

Regions in the G Protein γ Subunit Important for Interaction with Receptors and Effectors*

Chang-Seon Myung, William K. Lim, Joseph M. DeFilippo, Hiroshi Yasuda,
Richard R. Neubig and James C. Garrison[†]

Laboratory in Pharmacology, College of Pharmacy, Chungnam National University
Daejeon, Korea 305-764 (C.-S. M.)

Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, 93150 Kuching,
Sarawak, Malaysia. (W. K. L.)

Department of Gastroenterology, Showa University, Yokohama, Japan. (H. Y.)

Departments of Pharmacology and Medicine, University of Michigan, Ann Arbor,
Michigan, 48109 (R. R. N.)

Department of Pharmacology, University of Virginia Health Sciences Center,
Charlottesville, Virginia 22908 (J. M. D., J. C. G.)

Running Title: Interaction of G Protein γ Subunit with Receptors and Effectors

Corresponding Author:

James C. Garrison
Department of Pharmacology
University of Virginia Health Sciences Center
P.O. Box 800735
1300 Jefferson Park Avenue
Charlottesville, Virginia 22908-0735
Phone: (804) 924-5618
Fax: (804) 982-3878
E-mail: jcg8w@virginia.edu

Number of Text Pages: 27

Number of Tables: 3

Number of Words in Abstract: 250
Number of Words in Introduction 750
Number of Words in Discussion 1500

Abbreviations

¹The abbreviations used are: G proteins, guanine nucleotide-binding regulatory proteins; Sf9 cells, *Spodoptera frugiperda* cells (ATCC no. CRL 1711); PLC- β ; phosphatidylinositol-specific phospholipase C- β isoform; PtdIns 3-kinase, phosphatidylinositol (4,5) bisphosphate 3-kinase, CHAPS, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate; Genapol C-100, polyoxyethylene (10) dodecyl ether. ¹²⁵I-ABA, N⁶-(4-amino-3-¹²⁵iodo-benzyl)adenosine; DTT, dithiothreitol, EDTA, ethylenediamine tetraacetic acid, GTP- γ -S, Guanosine 5'-(3-O-thio) triphosphate.

Abstract

G $\beta\gamma$ dimers containing the γ_{11} or γ_1 subunits are often less potent and effective in their ability to regulate effectors as compared to dimers containing the γ_2 subunit. To explore the regions of the γ subunit which affect the activity of the $\beta\gamma$ dimer, we constructed 8 chimeric γ subunits from the γ_1 and γ_2 subunits. Two chimeras were made in which the N-terminal regions of γ_1 and γ_2 were exchanged and two in which the C-terminal regions were transposed. Another set of chimeras was made in which the CAAX motifs of the chimeras were altered to direct modification with different prenyl groups. All 8 γ chimeras were expressed in Sf9 cells with the β_1 subunit, G $\beta\gamma$ dimers purified, and assayed *in vitro* for their ability to bind to the G α_{i1} subunit, to couple G α_{i1} to the A1 adenosine receptor, to stimulate phospholipase C- β and to regulate type I or type II adenylyl cyclases. Dimers containing the C-terminal sequence of the γ_2 subunit modified with the geranylgeranyl lipid had the highest affinity for G α_{i1} (range 0.5-1.2 nM) and were most effective at coupling the G α_{i1} subunit to receptor. These dimers were most effective at stimulating PLC- β and inhibiting type I adenylyl cyclase. In contrast, $\beta\gamma$ dimers containing the N-terminal sequence of the γ_2 subunit and a geranylgeranyl group are most effective at activating type II adenylyl cyclase. The results indicate both the N and C terminal regions of the γ subunit impart specificity to receptor and effector interactions.

Among the best characterized signal transduction systems are the pathways used by receptors coupled to heterotrimeric G proteins¹. The G protein $\beta\gamma$ subunit plays an important role in these pathways. It is required for the interaction between the receptor and the α subunit to initiate GDP/GTP exchange and also stabilizes the basal state of the receptor- α -GDP- $\beta\gamma$ complex which forms the high affinity, ligand binding conformation of receptors (Cabrera-Vera *et al.*, 2003). Once released from the activated α subunit, the $\beta\gamma$ subunit can regulate over 20 effectors including PLC- β , adenylyl cyclase, ion channels (Cabrera-Vera *et al.*, 2003;Hildebrandt, 1997), PtdIns 3-kinase (Kerchner *et al.*, 2004) and guanine nucleotide exchangers for small GTP binding proteins (Welch *et al.*, 2002). The free $\beta\gamma$ dimer can also participate in regulatory events by binding to cytoplasmic proteins such as the β -adrenergic receptor kinase or phosducin (Cabrera-Vera *et al.*, 2003). Importantly, the multiple isoforms of the $\beta\gamma$ dimer can differentially regulate effectors (Cabrera-Vera *et al.*, 2003;Hildebrandt, 1997), making it necessary to understand the regions of these subunits which interact with their targets.

To date, seven β and twelve γ subunits have been identified in mammalian systems (Downes and Gautam, 1999); thus the $\beta\gamma$ dimers which can be assembled from the known subunits yield a large number of potentially unique complexes which differ in their interactions with receptors and effectors (Cabrera-Vera *et al.*, 2003;Hildebrandt, 1997). While the β_1 - β_4 subunits are more than 85% identical in amino acid sequence, the sequence identity of the γ subunits ranges from 10-70 % (Downes and Gautam, 1999). Interestingly, the diversity of either the β or the γ subunit can impart specificity to the interactions of the dimer with receptors (Richardson and Robishaw, 1999;McIntire *et al.*, 2001;Hou *et al.*, 2000) or effectors (Cabrera-Vera *et al.*, 2003) and the type of prenyl group on the γ subunit is clearly important in the interactions with both receptors (Yasuda *et al.*, 1996;Kisselev *et al.*, 1995) and effectors (Myung *et al.*, 1999). Multiple experiments suggest that the γ subunit is divided into three functionally different regions. The central region of all γ subunits is thought to be involved in the interactions between the β and γ subunits. For example, mutagenesis data indicate that a 14 amino acid sequence (amino acids 36-49 on γ_1 or 33-46 on γ_2) is critical for the interaction with the

β_1 subunit (Lee *et al.*, 1995) and the crystal structure of the $\beta\gamma$ dimer confirms that 17 of the 27 residues in the γ subunit which interact with the β subunit are centrally located (Sondek *et al.*, 1996). The N- and C-terminal regions of the γ subunits are the least conserved (Downes and Gautam, 1999;Sondek *et al.*, 1996), and several lines of evidence suggest that these regions are important for the interaction with receptors (Yasuda *et al.*, 1996;Hou *et al.*, 2000) and effectors (Cabrera-Vera *et al.*, 2003;Hildebrandt, 1997;Yasuda *et al.*, 1996;Yasuda *et al.*, 1998;Akgoz *et al.*, 2002).

The γ subunits segregate into five groups with γ_1 , γ_{11} and γ_8 comprising subfamily I, γ_2 , γ_3 , γ_4 and γ_9 subfamily II, γ_7 and γ_{12} subfamily III, γ_5 and γ_{10} subfamily IV and γ_{13} forming its own family (Downes and Gautam, 1999). Experiments show that $\beta\gamma$ dimers containing the γ_1 or the γ_{11} subunit are less potent in activating PLC- β and type II adenylyl cyclase than dimers containing the γ_2 subunit (Myung *et al.*, 1999). Whereas the expression of γ_1 is restricted to the visual system, γ_{11} and γ_8 are widely expressed (Morishita *et al.*, 1998;Downes and Gautam, 1999). These three γ subunits of subfamily I are modified with the farnesyl lipid (Downes and Gautam, 1999) and the distribution of charged amino acids in the N and C terminus is quite different from the other nine γ subunits.

To examine how the N- and C-terminal domains of the γ subunit and its prenyl group affect the interaction with α subunits, receptors and effectors, we constructed 8 chimeric γ subunits from the γ_1 and the γ_2 subunits. These two γ subunits were used for the chimeras as the N and C termini of these proteins are representative of the major differences between the γ subunits in subfamily I and the other nine γ subunits. Two chimeras were made in which the N-terminal amino acids of γ_1 and γ_2 were exchanged. Two other chimeras were made in which the C-terminal 23 amino acids of γ_1 and γ_2 were exchanged. Finally, another set of chimeras was made in which the CAAX motifs in the four chimeras were altered to direct modification with different prenyl groups. All 8 γ chimeras were expressed in Sf9 cells with the β_1 subunit and purified. Each dimer was tested using five *in vitro* assays chosen to probe major facets of $\beta\gamma$ activity; the ability to bind to the $G\alpha_{i1}$ subunit, to couple $G\alpha_{i1}$ to the A1 adenosine receptor, to stimulate PLC- β or to regulate type I and II adenylyl cyclases. The *in vitro* results indicate the γ subunit's C terminus and its

prenyl group are important for coupling α subunits to the A1 adenosine receptor, for the activation of PLC- β and for the inhibition of type I adenylyl cyclase. In contrast, the γ subunit's N terminus is very important for the activation of type II adenylyl cyclase.

