% of particulate protein [95, 96]. A specific target(s) for α_o is strongly suggested by the positive effects of constitutively active α_o mutants expressed in either oocytes [97] or NIH-3T3 cells [98], but what this target might be is unclear. One class of viable targets for $G_o\alpha$ are neuronal potassium channels [99]. An additional or alternative hypothesis is that α_o participates in vesicular trafficking [100].

Possibly, a major role of G_o is similar to that of the G proteins in the yeast pheromone response pathway [101, 102]. There the major downstream regulator is βγ rather than α. Since βγ does not change conformation upon binding α [103, 104], its downstream actions appear dependent upon α activation and subsequent dissociation. In other words, a major role of α_o may be as an inhibitor of βγ effects (Fig. 4B). This is the reverse of the pathway suggested as a mechanism of inhibition of AC (Fig. 3). Experimentally, this effect has been demonstrated by α subunit suppression of βγ regulation of AC2 [105], PLCβ [106–108], GIRK [109–111], PI3 kinase-γ [112, 113], and calcium channels [87].

Even if targets for α_o exist, it is plausible that βγ dimer signaling can be suppressed by excess α subunits. This could apply to other α subunits too, but this is particularly germane to α_o because of its abundance in brain. This mechanism requires production of sufficient unactivated α subunits to force the equilibrium toward heterotrimer formation. For example, an excess of inactive αGDP could result, at least locally, from Gα GTPase activation by RGS

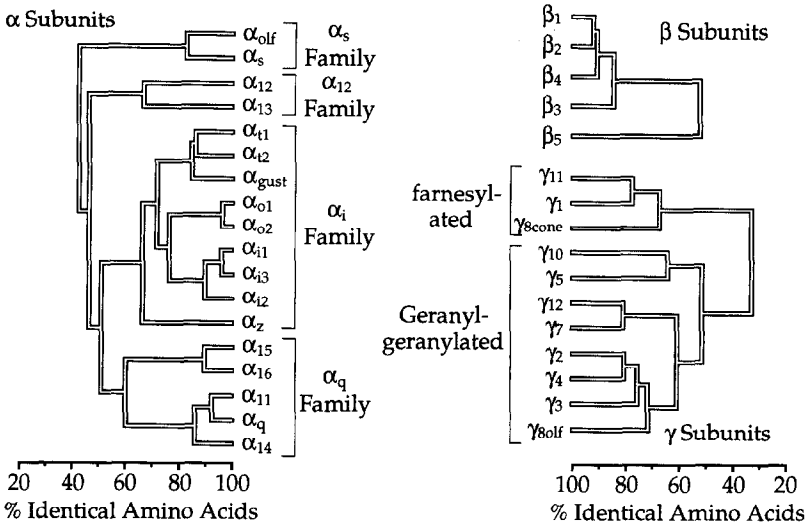

FIG. 5. Homology and diversity of G protein subunit isoforms. This figure is based upon modifications of previously published figures [33, 34, 63, 64, 117], with permission (see end of legend for copyright information). All isoforms are thought to be products of separate genes except α_{o1} and α_{o2}, which are splice variants of a single gene [118–123], and α_{15} (mouse) and α_{16} (human), which may be the same gene in different species [124]. Sequence alignments and tree diagram are based upon the algorithms in PILEUP [125, 126] in the Wisconsin GCG Programs [127]. The percent identities at any branch point are approximate, and the relationships shown are not meant to imply an evolutionary relationship. A different relationship for the α_{12} family, being evolutionarily closer to the α_q family, is found when apparent differences in the rate of mutation of different proteins are taken into account [128]. Sequences were human where available, and otherwise rodent or bovine. The GENEMBL accession numbers for the sequences used are (from the top): for α, P38405, P04895, P27600, P27601, P11488, P19087, P29348, P09471, P29777, P10824, P08754, P04899, P19086, P30678, P30679, P21278, P21279, and P30677; for β, P04901, P11016, P29387, P16520, and L34290; for γ, U31384, P02698, U20085, U31383, P30670, U37561, P30671, P16874, U31382, P29798, and L35921. This figure is a revised and updated version of figures previously published and reprinted with permission from: Ref. 33 [*Science* 252: 802–808, 1991. Copyright (1991) American Association for the Advancement of Science]; Ref. 34 [*Annual Review of Biochemistry* 60: 349–400, 1991. Copyright (1991) Annual Reviews Inc.]; Ref. 63 [*Cell* 71: 1069–1072, 1992. Copyright (1992) Cell Press]; Ref. 64 [*Trends in Cell Biology* 3: 230–236, 1993. Copyright (1993) Elsevier Science]; Ref. 117 [*Trends in Biochemical Sciences* 17: 383–387, 1992. Copyright (1992) Elsevier Science].