Materials and Methods

Strategy for Construction of the Chimeric γ Subunits — The γ_1 and γ_2 subunits have a conserved set of three residues about 20 amino acids from the N terminus (QLK) and the C terminus (DPL). The strategy for making the chimeras was based on creating new cDNAs for the γ_1 and γ_2 subunits which had restriction sites engineered into the QLK and DPL sequences and is similar to those published (Jian *et al.*, 2001). These sites are highlighted in Figure 1A. Also note the similarities in the N and C terminal regions of the γ_1 and γ_{11} subunits. Thus, while the chimeras were made with regions from γ_1 because the cDNA for γ_1 contained a convenient restriction site in the DPL sequence, the regions were selected to be representative of γ_{11} (see Figure 1). An *Afl*III site was used to create the QLK sequence and a *Bam*HI site to code for the DPL sequence in each molecule. The nomenclature used to describe these chimeric γ subunits is shown in Figure 1B. The protein was divided into three regions by the QLK and DPL sites: designated as AAA for γ_1 and BBB for γ_2 . If the C-terminal prenyl group was switched from the native modification, the amino acids in the mutant CAAX motif are indicated (Yasuda *et al.*, 1996). For example, γ_{2-L71S} indicates the native γ_2 subunit modified to change only the normal C-20 geranylgeranyl to the C-15 farnesyl. A designation of γ_{BBA} indicates a chimeric γ subunit where the C terminus of the γ_1 subunit (molecule A) is added to the N-terminal and middle regions of the γ_2 subunit (molecule B) at the conserved sequence. The prenyl group of this chimera would be farnesyl. A designation of $\gamma_{BBA-S74L}$ would indicate the same chimeric γ subunit mutated to code for addition of geranylgeranyl at the C terminus.

The γ_1 cDNA was excised from a pEV plasmid (*Hind*III/*Bam*HI) and subcloned into the pGem7zF+ plasmid utilizing the same restriction sites; it was then excised with *Hind*III/*Asp*700 for subcloning into the pAlter-1 mutagenesis plasmid at the *Hind*III and

*Sma*I sites. The γ_2 cDNA in pGem4z was excised by digestion with *Xba*I and *Sma*I and was subcloned into pAlter-1 using the same restriction sites. To construct the chimeras, identical restriction sites were created in the QLK and DPL sequences in both the γ_1 and γ_2 cDNAs; an *Afl*III site was engineered to code for the QLK sequence and a *Bam*HI site was engineered to code for the DPL sequence. As the *Bam*HI restriction site already existed in the γ_1 cDNA in the site corresponding to the DPL residues, a *Bam*HI site was added to the γ_2 cDNA through site-directed mutagenesis by altering one base in the γ_2 sequence (GAA to GAG, encoding Glu) using the Morph™ Site-Specific Plasmid DNA Mutagenesis Kit (5Prime -> 3Prime, Boulder, CO) taking care to conserve the protein sequence. The *Afl*III restriction site for the QLK sequence was engineered into both the γ_1 and γ_2 subunits using the same mutagenesis kit. The resulting γ_1 and γ_2 cDNAs in the pAlter-1 plasmid containing the new *Afl*III and *Bam*HI sites were used to make the C- or N-terminal chimeric γ subunits.

Construction of N- and C-terminal γ Chimeras and Production of Recombinant Baculoviruses — The γ_1 and γ_2 C-terminal chimeras (γ_{AAB} and γ_{BBA}) were constructed by digesting the γ_1 and γ_2 cDNAs in the pAlter-1 plasmid with *Bam*HI and *Nhe*I. The four resulting DNA fragments contain the C termini of γ_1 and γ_2 and cDNAs encoding for the intact N terminus and middle regions of the γ_1 and γ_2 proteins. The C-terminal cDNA fragments were gel purified and ligated back into either the cDNA encoding the N terminus and middle regions of the γ_1 or γ_2 as desired. This protocol generated the constructs containing the γ_{AAB} and γ_{BBA} chimeras. To exchange the N termini of the γ_1 and γ_2 molecules, the respective cDNAs in the pAlter-1 vector were digested with *Afl*III and *Nhe*I, the N-terminal cDNA fragments gel purified and ligated back into either the cDNA encoding the middle regions and C terminus of γ_1 or γ_2 as desired. This protocol generated the constructs containing γ_{BAA} and γ_{ABB} chimeras. It was desirable to subclone the γ cDNAs into the *Xba*I site in the baculovirus transfer vector, pVL1393. Thus, the four γ chimeras in the pAlter-1 plasmid were subcloned into the linear pCNTR plasmid which added the *Xba*I restriction sites to the end of each chimera. Each of the four chimeras was subcloned into the pVL1393 baculovirus transfer vector by excising them from pCNTR with *Xba*I and ligating the fragments into pVL1393 at its *Xba*I sites. The 4 γ

chimeras containing altered CAAX sequences, $\gamma_{\text{AAB-L71S}}$ (farnesyl), $\gamma_{\text{BBA-S74L}}$ (geranylgeranyl), $\gamma_{\text{BAA-S74L}}$ (geranylgeranyl) and $\gamma_{\text{ABB-L71S}}$ (farnesyl), were constructed using PCR on the cDNAs in pVL1393 as described (Lindorfer *et al.*, 1996). Each of the eight pVL1393 transfer vectors containing the cDNA for a chimeric γ subunit was sequenced to ensure fidelity. Recombinant baculoviruses encoding for the eight γ chimeras were produced by co-transfecting each recombinant plasmid DNA with linear wild type BaculoGold[®] viral DNA into Sf9 cells and purified by one round of plaque purification (Graber *et al.*, 1994). The recombinant baculoviruses encoding the α_s , α_{i1} , β_1 , γ_1 , γ_{1-S74L} , γ_2 and γ_{2-L71S} subunits have been described (Lindorfer *et al.*, 1996; Graber *et al.*, 1994).

Expression and Purification of G protein α and $\beta\gamma$ Subunits — G protein α subunits were overexpressed in bacteria (Sarvazyan *et al.*, 1998) and baculovirus-infected Sf9 insect cells (Graber *et al.*, 1994). Bacterially expressed, myristoylated α_{i1} was purified from *Escherichia coli* (BL21/DE3) following the method of Mumby and Linder (Mumby and Linder, 1994). The amount of α_{i1} protein was determined using the Bradford assay. The G_{i1} α subunits expressed in Sf9 insect cells were purified to homogeneity as described (Graber *et al.*, 1994) and the G_s α subunit used in the adenylyl cyclase assays was prepared from a 0.1% (w/v) CHAPS extract of crude cell lysates (McIntire *et al.*, 2001). The specific activity of GTP binding of these preparations was ~11-15 nmol/mg of α subunit as measured by [³⁵S]-GTP- γ -S binding (Sarvazyan *et al.*, 1998; Graber *et al.*, 1994). Bovine brain $\beta\gamma$ dimer was isolated from brain cortex synaptosomal membranes as described (Sarvazyan *et al.*, 1998). The membranes were a gift from Dr. T. Ueda, University of Michigan. Recombinant $\beta\gamma$ subunits were prepared from baculovirus infected Sf9 insect cells. Sf9 cells were coinfecting with the appropriate recombinant baculoviruses encoding the β_1 subunit and native or chimeric γ subunits in Sf9 cells at a multiplicity of infection of 3 and harvested 48 hr after infection. All $\beta\gamma$ subunits were extracted from frozen cell pellets with 0.1% Genapol C-100 and the detergent-extracted $\beta\gamma$ dimers purified on a DEAE column followed by affinity chromatography on a G_{i1} - α -agarose column (Graber *et al.*, 1996). The $\beta\gamma$ combinations used in this study are properly folded as they were purified on α subunit affinity column, have a high affinity for

the α subunit (see Figure 2) and were released following activation with AlF_4^- . The purified $\beta\gamma$ dimers were resolved on 12% polyacrylamide gels, stained with silver and the concentration of the purified $\beta\gamma$ dimers estimated using ovalbumin standards. All preparations of the dimers were highly pure, examples of their purity have been published (McIntire *et al.*, 2001). The experiments shown in *Results* were performed using at least two different preparations of each $\beta\gamma$ dimer with consistent results.

Analysis of the Post-Translational Processing of the γ Subunit by Mass Spectrometry —

To confirm that the proper chimeric γ subunits were being expressed and that their C termini were properly modified, the molecular masses of the γ subunits in the 12 $\beta\gamma$ dimers used in this study were determined using matrix-assisted laser desorption ionization (MALDI) mass spectrometry as described (Lindorfer *et al.*, 1996). The molecular mass of each chimeric γ subunit was estimated by the University of Wisconsin GCG program. The results summarized in Table I indicate that each chimeric γ subunit was faithfully expressed and had a fully processed C terminus. For example, the γ_{BAA} protein in the sample was comprised of one molecular weight species with a molecular mass of 7,818 Da. This result is consistent with the predicted amino acid sequence of the chimera and processing of the molecule by removal of the three C-terminal amino acids (-VIS), addition of a farnesyl lipid to the C-terminal cysteine, addition of a carboxymethyl group to the C terminus, removal of the N-terminal methionine and acetylation of the resulting N-terminal alanine. The observed prenyl modification of the γ subunits in this study is consistent with the electrospray mass spectrometric analysis of previous samples which show that over 90% of the purified protein is properly modified (Lindorfer *et al.*, 1996). Finally, the data in Table I, combined with that in Figures 2-5, show that use of the conserved QLK and DPL sites which are located in the two helical regions of the γ subunit (Sondek *et al.*, 1996) to construct the chimeras appears to generate fully functional molecules. These chimeras combine with β subunits, purify efficiently over an α subunit affinity column and interact well with receptors and effectors (see Figures 2-5).

Expression of A1 Adenosine Receptors, Phospholipase C- β , Type I and II Adenylyl Cyclase — A recombinant baculovirus encoding the A1 adenosine receptor was used to over-express the receptor in Sf9 insect cells and membranes were purified as described (Yasuda *et al.*, 1996). Sf9 insect cell membranes overexpressing recombinant type I or type II adenylyl cyclase were prepared as described (McIntire *et al.*, 2001). Recombinant turkey PLC- β was overexpressed in Sf9 cells and purified as described (Myung *et al.*, 1999).

Assay of the Activity of $\beta\gamma$ Dimers — Different facets of the activity of each $\beta\gamma$ dimer were measured in a set of *in vitro* assays chosen to probe the known protein-protein interaction domains of the dimer. Each of these assays was selected for its ability to highlight a known function of the $\beta\gamma$ dimer and because the outcome was directly dependent on $\beta\gamma$ activity. The affinity of the $\beta\gamma$ dimer for the α subunit reflects interactions of the top and sides of the β subunit with the switch II and C terminal regions of the α subunit (Cabrera-Vera *et al.*, 2003). The prenyl group on the C terminal region of the γ subunit is also important for binding the α subunit (Iniguez-Lluhi *et al.*, 1992). The affinity of the $\beta\gamma$ dimers for G α i1 was measured in solution with pure proteins by flow cytometry, using fluorescein-labeled bacterially-expressed myristoylated α i1 and biotinylated bovine brain $\beta\gamma$ as previously described (Sarvazyan *et al.*, 1998). Briefly, 1 nM of biotinylated brain $\beta\gamma$ was prebound to streptavidin-coated polystyrene beads (SVP-60-5, Spherotech Inc., Libertyville, IL) and incubated with 1 nM fluorescein-labeled, myristoylated α i1 together with varying amounts of recombinant $\beta\gamma$ in 20 mM Hepes, pH 8, 1 mM EDTA, 1 mM DTT, 100 mM NaCl, 1.2 mM MgCl₂, 0.1% Lubrol and 10 μ M GDP. After a 30 min incubation at room temperature, samples were analyzed with a Becton Dickinson FACScan capturing events on the forward scatter, side scatter, and fluorescein (FL-1) channels. Histograms of FL-1 fluorescence from singlet bead populations were obtained and mean channel numbers calculated using LYSIS II software (BD, San Jose, CA).

The ability of each particular β ₁ γ _x dimer to support coupling of the G_{i1} α subunit to the A1 adenosine receptor was used to monitor the interactions of the N terminal, central and C

terminal regions of the 10 γ subunit chimeras with the receptor. In this assay, the receptor is expressed in an Sf9 cell membrane in the absence of G proteins causing it to be in its low affinity, agonist binding conformation (Yasuda *et al.*, 1996). Reconstitution of the G protein heterotrimer into the membrane re-establishes the high affinity, agonist binding conformation of the receptor as measured by a radioligand binding assay using an agonist ligand (see Fig 2A and (McIntire *et al.*, 2002)). Alternatively, if an excess of α subunit and graded amounts of a $\beta\gamma$ dimer are reconstituted into the membrane, the amount of the receptor returned to the high affinity, agonist binding state as measured using a low concentration of the agonist ligand reflects the affinity of the receptor - α subunit complex for the $\beta\gamma$ dimer (McIntire *et al.*, 2002). As only the γ subunit was different in the experiments shown in Figure 2B, the assay provides a measure of the ability of the dimers containing the different chimeric γ subunits to interact with the receptor and α subunit. This value is expressed as EC₅₀ of receptor coupling in Fig. 2B. The ability of the three regions of the γ subunit to interact with PLC- β was measured by reconstituting dimers containing the chimeric γ subunits and pure PLC- β into synthetic lipid vesicles (Myung *et al.*, 1999). Sf9 cells infected with recombinant baculoviruses for adenylyl cyclases can be used to monitor the direct interaction of the $\beta\gamma$ dimer with type I and type II adenylyl cyclase (McIntire *et al.*, 2001; Taussig *et al.*, 1994).

To measure type I or II adenylyl cyclase activity, Sf9 membranes over-expressing the desired enzyme (10 μ g membrane protein/assay tube) were reconstituted with the purified GTP- γ -S-activated G_s α subunit and varying concentrations of pure $\beta\gamma$ dimers containing either a native or chimeric γ subunit and held on ice for 30 min (McIntire *et al.*, 2001). Each assay was incubated at 30 °C for 7 min and the reaction terminated with 1 ml 0.11 N HCl. The samples were prepared for the assay of cAMP and the assay performed as described (McIntire *et al.*, 2001). The activated G_s α subunit was used as the co-activator of type I and type II cyclase in these experiments because: (a) the $\beta\gamma$ dimer does not increase the activity of type II cyclase in the absence of G_s α or in the presence of forskolin (Taussig *et al.*, 1994); (b) the $\beta\gamma$ dimer only modestly inhibits type I cyclase if it is preactivated with forskolin or Ca²⁺/calmodulin (Taussig *et al.*, 1994). Pilot experiments with our Sf9 membranes confirmed both of these findings (data not shown).

Calculation and Expression of Results — Experiments presented under “*Results*” are the average of three or more similar experiments. Data expressed as concentration-response curves were fit to sigmoid curves using the fitting routines in the GraphPad Prism™ software. The EC₅₀ and V_{max} values shown in Tables II and II were taken from these fits. Statistical differences between the fitted curves were determined using all the individual data points from multiple experiments to calculate the F statistic (Motulsky and Ransnas, 1987).

Materials — All reagents used in the culture of Sf9 cells and for the expression and purification of G protein $\beta\gamma$ subunits have been described in detail (Graber *et al.*, 1996). The baculovirus transfer vector, pVL1393, was purchased from Invitrogen; BaculoGold® viral DNA from PharMingen; 10% Genapol C-100 and phosphatidylinositol 4,5-bisphosphate from Calbiochem®; phosphatidylethanolamine (bovine heart) from Avanti Polar Lipids, Inc.; [*inositol-2-³H*]phosphatidylinositol 4,5-bisphosphate from NEN Life Science Products; CHAPS from Roche Molecular Biochemicals; BSA (fatty acid-free) from Sigma; the pCNTR shuttle vector and the Morph™ Site-Specific Plasmid DNA Mutagenesis Kit from 5 Prime → 3 Prime, Inc. (Boulder, CO). All other reagents were of the highest purity available.

Results

The $\beta_1\gamma_1$ and $\beta_1\gamma_{11}$ dimers are measurably less active than the $\beta_1\gamma_2$ dimer in its ability to activate K⁺ channels (Cabrera-Vera *et al.*, 2003), PLC- β (Myung *et al.*, 1999), type II adenylyl cyclase (Myung *et al.*, 1999) and PtdIns 3-kinase (Kerchner *et al.*, 2004), or to inhibit type I adenylyl cyclase (McIntire *et al.*, 2001). As the dimers tested in these experiments all contained the β_1 subunit, differences in the primary amino acid sequence of the γ subunits, in their prenyl modification or both must explain the differences in activity. While it was initially attractive to ascribe the unique properties of the γ_1 subunit to its specialized role in visual transduction (Cabrera-Vera *et al.*, 2003), the fact that γ_{11} and γ_8 , have similar amino acid sequences and prenyl modifications (see Figure 1A) and

are widely expressed (Downes and Gautam, 1999), makes it important to understand the reasons for the differential activity of this subfamily of γ subunits. Figure 1A presents the differences in the N and C termini of the γ_1 , γ_{11} and γ_2 subunits. Note that the N-terminal 20 amino acids of the very similar γ_1 and γ_{11} subunits have six negatively charged amino acids whereas this region of the γ_2 subunit has only one negative charge and that the C-terminal amino acids of the γ subunits also show marked differences in charge distribution. Thus, differences in the amino acid sequences and charges in the N- and C-terminal regions of these proteins may be important for the signaling-specificity of the $\beta\gamma$ dimer. To address this issue, we constructed a set of 4 chimeric γ subunits in which the N- and C-terminal amino acids of γ_1 and γ_2 were exchanged. To complete the set, an additional 4 mutants with altered prenyl modifications was prepared. Figure 1B presents the 8 chimeras which were made and the nomenclature used to describe them. The 12 γ subunits shown in Figure 1B were expressed with β_1 subunit and the dimers purified from baculovirus-infected Sf9 insect cells by α_{i1} -agarose affinity chromatography. The activity of the complete set of $\beta\gamma$ dimers was examined in a panel of *in vitro* assays measuring the affinity of the dimer for the G_{i1} α subunit, the ability to support coupling of the α subunit to the bovine A1 adenosine receptor, to activate PLC- β and to regulate type I or type II adenylyl cyclases.