proteins, as suggested by Iyengar [14]. This could actually be one major function of such RGS proteins.

There may also be physiological conditions where α subunits predominate over βγ dimers, suppressing βγ signaling by heterotrimer formation. One such case may be during early mouse embryonic development, where there is a specific decline in βγ dimers compared to α_i as oocytes begin maturation [114]. Another condition where there is a selective decline in βγ levels is after pertussis toxin treatment of cells [115]. Although this is a pathologic condition, this too suggests that regulation of the ratio of α to βγ has functional consequences. It is perhaps significant that often in transient expression experiments increased α subunit expression is not compensated for by increased βγ levels, but is in stable transfections [116]. This suggests specific control over levels of α and βγ expression, and the possibility that their altered ratio has physiological consequences.

The role that α subunit suppression of βγ signaling plays in cell regulation requires further elucidation. Besides the requirement of a critical level of unactivated α subunits, this mechanism will depend upon the specificity of α subunits for different βγ dimers. It is still not clear whether α subunit isoforms prefer specific βγ dimers. Whether or not they do will greatly influence signaling specificity mediated through heterotrimeric G proteins.

G PROTEIN HETEROTRIMER SUBUNIT COMPOSITION AND HETEROGENEITY

Generally, G proteins are named for their α subunits. There are at least sixteen α subunit genes (Fig. 5). They can be grouped into four families represented by the α subunits of G_s, G_i, G_q, and G_{12} [128, 129]. There may be additional subfamilies of α subunits [130, 131], and at least three α subunits have multiple splice variants, including $G_s\alpha$ [132–135], $G_{i2}\alpha$ [136], and $G_o\alpha$ [118–123]. In addition to this substantial α subunit variability, there are five β subunit and eleven γ subunit genes, with a recently described splice variant of β_5 [137]. There may also be splice variants of some γ subunits [138]. Based upon known α, β, and γ subunit isoforms, there are over a thousand possible G protein heterotrimer combinations. Clearly some isoforms are found only in specialized cells, including α_{olf} [139], α_{t1} [140], α_{t2} [140], α_{gust} [141], γ_1 [142], γ_{8cone} [143], and γ_{8olf} [144]. In addition, not all βγ combinations form functional dimers [145–147]. Even accounting for these limitations, though, there are still hundreds of possible heterotrimers.

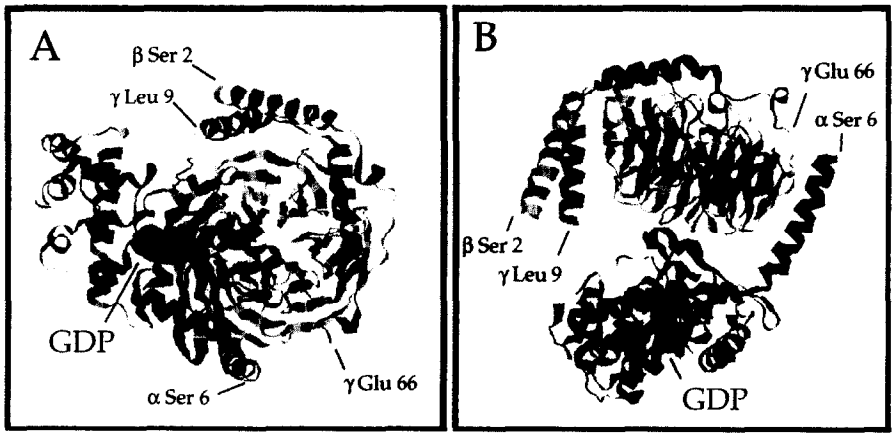

FIG. 6. Structure of the G_t heterotrimer. Shown are ribbon diagrams of G_t based upon the data of Lambright *et al.* [103]. (A) From the top of β looking down toward the α subunit. (B) From the side. The α subunit is in blue, the β subunit in yellow, and the γ subunit in green. The terminal residues definable in the X-ray structure and clearly evident in the figure are marked. Of these, the Glu 66 of γ and the Ser 6 of α are within 18 Å of each other. The actual termini would potentially be closer by additional amino acids and by terminal modifications of the proteins.