Receptor Coupling — The C-terminal domain of the mammalian γ subunit and its prenyl group are known to be important for the interaction of the $\beta\gamma$ dimer with α subunits and receptors (Yasuda *et al.*, 1996; Azpiazu *et al.*, 1999). Interestingly, the affinity of the α subunit for the $\beta\gamma$ dimer is an important component of the interaction of the heterotrimer with the receptor (Sarvazyan *et al.*, 1998) but this parameter is not commonly measured. Moreover, the differences noted with the different forms of the γ subunit can be minimized when high concentrations of the α and $\beta\gamma$ subunits are reconstituted into vesicles or membranes (see Figure 2A). For these reasons, we performed experiments to compare the affinity of dimers containing the chimeric γ subunits for the G_{i1} α subunit with the EC₅₀ values determined for the ability of the dimer to support the high affinity, agonist binding state of the A1 adenosine receptor. Figure 2A shows ability of various concentrations of the $G_{i1} : \beta_1\gamma_2$ heterotrimer to reconstitute the high affinity, agonist

binding conformation of the adenosine A1 receptor over-expressed in Sf9 cell membranes (Yasuda *et al.*, 1996). Four sets of membranes were reconstituted with increasing amounts of heterotrimer and the amount of total agonist ligand bound plotted as a function of ligand concentration. Note that concentrations of heterotrimer in the 40 nM range shift the affinity of the receptor for the agonist (amino-benzyladenosine – ABA) from about 30 nM (open circles - no G protein) to 0.3 nM (inverted triangles - 40 nM $G\alpha_{i1} : \beta_1\gamma_2$). An intermediate shift in the affinity of the receptor is caused by 2-6 nM heterotrimer. When the concentration of the heterotrimer is in the the 2-6 nM range, it is possible to measure differences in the ability of various $\beta\gamma$ combinations to interact with the receptor - α subunit complex and support high affinity binding (McIntire *et al.*, 2002). Therefore, we measured the ability of the 6 C-terminal chimeras to support the establishment of the high affinity, agonist binding conformation of bovine A1 adenosine receptors at 6 nM heterotrimer. The results were expressed as the EC₅₀ of the interaction and compared with the results with the affinity of the $\beta\gamma$ dimers for the G_{i1} α subunit measured in solution. The data in Figure 2B show that the K_d (x axis) of the $\beta\gamma$ dimer for the G_{i1} α subunit varies about 20 fold from 0.4 nM ($\beta_1\gamma_2$) to 7.7 nM ($\beta_1\gamma_{BAA}$). Note that the EC₅₀ for supporting coupling of the G_{i1} α subunit to the receptor (y axis) varies 5-6 fold from 0.4 nM ($\beta_1\gamma_2$) to about 2.2 nM ($\beta_1\gamma_{BAA}$). All $\beta\gamma$ dimers containing γ subunits modified with the farnesyl group (open squares) had lower affinities for the G_{i1} α subunit and lower EC₅₀'s for supporting receptor coupling than did those containing the geranylgeranyl moiety. These results confirm and extend the concept that the C-terminal region of the γ subunit and its prenyl group are very important for the interaction of the dimer with α subunits and receptors in this assay. Moreover, the data show that there is a rough correlation between the EC₅₀ for receptor coupling and the affinity of the α and $\beta\gamma$ subunits themselves.

Phospholipase C- β — Our previous experiments show that the activity of the $\beta_1\gamma_1$ and $\beta_1\gamma_{11}$ dimers on PLC- β is significantly less than that of the $\beta_1\gamma_2$ dimer and the activity is affected by the type of prenyl group on the γ subunit (Myung *et al.*, 1999). To determine how the N and C terminal regions of the γ subunit affected the interaction of the $\beta\gamma$ dimer with PLC- β , dimers containing the 12 γ subunits shown in Figure 1B were tested for their

ability to stimulate avian PLC- β *in vitro*. The data in Figure 3A show that $\beta_1\gamma_2$ markedly activates PLC- β with an EC₅₀ of about 5 nM (closed squares) and that $\beta_1\gamma_1$ (open circles) is about 5 fold less potent (EC₅₀ of 25 nM – see Table II). The activity of the $\beta_1\gamma_1$ dimer can be made equal to the $\beta_1\gamma_2$ dimer by replacing the central and C-terminal amino acids of the γ_1 subunit with those from the γ_2 subunit (open triangles - $\beta_1\gamma_{ABB}$). A chimeric γ subunit containing the first 50 amino acids of the γ_1 subunit and the C terminus of the γ_2 subunit ($\beta_1\gamma_{AAB}$) is also equally effective as $\beta_1\gamma_{ABB}$ (see Table II). These results suggest that the C terminal 23 amino acids of the γ_2 subunit are important for potent activation PLC- β *in vitro*, a result confirmed by the data in Figure 3C which shows replacing the last 23 amino acids and the geranylgeranyl group of γ_2 with the amino acids and farnesyl group of γ_1 ($\beta_1\gamma_{BBA}$) yields a dimer with activity very similar to $\beta_1\gamma_1$. The amino acids in the central region do not seem to affect the interaction with PLC- β , as $\beta_1\gamma_{BAA}$ has an activity equal to $\beta_1\gamma_{BBA}$ and $\beta_1\gamma_1$ (see Table II).

Chimeric γ subunits with switched prenyl groups indicate that the differences the activity of the dimers shown in Figures 3A and C are due both to the differences in the C terminal 23 residues and the composition of the prenyl group. Note that switching the prenyl group on the $\beta_1\gamma_2$ dimer from farnesyl to geranylgeranyl ($\beta_1\gamma_{BAA-S74L}$) provides a dimer with intermediate activity on PLC- β (closed diamonds – Figure 3B). Exchanging the prenyl group on $\beta_1\gamma_1$ ($\beta_1\gamma_1-S74L$) produces a similar result (closed circles). In fact, all dimers containing γ subunits with exchanged prenyl groups were intermediate in either their EC₅₀ or their maximal activity on PLC- β (see Table II). These results indicate that the $\beta\gamma$ dimers containing the C-terminal sequence of the γ_2 subunit modified with a geranylgeranyl group are more potent and effective in activating PLC- β *in vitro* than those with the C-terminal region of the γ_1 subunit and/or modified with farnesyl group.

Type I Adenylyl Cyclase — The data in Figure 4 present the ability of five representative $\beta\gamma$ dimers to inhibit type I adenylyl cyclase. Figure 4A shows that the $\beta_1\gamma_2$ dimer inhibited type I adenylyl cyclase with an IC₅₀ value of 15 nM (closed squares) and was about 2-fold more potent and effective than the $\beta_1\gamma_1$ dimer (IC₅₀ of 36 nM - see Table II). However, a

chimeric γ subunit with the C terminal 23 amino acids of the γ_1 subunit and its prenyl group exchanged with those of the γ_2 subunit (γ_{AAB}) produces a γ subunit with activity equal to that of the γ_2 subunit (solid triangles – Figure 4A). In contrast, replacing the C terminus of γ_2 and the geranylgeranyl group of γ_2 with the C terminus of γ_1 and the farnesyl group (γ_{BBA}) produces a dimer with activity similar to that of $\beta_1\gamma_1$ (open triangles - Figure 4C). The data in Table II indicate that other dimers in which the C terminus of the γ_1 subunit is intact and modified with farnesyl were roughly equal to the native $\beta_1\gamma_1$ dimer in their ability to inhibit type I adenylyl cyclase (e.g. $\beta_1\gamma_{BAA}$ or $\beta_1\gamma_{BBA}$). These observations indicate that the C-terminal region of γ_2 is most critical for the inhibition of type I adenylyl cyclase *in vitro*. As was the case with PLC- β , both the C terminal amino acids and the prenyl group appear to be important for the interaction of the $\beta\gamma$ dimer with Type I cyclase. Note that if the prenyl group on the $\beta_1\gamma_{AAB}$ dimer is changed to farnesyl ($\beta_1\gamma_{AAB-L71S}$), the activity was intermediate between that of $\beta_1\gamma_2$ and $\beta_1\gamma_1$ (closed triangles – Figure 4B). Similar data was obtained with the 6 other dimers containing γ subunits with modified prenyl groups (see Table II). Overall, the data in Figures 3-4 demonstrate that the C-terminal amino acid sequence of the γ_2 subunit and its native geranylgeranyl group are important determinants for the interaction of the $\beta\gamma$ subunit with effectors such as PLC- β and type I adenylyl cyclase.

Type II Adenylyl Cyclase — The $\beta_1\gamma_2$ subunit activates type II adenylyl cyclase more than ten-fold with a nanomolar EC_{50} and surprisingly, neither the $\beta_1\gamma_1$ nor the $\beta_1\gamma_{11}$ dimer activate type II adenylyl cyclase well (Myung *et al.*, 1999). In addition, phosphorylation of the γ_{12} subunit in the $\beta_1\gamma_{12}$ dimer significantly inhibits its ability to stimulate type II adenylyl cyclase (Yasuda *et al.*, 1998). The phosphorylation site has been determined to be at Ser¹ in the N-terminus of the molecule (Asano *et al.*, 1998). There are more negative charges in the N-terminal region of γ_1 or γ_{11} as compared to the γ_2 subunit (Figure 1A), potentially explaining the inability of dimers containing the γ_1 or γ_{11} subunits to activate type II adenylyl cyclase. Taken together, these results suggest that introduction of negative charges in the N-terminal regions of the γ subunit inhibit the interaction of the dimer with the type II adenylyl cyclase. Thus, we examined the ability

of $\beta\gamma$ dimers containing N-terminal chimeric γ subunits to activate type II adenylyl cyclase.

The data in Figure 5 present the ability of 6 representative $\beta\gamma$ dimers to activate type II adenylyl cyclase performed using concentrations of dimers ranging from 0.1 nM to 100 nM. As expected (Yasuda *et al.*, 1998), the $\beta_1\gamma_2$ dimer (closed squares) activated type II adenylyl cyclase with an estimated EC_{50} value of 13 nM and was far more potent and effective in activating this effector enzyme than the $\beta_1\gamma_1$ dimer (Figure 5A). Adding the N-terminal region of the γ_2 subunit to the γ_1 subunit and changing its prenyl group to geranylgeranyl ($\beta_1\gamma_{BAA-S74L}$) generated a molecule with activity equal to $\beta_1\gamma_2$ (closed diamonds – Figure 5A). A similar result was observed with $\beta_1\gamma_{BBA-S74L}$ (see Table III). In contrast, a dimer with the poor activity of $\beta_1\gamma_1$ could be created using a chimeric γ subunit containing the N terminal 23 amino acids of γ_1 and a farnesyl group ($\beta_1\gamma_{AAB-L71S}$ - closed circles – Figure 5C). These results suggest that both the N-terminal region and the geranylgeranyl group are very important for the activation of type II adenylyl cyclase *in vitro*. Indeed, Figure 5B shows that the $\beta_1\gamma_{BAA}$ dimer which contained the N-terminal amino acids of γ_2 and the farnesyl modification was intermediate in the activation of type II adenylyl cyclase. Indeed, the data in Table III show that every chimeric γ subunit which is intermediate in activity on type II cyclase is missing either the N terminal amino acids of γ_2 and or the geranylgeranyl group. These results indicate that the $\beta\gamma$ dimers containing the N-terminal sequence of the γ_2 subunit which has one negatively charged amino acid are more potent and effective in activating type II adenylyl cyclase than those with the N-terminal region of the γ_1 subunit which has six negatively charged amino acids. A C terminus modified with the geranylgeranyl group is also important. Similar results are observed with the effector PtdIns 3-kinase in that dimers containing γ_1 or γ_{11} weakly activate the enzyme (Kerchner *et al.*, 2004).

Discussion

There are three major findings of this *in vitro* study. First, $\beta\gamma$ dimers with a γ subunit containing the C-terminal 23 amino acid sequence of the γ_2 subunit and modified with a geranylgeranyl group have the highest affinity for the G_{i1} α subunit and are the most effective in coupling of the G_{i1} α subunit to bovine A1 adenosine receptors (Figure 2). These data provides an important demonstration of the correlation between the affinity of the α subunit for the $\beta\gamma$ dimer and the ability of the complex to support high affinity receptor – G protein coupling. Second, dimers with these attributes are most effective at activating PLC- β and inhibiting type I adenylyl cyclase *in vitro* (Figures 3-4). Finally, $\beta\gamma$ dimers containing the N-terminal 19 residues of the γ_2 subunit are far more effective at activating type II adenylyl cyclase than those containing the N-terminal 22 residues of the γ_1 subunit. The full activity on type II adenylyl cyclase also required modification of the γ subunit with a geranylgeranyl group, but experiments with dimers such as $\beta_1\gamma_{1-S74L}$ and $\beta_1\gamma_{11-S73L}$ (Table II) which are modified with the geranylgeranyl lipid, indicate that the C-terminal prenyl group is of secondary importance to the N terminal amino acid residues in the activation of this effector (Myung *et al.*, 1999).

The finding that the C-terminal region of the γ subunit and its prenyl group is much more important than the N terminus for the interaction with α subunits and receptors confirms and extends a large amount of data obtained with rhodopsin in the visual system (Jian *et al.*, 2001), the A1 adenosine receptor (Yasuda *et al.*, 1996), the 5-HT_{1A} receptor (Butkerait *et al.*, 1995), the α_{2A} receptor (Richardson and Robishaw, 1999; Lim *et al.*, 2001) and bombesin receptors (Jian *et al.*, 1999). Experiments performed with a similar set of chimeric γ subunits made between γ_1 and γ_2 were tested for their ability to support coupling between rhodopsin and the Gt α subunit (Jian *et al.*, 2001). These experiments also indicated that the C terminus of the γ_2 subunit and its geranylgeranyl group contribute to better coupling between receptor and the heterotrimer (Jian *et al.*, 2001).

Moreover, experiments using synthetic peptides to probe the rhodopsin : γ subunit interaction found that the C-terminal 12 amino acids of the γ subunit (DKNPFKELKGGC in γ_1 or SENPFREKKFFC in γ_2) are clearly involved in interacting with α subunits and the receptor. When the γ_1 peptide was altered to change Phe⁶⁴ to Thr and Leu⁶⁷ to Ser, it markedly decreased the interaction with rhodopsin (Azpiazu *et al.*, 1999). Taken together, the results indicate that the C terminus of the mammalian γ subunit is very important for the interaction between G protein-coupled receptors and the heterotrimer. Interestingly, the receptor also appears to contact multiple domains in the α subunit and a region near the C terminus of the β subunit (Cabrera-Vera *et al.*, 2003). Thus, each subunit in the heterotrimer is used for efficient activation of the α subunit.

While data obtained with mammalian cells point to a role for the C terminus of the γ subunit in coupling $\beta\gamma$ dimers to receptors, studies with the *S. cerevisiae* pheromone mating pathway provide evidence that the C-terminal region of the yeast γ subunit is not important in receptor coupling. Using yeast strains in which the Ste18 locus (the only yeast γ subunit) was disrupted and pheromone signaling inhibited, a variety of mutated γ subunits were tested for their ability to rescue signaling. Yeast γ subunits with a variety of C-terminal sequences, mutated by alanine scanning or by deletion of up to 8 amino acids immediately preceding the CAAX box were all able to rescue pheromone signaling (Chinault and Blumer, 2003). These data suggests that this region of the yeast molecule was not important for signaling through yeast G protein coupled receptors *in vivo* (Chinault and Blumer, 2003). More studies with mammalian cells are needed to reconcile these differences, although the diversity of the $G\beta$ and $G\gamma$ subunits expressed in the mammalian genome will complicate these studies.

While the C termini of γ subunits contain sequences capable of interacting with multiple receptors, subtle differences in the residues and charges must be important in determining specificity. For example, the $\beta_1\gamma_1$ dimer couples the G_{i1} α subunit to the α_2 -adrenergic receptor poorly (Richardson and Robishaw, 1999), whereas the $\beta_1\gamma_{11}$ dimer couples well (Lim *et al.*, 2001), yet there are only two differences in the C-terminal 12

amino acids of γ_1 and γ_{11} (see Figure 1A). Experiments performed with receptors reconstituted with G protein heterotrimers using various protocols show relatively modest differences in the interaction of the various γ subunits with receptors (Hou *et al.*, 2001; Akgoz *et al.*, 2002; Lim *et al.*, 2001; Yasuda *et al.*, 1996). However, antisense experiments performed with intact cells suggest an exquisite selectivity between receptors, α subunits and certain $\beta\gamma$ combinations (Kleuss *et al.*, 1993). Thus, in spite of considerable effort, the factors dictating the specificity that must exist in intact cells remain elusive. These data, combined with the studies performed in yeast, suggest that additional work with mammalian cells is needed to reconcile these differences. The diversity of the G β and G γ subunits in the mammalian genome will make these studies complex, but the observation that mice with the γ_7 subunit knocked out have an altered phenotype (Schwindinger *et al.*, 2003) offers hope that these studies will provide interesting results.

The data in Figure 3 indicate that, as with receptors, both the prenyl group and the C-terminal amino acids of the γ subunit contribute significantly to the interaction of the dimer with PLC- β in vesicles. This finding is consistent with experiments showing that native dimers containing the γ_1 or γ_{11} subunits have reduced activity on this effector (Myung *et al.*, 1999). In addition, experiments performed with dimers containing the γ_5 subunit modified to contain a shortened or extended C terminus are less effective at activating PLC- β (Akgoz *et al.*, 2002). A possible explanation for the role of the prenyl group in the activation of effectors may be provided by the hypothesis that the prenyl group on the γ subunit interacts with the C-terminal region of the β subunit and participates in a conformational change that, in part, determines the activity of the dimer at effectors (Myung and Garrison, 2000). Alternatively, the prenyl group may interact directly with PLC- β as suggested by experiments with prenylated peptides (Akgoz *et al.*, 2002). It is also possible that both mechanisms come into play. A more complete understanding of the role of this region of the β and γ subunits in these interactions awaits structural information or biophysical experiments determining the exact sites of contact between these regions of the dimer and receptors or effectors.

A surprising finding is that the C-terminal region of the γ subunit appears to predominate in the inhibition of type I adenylyl cyclase whereas the N-terminal region of the molecule is the most important for the activation of type II adenylyl cyclase. However, the regulation of adenylyl cyclase is complex, with multiple binding sites identified for the known regulatory molecules (Taussig *et al.*, 1994). The differences identified for regulation by the γ subunit in the dimer are in keeping with these findings. The observations that the N-terminal domain of γ is important for the activation of type II cyclase is consistent with earlier experiments showing that phosphorylation of the γ_{12} subunit in the $\beta_1\gamma_{12}$ dimer with protein kinase C decreases its ability to stimulate type II adenylyl cyclase (Yasuda *et al.*, 1998). The phosphorylation site in γ_{12} is Ser¹ at the N terminus (Asano *et al.*, 1998), suggesting that negative charges in this region decrease the interaction of the dimer with type II cyclase. Interestingly, the γ_1 , γ_8 and γ_{11} subunits contain six negatively charged amino acids in their N terminus (Figure 1A) and thus dimers containing these γ 's might be predicted to interact poorly with type II cyclase. Indeed, dimers containing γ_1 , γ_{11} or chimeras such as $\gamma_{ABB-L71S}$ or γ_{ABB} are not able to activate the enzyme well (see Table III and (Myung *et al.*, 1999)). Together, these results strongly suggest that negatively charged amino acids in the N terminus of the γ subunit lead to an inability to activate type II adenylyl cyclase *in vitro*. The observation that inhibition of the type I isoform of adenylyl cyclase is very dependent on the sequences in the opposite end of the γ subunit indicates that the different isoforms of adenylyl cyclase must bind to different regions of the β and γ subunits in the $\beta\gamma$ dimer. In keeping with this finding, mutations on the top surface of the β subunit that dramatically inhibit activation of type II cyclase do not alter the inhibition of type I cyclase (Li *et al.*, 1998). Thus, the precise determinants of the interaction of the type I and type II cyclases with $\beta\gamma$ dimers appear to be different.