Although there are many possible subunit isoforms, any given cell might contain only a few of these, and thus only a few potential heterotrimers. Generally, this may not be true. Early characterization of the γ subunits compared those of bovine brain and human red blood cells (RBCs) [148]. That work was done prior to knowledge of the diversity of the γ subunits or their sequences. It used peptide mapping of radioiodinated peptides and found that the γ subunit(s) of human RBCs is similar to those of bovine brain. A not easily explained result at that time was the complexity of the γ subunit maps from a homogeneous tissue such as human RBCs, especially as compared with the simple maps of $G_t\gamma$. From the now known eleven γ subunit sequences, it is apparent that the method used would label a single peptide in all but one γ isoform. Thus, that data suggested that there are just as many γ subunit isoforms in human RBCs, a homogeneous cell population, as are found in bovine brain. The brain contains a very large number of different γ subunits (Cook LA, Wilcox M, Schey KL and Hildebrandt JD, unpublished observations). This suggests the possibility of extreme G protein heterotrimer variability even in a single cell.

Surprisingly, analyses of purified G proteins, based on their α subunits, indicate that even the generally expressed subunits do not form random heterotrimer combinations [149–151]. For example, different splice variants of a single isoform, α_{OA} versus α_{OB}, or isoforms thought to differ because of protein processing, α_{OA} versus α_{OC}, can have very different γ subunits associated with them [151]. Specific combinations of subunits could result from specificity of α and βγ subunit interactions, tissue specific expression of subunit isoforms, targeting of specific combinations of subunits within cells, or regulation of subunit composition by intracellular processes (see below). All of these may play some role in determining G protein subunit composition.

Generally, regardless of any observed specificity, individual α subunits associate with multiple β and γ isoforms [149–151]. In other words, there are not unique G protein heterotrimer combinations. Further, different α subunits, such as α_o, α_{i1}, and α_{i2}, can share many dimers with the same β and γ subunits [149]. This suggests access of α subunits to a common pool of βγ dimers, and could argue for exchange of subunits during activation and deactivation in the cell.

Gα and γ subunit isoforms, in particular, seem to be coupled somehow [145–147]. It is interesting therefore that in tissues with unique G proteins there are often both specialized α and γ isoforms. Biochemical evidence suggests direct binding of isolated γ subunits to α subunits [152, 153]. These interactions are sensitive to α subunit activation by aluminum fluoride [152] and are related to the highly variable N-terminal sequences of γ [153]. Involvement of the N-terminus of γ is also suggested by increased affinity for α subunits of βγ dimers containing γ_{12} phosphorylated at the N-terminus by protein kinase C [154].

The X-ray structures of intact heterotrimers [103, 155] identify the N-terminus of α and its Switch II region as sites on α that bind βγ. The switch regions are loops of α with conformations sensitive to guanine nucleotides. The α subunit appears to contact only β in the dimer, lying on the opposite face from γ (Fig. 6 A and B). The contacting amino acids of α and β are mostly conserved residues, and the highly variable N-terminus of γ is not even in close contact with α. This does not immediately suggest how α and γ subunits might specifically interact with one another. Further, recombinant βγ dimers with N-terminal hexahistidine tags interact with α subunits [156]. Although X-ray structures do not support specific association of α and γ subunits, crystals thus far analyzed are from proteins lacking the modifications at the C-terminus of γ and the N-terminus of α. Further, terminal residues in the subunits often do not form well-defined structures even when present.