The isoform of the β subunit is also important in the interaction of $\beta\gamma$ dimers with effectors and certain regions on the top surface of the β subunit have been identified as critical for interaction of the $\beta\gamma$ dimer with effectors (Ford *et al.*, 1998). A major concept arising from these studies is that while different effectors may interact with distinct or overlapping regions of the β subunit, certain regions in the β subunit interacting with

effectors are in the domain covered by the α subunit in the heterotrimer (Ford *et al.*, 1998). Thus, formation of the basal state of the α : $\beta\gamma$ -receptor complex is an efficient mechanism to terminate $\beta\gamma$ signaling. The data in Figure 2B shows that the C terminus of the γ subunit and its prenyl group are important determinants of the affinity of the α subunit for the $\beta\gamma$ dimer. Thus, this domain of the γ subunit may be important for the rapid formation of a high affinity, α : $\beta\gamma$ complex and which speeds reversal of the effects of the $\beta\gamma$ dimer on targets such as PLC- β or adenylyl cyclase. The present study also demonstrates that the nature of the N and C terminal regions of the γ subunit imparts significant specificity to $\beta\gamma$ signaling. Thus, $\beta\gamma$ dimers containing γ subunits from subfamily I such as γ_1 , or γ_{11} (and probably γ_8 , although it has not been studied extensively) appear to have unique signaling properties in that they are unable to activate type II adenylyl cyclase or other important effectors such as phosphatidylinositol (4,5) 3-kinase (Kerchner *et al.*, 2004). The distribution of γ_1 is restricted to retinal rods (Fung, 1983) and the original report of γ_8 (termed γ_c) suggested that it was restricted to cone cells (Ong *et al.*, 1995). However, the γ_{11} subunit is widely expressed (Morishita *et al.*, 1998) and it now appears that γ_8 is also widely expressed (Downes and Gautam, 1999). Thus, it is important to define the signaling properties of dimers containing these farnesylated γ subunits in multiple cellular systems. The appealing concept that certain $\beta\gamma$ dimers may associate selectively with distinct α subunits and/or receptors has been employed to account for the diversity of these subunits in the G protein family (McIntire *et al.*, 2001; Richardson and Robishaw, 1999; Cabrera-Vera *et al.*, 2003; Hildebrandt, 1997). This hypothesis also predicts that unique $\beta\gamma$ dimers will be released upon receptor activation in intact cells, allowing a given receptor to release a $\beta\gamma$ dimer with selective signaling properties. If the $\beta\gamma$ dimers released were from subfamily I, they might have very selective signaling properties at effectors. The data in this report support this idea, however future experiments will need to concentrate on the functions of this diverse family of proteins in intact cellular systems.

Acknowledgments

We would like to thank Dr. Ravi Iyengar for the baculoviruses encoding type I and type II adenylyl cyclases, Dr. T. K. Harden for the purified recombinant turkey phospholipase C- β , the University of Virginia Diabetes Core Facility for cyclic AMP assays and the Biomolecular Research Facility for DNA sequencing and mass spectrometric analysis. We also acknowledge Warren J. Clingan, III for excellent technical assistance.

References

- Akgoz M, Azpiazu I, Kalyanaraman V and Gautam N (2002) Role of the G Protein Gamma Subunit in Beta Gamma Complex Modulation of Phospholipase C β Function. *J Biol Chem* **277**: 19573-19578.
- Asano T, Morishita R, Ueda H, Asano M and Kato K (1998) GTP-Binding Protein γ 12 Subunit Phosphorylation by Protein Kinase C--Identification of the Phosphorylation Site and Factors Involved in Cultured Cells and Rat Tissues in Vivo. *Eur J Biochem* **251**: 314-319.
- Azpiazu I, Cruzblanca H, Li P, Linder M, Zhuo M and Gautam N (1999) A G Protein Gamma Subunit-Specific Peptide Inhibits Muscarinic Receptor Signaling. *J Biol Chem* **274**: 35305-35308.
- Butkerait P, Zheng Y, Hallak H, Graham T E, Miller H A, Burris K D, Molinoff P B and Manning D R (1995) Expression of the Human 5-Hydroxytryptamine $1A$ Receptor in Sf9 Cells. Reconstitution of a Coupled Phenotype by Co-Expression of Mammalian G Protein Subunits. *J Biol Chem* **270**: 18691-18699.
- Cabrera-Vera TM, Vanhauwe J, Thomas T O, Medkova M, Preininger A, Mazzoni M R and Hamm H E (2003) Insights into G Protein Structure, Function, and Regulation. *Endocr Rev* **24**: 765-781.
- Chinault SL and Blumer K J (2003) The C-Terminal Tail Preceding the CAAX Box of a Yeast G Protein Gamma Subunit Is Dispensable for Receptor-Mediated G Protein Activation in Vivo. *J Biol Chem* **278**: 20638-20644.
- Downes GB and Gautam N (1999) The G Protein Subunit Gene Families. *Genomics* **62**: 544-552.
- Ford CE, Skiba N P, Bae H, Daaka Y, Reuveny E, Shekter L R, Rosal R, Weng G, Yang C S, Iyengar R, Miller R J, Jan L Y, Lefkowitz R J and Hamm H E (1998) Molecular Basis for Interactions of G Protein $\beta\gamma$ Subunits With Effectors. *Science* **280**: 1271-1274.
- Fung BK (1983) Characterization of Transducin From Bovine Retinal Rod Outer Segments. I. Separation and Reconstitution of the Subunits. *J Biol Chem* **258**: 10495-10502.
- Graber SG, Figler R A and Garrison J C (1994) Expression and Purification of G-Protein Alpha Subunits Using Baculovirus Expression System. *Methods Enzymol* **237**: 212-226.
- Graber SG, Lindorfer M A and Garrison J C (1996) Expression and Purification of G Protein Beta Gamma Subunits Using a Baculovirus Expression System. *Methods Neurosci* **29**: 207-226.

- Hildebrandt JD (1997) Role of Subunit Diversity in Signaling by Heterotrimeric G Proteins. *Biochem Pharmacol* **54**: 325-339.
- Hou Y, Azpiazu I, Smrcka A and Gautam N (2000) Selective Role of G Protein Gamma Subunits in Receptor Interaction. *J Biol Chem* **275**: 38961-38964.
- Hou Y, Chang V, Capper A B, Taussig R and Gautam N (2001) G Protein Beta Subunit Types Differentially Interact With a Muscarinic Receptor but Not Adenylyl Cyclase Type II or Phospholipase C-Beta 2/3. *J Biol Chem* **276**: 19982-19988.
- Iniguez-Lluhi JA, Simon M I, Robishaw J D and Gilman A G (1992) G Protein Beta Gamma Subunits Synthesized in Sf9 Cells. Functional Characterization and the Significance of Prenylation of Gamma. *J Biol Chem* **267**: 23409-23417.
- Jian X, Clark W A, Kowalak J, Markey S P, Simonds W F and Northup J K (2001) Gbeta Gamma Affinity for Bovine Rhodopsin Is Determined by the Carboxyl- Terminal Sequences of the Gamma Subunit. *J Biol Chem* **276**: 48518-48525.
- Jian X, Sainz E, Clark W A, Jensen R T, Battey J F and Northup J K (1999) The Bombesin Receptor Subtypes Have Distinct G Protein Specificities. *J Biol Chem* **274**: 11573-11581.
- Kerchner KR, Clay R L, McCleery G, Watson N, McIntire W E, Myung C S and Garrison J C (2004) Differential Sensitivity of Phosphatidylinositol 3-Kinase P110gamma to Isoforms of G Protein Beta/Gamma Dimers. *J Biol Chem* **279**: 44554-44562.
- Kisselev O, Ermolaeva M and Gautam N (1995) Efficient Interaction With a Receptor Requires a Specific Type of Prenyl Group on the G Protein Gamma Subunit. *J Biol Chem* **270**: 25356-25358.
- Kleuss C, Scherubl H, Hescheler J, Schultz G and Wittig B (1993) Selectivity in Signal Transduction Determined by Gamma Subunits of Heterotrimeric G Proteins. *Science* **259**: 832-834.
- Lee C, Murakami T and Simonds W F (1995) Identification of a Discrete Region of the G Protein Gamma Subunit Conferring Selectivity in Beta Gamma Complex Formation. *J Biol Chem* **270**: 8779-8784.
- Li Y, Sternweis P M, Charnecki S, Smith T F, Gilman A G, Neer E J and Kozasa T (1998) Sites for Galpha Binding on the G Protein Beta Subunit Overlap With Sites for Regulation of Phospholipase Cbeta and Adenylyl Cyclase. *J Biol Chem* **273**: 16265-16272.
- Lim WK, Myung C S, Garrison J C and Neubig R R (2001) Receptor-G Protein Gamma Specificity: Gamma11 Shows Unique Potency for A(1) Adenosine and 5-HT(1A) Receptors. *Biochemistry* **40**: 10532-10541.
- Lindorfer MA, Sherman N E, Woodfork K A, Fletcher J E, Hunt D F and Garrison J C (1996) G Protein Gamma Subunits With Altered Prenylation Sequences Are Properly Modified When Expressed in Sf9 Cells. *J Biol Chem* **271**: 18582-18587.