It is not yet clear to what degree preferences of α subunits for specific βγ dimers determine or limit the number of possible heterotrimer combinations. Association of α subunits with specific γ subunits could support the idea of preferential associations, or this could reflect cellular processes involved in coordinating or targeting the expression of specific combinations of subunits. Whichever is the case, heterotrimer formation is not entirely random and presumably plays a role in signaling by G proteins. The importance

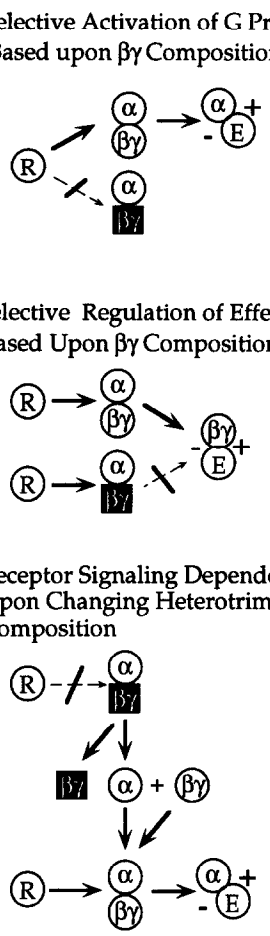

FIG. 7. Signaling mechanisms based upon G protein subunit heterogeneity.

of this depends upon the functional significance of subunit variability (Fig. 7).

RECEPTOR RECOGNITION OF SPECIFIC G PROTEIN HETEROTRIMER COMBINATIONS

One role of βγ is to facilitate receptor activation of G proteins. First shown by Fung for G_t activation by rhodopsin [157], in general, βγ is required for, or greatly facilitates, receptor coupling to G protein α subunits [158–165]. The interactions of βγ with rhodopsin, in the absence of α, have been monitored directly by fluorescence energy transfer [166], and isolated βγ blocks rhodopsin kinase phosphorylation of rhodopsin [159]. βγ associates with β-adrenergic receptors in liposomes or in nondenaturing gels [167, 168], and receptor peptides can be specifically cross-linked to βγ dimers [169]. Further, receptors can be co-immunoprecipitated with anti-β subunit antibodies [170]. These studies indicate a direct interaction between receptors and βγ dimers. Interestingly, antisera against βγ can contain antibodies recognizing rhodopsin, suggesting production of anti-idiotypic antibodies to specific receptor sites for βγ [171].

Plausibly, βγ participates in receptor/G protein interactions. Studies by Kleuss *et al.* [172–174] suggest association of specific heterotrimers with receptor regulation of calcium currents. In those studies, microinjection into GH3 cells of antisense for specific α, β, and γ subunit isoforms blocked somatostatin and carbachol inhibition of calcium currents [175]. Even though both compounds appeared to regulate the same target, they appeared to use entirely different heterotrimers: $\alpha_{o2}\beta_1\gamma_3$ for somatostatin, and $\alpha_{o1}\beta_3\gamma_4$ for carbachol (m_4 receptor). This same heterotrimer requirement was also found for somatostatin effects in RINm5F cells [176], and its specificity for α_{o2} was demonstrated by others in pituitary somatotrophs [177]. The specificity implied by these studies is surprising and highly significant. Subsequent studies extended these observations, perhaps with somewhat less specificity implied. Thus, the m_1-muscarinic receptor increased cytosolic calcium in RBL-2H3-hm1 cells via phosphatidylinositol turnover through G proteins containing either α_q or α_{11} in association with $\beta_1\gamma_4$ or $\beta_4\gamma_4$ dimers [178]. Curiously, antisense to either α_q or α_{11} essentially eliminated signaling, suggesting that both were simultaneously required. In addition, in two different cell types, GH3 cells and RINm5F cells, the galanin receptor inhibited a calcium current through G proteins deduced to be $\alpha_{o1}\beta_2\gamma_2$, as the major one, or $\alpha_{o1}\beta_3\gamma_4$, as a minor one [179].

It is unclear how the specificity of receptor G protein signaling implied by studies with antisense [175] relates to receptor/G protein promiscuity [38]. This is an extremely important point. Very different approaches were used in these two studies, perhaps leading to different conclusions. For example, microinjection studies rely upon both receptor/G protein and G protein/effector coupling. However, since neither the α nor βγ isoforms implicated in the original studies were similar [172–174], presumably the effector was not very selective, and the receptor itself required specific heterotrimers. Possibly these observations are receptor specific, and comparison of the same receptor by the two methods will give the same result. This seemed to be true for muscarinic receptors in RBL-2H3-hm1 cells [178]. Alternatively, signaling specificity may depend upon cellular integrity and the organization of signaling components within cells [175]. Such restrictive localization of G proteins in specific domains [180], perhaps in association with the cytoskeleton [181, 182], is widely recognized and could be reflected in the longstanding implications of precoupling of G proteins with effectors [183]. This could relate to α subunit or intact heterotrimer association with potassium channels [184] and calcium channels [92] thought to be regulated by βγ. Compartmentalization must not completely limit access to G proteins, however, since multiple receptors can regulate a single pool of AC [20].

Receptor specificity for βγ dimers may be only indirectly related to G protein subunit isoforms. Clearly, rhodopsin prefers G_t βγ to that from bovine brain [160]. Although the sequence of γ is important [185], major determinants of receptor interactions are the modifications of the subunits. Both Gα and Gγ contain varying patterns of protein

processing [186–191]. Many α subunits contain covalently attached myristate at the N-terminus, or palmitate on one or more Cys residues near the N-terminus [187–189]. The γ subunits are isoprenylated with either farnesyl or geranylgeranyl groups on a Cys four residues from the C-terminus of the coded protein. Interestingly, receptor activation causes turnover of the modifications of α [192–194] and γ [195–197], possibly changing the pattern of their modifications.

For βγ to interact with rhodopsin, its γ subunit must be isoprenylated [198], and farnesylation is more effective than geranylgeranylation [160, 199, 200]. Peptides homologous to the C-terminus of γ_1 have a similar specificity [185]. Adenosine A1 receptors also discriminate between βγ dimers containing γ_1 or γ_2, primarily due to their C-terminal modifications [162]. Gα subunit modifications may also be involved. The palmitoylation site of α_q is important for receptor coupling, although not clearly due to the modification itself [59]. N-terminal lipid could at least indirectly affect receptor coupling since myristoylation increases the affinity of α for βγ [201]. In addition, some α subunits contain variable N-terminal fatty acids [202–204], which may also be functionally significant [205].

The subunit composition of βγ dimers likely affects receptor specificity for G proteins. This could result from specificity in receptor/G protein interactions, the organization of signaling molecules in cells, or some combination of these two. Whether specificity of direct interactions resides in amino acid sequences of the subunits, or their associated lipid modifications, is not clear. One idea about lipid modifications of G proteins is that they provide a membrane anchor [5, 186]. Additionally or alternatively these modifications may provide sites of protein–protein interactions [206, 207]. Regardless, receptors may only be able to activate some heterotrimers containing specific α subunits, and based upon their βγ composition (Fig. 7A). The physiological significance of such phenomena is not yet proven (see below), but could account for restricted access of receptors for the pool of G proteins in cells [180].

EFFECTOR SPECIFICITY OF G PROTEIN βγ DIMERS

G protein βγ dimers regulate an increasing number of downstream effectors. The existence of five β and eleven γ subunit isoforms suggests, with some support [147, 208], specificity of βγ effects (Fig. 7B). However, this is yet to be definitively established. βγ dimers containing β_1 or β_2 are essentially equivalent for effects on AC1, AC2 and PLCβ$_3$ [209, 210]. There are more selective effects of βγ dimers on βARK, but mostly alternative combinations are more effective than $\beta_1\gamma_1$ [211]. Conversely, phosducin interacts more effectively with $\beta_1\gamma_1$ than with other dimers [211]. Nonspecific effector interaction of βγ dimers has been used to explain why many different α subunits, including those of G_o, G_{i1}, $G_{i2/3}$, G_q, G_{11}, and G_s, have been implicated to stimulate PLCβ (reviewed in Ref. 93). Generally, G_t βγ is different from other dimers, perhaps emphasizing its different prenyl group. As for receptor interactions, protein modifications of βγ are important for effector interactions, and prenylation of γ appears required for effector regulation [209, 212, 213].