- McIntire WE, MacCleery G and Garrison J C (2001) The G Protein Beta Subunit Is a Determinant in the Coupling of Gs to the Beta 1-Adrenergic and A2a Adenosine Receptors. *J Biol Chem* **276**: 15801-15809.
- McIntire WE, Myung C S, MacCleery G, Wang Q and Garrison J C (2002) Reconstitution of G Protein-Coupled Receptors With Recombinant G Protein Alpha and Beta Gamma Subunits. *Methods Enzymol* **343**: 372-393.
- Morishita R, Ueda H, Kato K and Asano T (1998) Identification of Two Forms of the Gamma Subunit of G Protein, Gamma10 and Gamma11, in Bovine Lung and Their Tissue Distribution in the Rat. *FEBS Lett* **428**: 85-88.
- Motulsky HJ and Ransnas L A (1987) Fitting Curves to Data Using Nonlinear Regression: a Practical and Nonmathematical Review. *FASEB J* **1**: 365-374.
- Mumby SM and Linder M E (1994) Myristoylation of G-Protein Alpha Subunits. *Methods Enzymol* **237**: 254-268.
- Myung CS and Garrison J C (2000) Role of C-Terminal Domains of the G Protein Beta Subunit in the Activation of Effectors. *Proc Natl Acad Sci U S A* **97**: 9311-9316.
- Myung CS, Yasuda H, Liu W W, Harden T K and Garrison J C (1999) Role of Isoprenoid Lipids on the Heterotrimeric G Protein Gamma Subunit in Determining Effector Activation. *J Biol Chem* **274**: 16595-16603.
- Ong OC, Yamane H K, Phan K B, Fong H K, Bok D, Lee R H and Fung B K (1995) Molecular Cloning and Characterization of the G Protein Gamma Subunit of Cone Photoreceptors. *J Biol Chem* **270**: 8495-8500.
- Richardson M and Robishaw J D (1999) The Alpha2A-Adrenergic Receptor Discriminates Between Gi Heterotrimers of Different Betagamma Subunit Composition in Sf9 Insect Cell Membranes. *J Biol Chem* **274**: 13525-13533.
- Sarvazyan NA, Remmers A E and Neubig R R (1998) Determinants of Gi1alpha and Beta Gamma Binding. Measuring High Affinity Interactions in a Lipid Environment Using Flow Cytometry. *J Biol Chem* **273**: 7934-7940.
- Schwindinger WF, Betz K S, Giger K E, Sabol A, Bronson S K and Robishaw J D (2003) Loss of G Protein Gamma 7 Alters Behavior and Reduces Striatal Alpha(Olf) Level and CAMP Production. *J Biol Chem* **278**: 6575-6579.
- Sondek J, Bohm A, Lambright D G, Hamm H E and Sigler P B (1996) Crystal Structure of a G-Protein Beta Gamma Dimer at 2.1A Resolution. *Nature* **379**: 369-374.
- Taussig R, Tang W J, Hepler J R and Gilman A G (1994) Distinct Patterns of Bidirectional Regulation of Mammalian Adenylyl Cyclases. *J Biol Chem* **269**: 6093-6100.
- Welch HC, Coadwell W J, Ellson C D, Ferguson G J, Andrews S R, Erdjument-Bromage H, Tempst P, Hawkins P T and Stephens L R (2002) P-Rex1, a PtdIns(3,4,5)P3- and

Gbetagamma-Regulated Guanine-Nucleotide Exchange Factor for Rac. *Cell* **108**: 809-821.

Yasuda H, Lindorfer M A, Myung C S and Garrison J C (1998) Phosphorylation of the G Protein Gamma12 Subunit Regulates Effector Specificity. *J Biol Chem* **273**: 21958-21965.

Yasuda H, Lindorfer M A, Woodfork K A, Fletcher J E and Garrison J C (1996) Role of the Prenyl Group on the G Protein Gamma Subunit in Coupling Trimeric G Proteins to A1 Adenosine Receptors. *J Biol Chem* **271**: 18588-18595.

Footnotes

*This work was supported by a Postdoctoral Fellowship from the Virginia Affiliate of the American Heart Association (to C.-S. Myung), a training grant from the Universiti Malaysia Sarawak (to W.K. Lim) and the National Institutes of Health Grants CA40042, DK19952 (to J.C. Garrison) and HL46417 (to R.R. Neubig). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be marked "*advertisement*" in accordance with 18 U.S.C section 1734 solely to indicate this fact.

‡ Address reprint requests to:

James C. Garrison
Department of Pharmacology
University of Virginia Health Sciences Center
P.O. Box 800735
1300 Jefferson Park Avenue
Charlottesville, Virginia 22908-0735
Phone: (804) 924-5618
E-mail: jcg8w@virginia.edu

Figure Legends

Fig. 1. Strategy for constructing the chimeric γ subunits. **(A)**, Comparison of the primary sequences and charged amino acids in the N- and C-terminal regions of the γ_1 and γ_{11} subunits with those of γ_2 . The QLK and DPL sites and the C-terminal CAAX motif are highlighted. The center section of the molecules is omitted. **(B)**, Schematic view of the chimeric γ subunits. The sequence of the γ_1 subunit is indicated in *white*, and the γ_2 subunit in *grey*. Both the γ_1 and γ_2 subunits are divided into three regions at the QLK and DPL sites. The molecules were designated as AAA for the γ_1 subunit and BBB for the γ_2 subunit. The farnesyl group (C-15) on the γ_1 subunit is indicated in *light grey*, and the geranylgeranyl group (C-20) on the γ_2 subunit is indicated in *dark grey*. The nomenclature used in the text to identify each chimera is indicated above the schematic representation.

Fig. 2. Comparison of the ability of native $\beta\gamma$ dimers and those containing chimeric γ subunits to support the high affinity, agonist binding state of the A1 adenosine receptor. **(A)**, Sf9 cell membranes expressing recombinant, bovine A1 adenosine receptors were reconstituted with the Gi1 α subunit and the $\beta\gamma$ dimer at concentrations of 0 nM, 2 nM, 6 nM or 40 nM. The ratio of receptor : α : $\beta\gamma$ was approximately 1:25. The amount of agonist binding to the receptor was measured with graded concentrations of ^{125}I -ABA as described in *Experimental Procedures*. The figure indicates the recovery of high-affinity, agonist binding conformation of the receptor as a function of the total amount of G protein heterotrimer reconstituted into the membrane. Each data point is an average of three similar experiments performed in triplicate. **(B)**, A plot of the affinity of the Gi1 α subunit for eight different $\beta\gamma$ dimers measured in the flow cytometer against the EC₅₀'s for formation of the high affinity, agonist binding state of the receptor measured at 6 nM Gi1 α and concentrations of $\beta\gamma$ ranging from 0-100 nM. Each data point is an average of three similar experiments performed in triplicate. Details of both assays are found in *Experimental Procedures*. The open squares represent dimers with farnesyl moieties and the closed squares represent dimers with geranylgeranyl moieties.

Fig. 3. Comparison of the ability of native $\beta\gamma$ dimers and those containing chimeric γ subunits to stimulate PLC- β . **(A)**, The indicated concentrations of $\beta\gamma$ dimers were reconstituted with recombinant, turkey PLC- β in phospholipid vesicles containing [3 H]-PIP $_2$ and PLC- β activity was measured as described in *Experimental Procedures*. The activity of $\beta 1\gamma$ ABB (*open triangles*) was compared with $\beta 1\gamma 2$ (*closed squares, thick solid line*) and $\beta 1\gamma 1$ (*open circles, thick dotted line*). The effect of the $\beta 1\gamma$ ABB dimer was statistically different from $\beta 1\gamma 1$ ($p < 0.0001$; see Table II), but not from $\beta 1\gamma 2$. **(B)**, An analogous experiment performed with $\beta 1\gamma 1$ -S74L and $\beta 1\gamma$ BAA-S74L and compared with the effects of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. See Table II for the activity of dimers containing other chimeric γ subunits. **(C)**, An analogous experiment performed with $\beta 1\gamma$ BBA and compared with the effect of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. The effect of the $\beta 1\gamma$ BAA dimer was statistically different from $\beta 1\gamma 2$, but not from $\beta 1\gamma 1$ ($p < 0.0001$; see Table II). Each data point is an average of three independent experiments, each performed in duplicate.

Fig. 4. Comparison of the ability of native $\beta\gamma$ dimers and those containing chimeric γ subunits to inhibit type I adenylyl cyclase. **(A)**, Sf9 cells were infected with a recombinant baculovirus encoding the type I adenylyl cyclase, membranes prepared, and the cyclase reaction performed with the indicated concentrations of $\beta\gamma$ dimers. Cyclic AMP was measured as described in *Experimental Procedures*. The ability of $\beta 1\gamma$ AAB (*closed triangles*) to inhibit type I cyclase was compared with $\beta 1\gamma 2$ (*closed squares, thick solid line*) and $\beta 1\gamma 1$ (*open circles, thick dotted line*). The effect of $\beta 1\gamma$ AAB was statistically significant different from $\beta 1\gamma 1$ but not from $\beta 1\gamma 2$ (see Table II). Each data point is an average of three independent experiments, each performed in duplicate. **(B)** An analogous experiment performed with $\beta 1\gamma$ AAB-L71S (*closed triangles*) and compared with the effect of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. Each data point is an average of three independent experiments, each performed in duplicate. **(C)**, An analogous experiment performed with $\beta 1\gamma$ BBA (*open triangles*) and compared with the effect of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. The effect of the $\beta 1\gamma$ BBA dimer was statistically different from $\beta 1\gamma 2$, but not from $\beta 1\gamma 1$ ($p < 0.0001$; see Table II). Each data point is an average of three independent experiments, each performed in duplicate.