Nonspecific βγ effects are perplexing, since all G protein dimers would be equivalent and regulate an ever increasing number of effectors. Sternweis [66] has discussed possible explanations for this, noting, in particular, that βγ effects often require higher concentrations than α effects. Maybe βγ provides a general readout for the cell of the total incoming information transmitted by G proteins. Alternatively, βγ may become "activated" just as α is activated by GTP. βγ dimers are phosphorylated at a novel histidine site [214–216], and this may be an activation step providing some kind of effector specificity. The fact that co-expression of βγ dimers with PLCβ$_2$ increases both basal and receptor-stimulated inositol triphosphate (IP_3) levels for many different receptors might support such a mechanism [217].

Recently, Yan and Gautam [218] suggested that βγ dimer effector specificity resides in their β subunits. This is surprising because most β subunits are similar, unlike the very diverse γ subunits. They suggested that previous lack of specificity resulted from focusing on different γ subunits in dimers containing β_1 and β_2. These β subunits are the most similar. In contrast, β_3, β_4, and β_5 differed from β_1 and β_2 in their interactions with fragments of AC2 and the GIRK1 potassium channel in the yeast two-hybrid screen [218]. The results of those studies showed unequivocal specificity for β interactions. These results are important and encouraging, although such artificial binding studies must be confirmed with intact functional proteins under native conditions. Subsequent studies suggested that different effectors interact with different determinants on the β subunit [219]. It was also shown recently that β_5 is more specific for stimulation of PLCβ than for MAP kinase [208]. This too is an important observation, but the regulation of these two events is very different. PLCβ is directly regulated by βγ, whereas MAP kinase appears to be many steps downstream from βγ effects.

Presumably, if βγ dimers have specific effects, this would be reflected somehow in the sites on the dimer that bind effectors. Chen *et al.* [220] identified a peptide homologous to a region of AC2 that blocks its stimulation by βγ (Fig. 8). This peptide, called QEHA, also blocks βγ effects on calmodulin-sensitive brain AC (presumably AC1), PLCβ$_3$, GIRK1, and βARK, although it does not block α binding to βγ [220]. These results could suggest a common site on βγ for effectors, although QEHA could also induce a dimer conformation incompatible with simultaneous interactions with effectors. βγ interaction with calcium channels at a sequence, QXXER, also found in QEHA, supports the idea that this is a βγ binding motif in effectors [88]. Molecular modeling of QEHA binding to β, based upon its cross-linking to specific sites on β, suggests that it binds the second blade of the β propeller, opposite the side to which

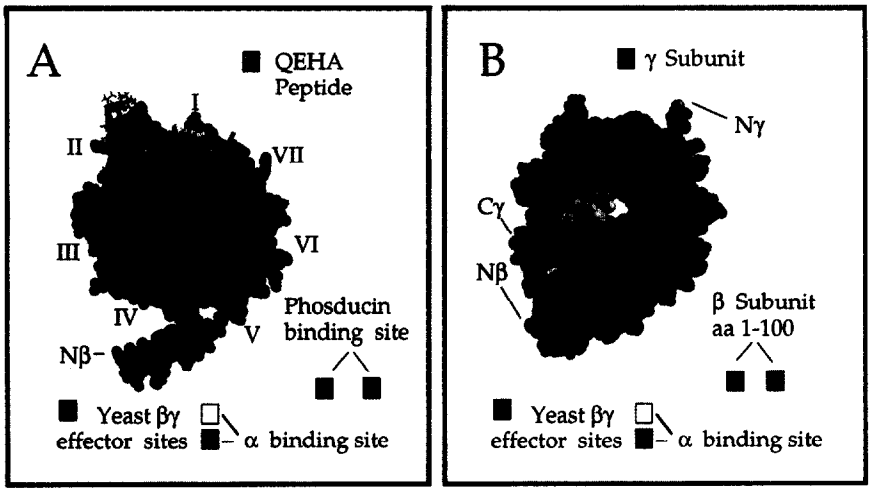

FIG. 8. Effector binding sites on the βγ dimer. Shown are space filling models of the β subunit complexed with (A) the QEHA peptide of AC2 as a stick figure and taken from the data of Weng *et al.* [221], and (B) the γ subunit, taken from the data of Sondek *et al.* [104]. The red dotted lines in (A) mark the approximate locations of the seven blades of the β propeller.

γ binds [221]. This site includes residues identified from dominant negative mutations of the yeast β that are thought to identify its effector binding region [222]. This region includes some important residues involved in β interactions with α, but by-and-large is adjacent to the α subunit binding site. Most residues in this contact region are conserved among β subunits, and would not suggest isoform specific interactions at this site.

There are other possible or additional regions of βγ implicated in effector interactions. Because βγ does not change conformation upon binding α [103, 104], the α binding site of βγ may be implicated in effector interactions, although, as noted [103], steric constraints could extend this region. The proposed QEHA binding site is compatible with this idea. The QEHA site is removed, however, from the phosducin C-terminal binding site (blades 6 and 7) [223], also thought to be the site of membrane association of β. Whether phosducin is analogous to an effector or not is uncertain, and the QEHA site is adjacent to the phosducin site that blocks α binding to βγ [223]. The proposed QEHA site is also adjacent to the region identified by Yan and Gautam [218] as binding QEHA based upon the yeast two-hybrid screen, the first 100 residues of β. Only a few residues at the end of this region overlap both sites. It is likely, though, that there are two regions of βγ binding to effectors [224–227]. One of these is the region identified by Weng *et al.* [221], particularly regions overlapping the original β fragments identified by Yan and Gautam [218]. The other is likely to be associated with the N-terminus of β and with neighboring sequences in γ. Dominant negative mutations of yeast identify N-terminal regions of both β [222] and γ [228] in effector interactions. Involvement of γ in effector interactions is consistent with its required posttranslational modification for effector interactions [209, 212, 213]. The N-terminal regions of γ or nearby residues of β could provide a likely basis for βγ effector specificity.

The data of Yan and Gautam [218, 219] and Zhang *et al.* [208] provide the first glimpse of the possibility that βγ dimers differentially regulate downstream effectors. In effect, βγ specificity for both receptor recognition of the heterotrimer (Fig. 7A) and for downstream effects of both the resulting activated α subunit and the βγ dimer (Fig. 7B) would go a long way to explaining the exquisite specificity suggested by the antisense studies of Kleuss *et al.* [172–174]. A challenge for the future will be to integrate any specificity of these events into the physiological mechanisms by which G proteins mediate signaling events in intact cells.

SUBUNIT EXCHANGE AS A MECHANISM OF G PROTEIN SIGNALING

G protein activation by GTP binding and subunit dissociation is supported by a large amount of biochemical, genetic, and phenomenological data [1], and still remains the most viable explanation for how these proteins work in cells. This mechanism has, in fact, withstood many challenges, and additional circumstantial data continue to support this idea. Most recently this would include the suggestion that βγ interactions with effectors requires subunit dissociation [103, 104]. Nevertheless, it was pointed out several years ago that the final proof of this mechanism is lacking, i.e. subunit dissociation has never been demonstrated in intact membranes or cells [229]. This is still true.

In the most extreme case, it is possible that all of the *in vitro* biochemical data supporting subunit dissociation result from a conformational change in the protein upon activation, which has physically different consequences *in vitro* compared to what actually happens in intact membranes or cells. Does this mechanism of activation have physiological consequences for how cells are regulated by G protein-coupled receptors? Given the immense number of possible G protein heterotrimers and the equally immense number of probable G protein-coupled receptors, this mechanism suggests that heterotrimer composition will affect what G proteins a receptor can activate (Fig. 7A). In addition, if there is effector specificity of the effects of βγ dimers, heterotrimer composition could also affect the downstream

targets for any given receptor (Fig. 7B). In the ultimate case, subunit dissociation and recombination upon deactivation will alter in the cell the complement of G protein heterotrimers present (Fig. 7C). Experiments that demonstrate that this kind of effect occurs could provide the final proof of the idea that G proteins are activated in and function within cells by subunit dissociation. At the same time, such experiments would establish a physiological principle about how signaling through G proteins can influence cell behavior not only in a variable way, but also in a way that would depend upon the past history of the cell. All of these ideas, however, are based upon the role of subunit heterogeneity in signaling by G proteins. This role is yet to be fully defined, but offers immense opportunities for future research.

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