Fig. 5. Comparison of the ability of native $\beta\gamma$ dimers and those containing chimeric γ subunits to stimulate type II adenylyl cyclase. **(A)**, Sf9 cells were infected with a recombinant baculovirus encoding the type II adenylyl cyclase, membranes prepared, and the cyclase reaction performed with the indicated concentrations of $\beta\gamma$ dimers as described in *Experimental Procedures*. The ability of $\beta 1\gamma$ BAA-S74L (*closed diamonds*) to activate type II adenylyl cyclase was compared with $\beta 1\gamma 2$ (*closed squares, thick solid line*) and $\beta 1\gamma 1$ (*open circles, thick dotted line*). The effect of $\beta 1\gamma$ BAA-S74L was statistically significant different from $\beta 1\gamma 1$ (see Table III). **(B)** An analogous experiment performed with $\beta 1\gamma$ BAA and $\beta 1\gamma$ BBA and compared with the effects of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. Each data point is an average of three independent experiments, each performed in duplicate. **(C)** An analogous experiment performed with $\beta 1\gamma$ ABB-L71S (*closed circles*) and compared with the effects of $\beta 1\gamma 1$ and $\beta 1\gamma 2$. The difference between the effect of $\beta 1\gamma$ ABB-L71S and $\beta 1\gamma 2$ was statistically significant (see Table III). Each data point is an average of three independent experiments, each performed in duplicate.

Table I
Molecular mass of recombinant, chimeric γ subunits.

The molecular weight of the γ subunits in the dimers used in this study was measured by mass spectrometry as described “*Experimental Procedures*”. Molecular masses were determined on two independent preparations of each $\beta\gamma$ dimer and were in agreement. The data presented are from one determination.

	γ Subunits	Calculated Mass (Da)	Observed Mass (Da)
Native	γ_1	8,332	8,330
	γ_2	7,751	7,751
Exchanged Prenyl Group	γ_{1-S74L}	8,400	8,400
	γ_{2-L71S}	7,683	7,681
C-Terminal Chimeras	γ_{AAB}	8,551	8,552
	$\gamma_{AAB-L71S}$	8,483	8,482
	γ_{BBA}	7,532	7,531
	$\gamma_{BBA-S74L}$	7,600	7,602
N-Terminal Chimeras	γ_{BAA}	7,818	7,818
	$\gamma_{BAA-S74L}$	7,886	7,888
	γ_{ABB}	8,265	8,265
	$\gamma_{ABB-L71S}$	8,197	8,199

Table II

Comparison of the ability of $\beta\gamma$ dimers containing native or chimeric γ subunits to activate PLC- β or to inhibit Type I adenylyl cyclase. The EC_{50} , IC_{50} , or V_{max} values for PLC obtained with the indicated $\beta\gamma$ dimers were determined by fitting each data set to sigmoid curves as described in "Experimental Procedures." V_{inhib} is the level of cyclic AMP production (as nmole cyclic AMP/mg membrane protein/min) observed with $10^{-7}M$ of the indicated $\beta\gamma$ dimer. Control levels of ACI activity were 0.9 nmoles cyclic AMP/mg protein/min. Values are the average of three similar experiments expressed as means \pm S.E.M.

Type	$\beta\gamma$ Dimers	Phospholipase C- β		Type I Adenylyl Cyclase	
		EC_{50} (nM)	V_{max} (μ mol/mg PLC/min)	IC_{50} (nM)	V_{inhib} (nmol/mg protein/min)
Chimeras with activity similar to $\beta_1\gamma_2$	$\beta_1\gamma_2$	5.2 \pm 1.1	3.40 \pm 0.07	15.4 \pm 1.4	0.26 \pm 0.06
	$\beta_1\gamma_{ABB}$	6.2 \pm 1.2 [†]	3.24 \pm 0.13 [†]	18.1 \pm 2.2 [†]	0.34 \pm 0.24 [†]
	$\beta_1\gamma_{AAB}$	7.8 \pm 1.1 [†]	3.16 \pm 0.09 [†]	14.2 \pm 1.3 [†]	0.39 \pm 0.03 [†]
Chimeras with activity similar to $\beta_1\gamma_1$	$\beta_1\gamma_1$	24.6 \pm 1.2	2.14 \pm 0.16	36.4 \pm 2.6	0.58 \pm 0.09
	$\beta_1\gamma_{BBA}$	51.2 \pm 1.3 [*]	2.57 \pm 0.29 [*]	96.0 \pm 15.1 [*]	0.48 \pm 0.28 [*]
	$\beta_1\gamma_{BAA}$	25.8 \pm 1.3 [*]	1.95 \pm 0.15 [*]	60.7 \pm 6.6 [*]	0.49 \pm 0.25 [*]
	$\beta_1\gamma_{AAB-L71S}$	19.1 \pm 1.3 [§]	2.32 \pm 0.13 [§]	24.6 \pm 1.5 [§]	0.54 \pm 0.03 [§]
Chimeras with Intermediate activity	$\beta_1\gamma_1$ -S74L	15.9 \pm 1.2 [§]	3.21 \pm 0.20 [§]	18.5 \pm 1.7 [§]	0.53 \pm 0.04 [§]
	$\beta_1\gamma_2$ -L71S	15.0 \pm 1.2 [§]	2.83 \pm 0.14 [§]	26.2 \pm 2.6 [§]	0.43 \pm 0.15 [§]
	$\beta_1\gamma_{BBA}$ -S74L	12.2 \pm 1.5 [§]	2.94 \pm 0.22 ^{§§}	16.2 \pm 2.2 [§]	0.51 \pm 0.08
	$\beta_1\gamma_{BAA}$ -S74L	12.2 \pm 1.2 [§]	2.34 \pm 0.22 ^{§§}	22.9 \pm 1.9 [§]	0.37 \pm 0.14
	$\beta_1\gamma_{ABB}$ -L71S	15.6 \pm 1.3 [§]	2.66 \pm 0.17 [§]	26.1 \pm 2.2 [§]	0.62 \pm 0.11 [§]

* Significant differences in responses to $\beta\gamma$ dimers in comparison with native $\beta_1\gamma_2$; * $p < 0.0001$.

[†] Significant differences in responses to $\beta\gamma$ dimers in comparison with native $\beta_1\gamma_1$; [†] $p < 0.0001$.

[§] Significant differences in responses to $\beta\gamma$ dimers containing γ subunits altered CAAX sequence to direct modification with different prenyl groups with those containing γ subunits with native prenyl groups; [§] $p < 0.0001$, ^{§§} $p < 0.001$.

Table III

Comparison of the ability of $\beta\gamma$ dimers containing native or chimeric γ subunits to activate Type II adenylyl cyclase. The EC_{50} and V_{max} values obtained with each $\beta\gamma$ dimer was determined by fitting each data set to sigmoid curves as described in "Experimental Procedures." Values are the average of three similar experiments expressed as means \pm S.E.M.

Type	$\beta\gamma$ Dimers	Type II Adenylyl Cyclase	
		EC_{50} (nM)	V_{max} ($\mu\text{mol}/\text{mg protein}/\text{min}$)
Chimeras with activity similar to $\beta_1\gamma_2$	$\beta_1\gamma_2$	13.3 \pm 1.2	29.05 \pm 1.72
	$\beta_1\gamma\text{BAA-S74L}$	15.6 \pm 1.2 [§]	27.8 \pm 1.74 [§]
	$\beta_1\gamma\text{BBA-S74L}$	18.2 \pm 1.4 [§]	24.07 \pm 1.85 [§]
Chimeras with activity similar to $\beta_1\gamma_1$	$\beta_1\gamma_1$	46.7 \pm 1.9	6.31 \pm 0.65
	$\beta_1\gamma\text{ABB-L71S}$	48.7 \pm 2.1 [§]	6.89 \pm 1.09 [§]
	$\beta_1\gamma\text{AAB}$	61.8 \pm 2.0 [*]	9.13 \pm 2.31 [*]
	$\beta_1\gamma\text{AAB-L71S}$	93.3 \pm 11.9 [§]	25.91 \pm 9.45 [§]
	$\beta_1\gamma_1\text{-S74L}$	33.1 \pm 1.6 ^{§§}	8.39 \pm 1.68
Chimeras with Intermediate activity	$\beta_1\gamma\text{BAA}$	24.6 \pm 1.3 [†]	13.52 \pm 1.11 [†]
	$\beta_1\gamma\text{BBA}$	35.7 \pm 1.2 ^{††}	14.05 \pm 1.07 [†]
	$\beta_1\gamma_2\text{-L71S}$	41.7 \pm 1.3 [§]	16.19 \pm 1.3 [§]
	$\beta_1\gamma\text{ABB}$	25.2 \pm 1.3 [*]	23.17 \pm 1.66 ^{††}

* Significant differences in responses to $\beta\gamma$ dimers in comparison with native $\beta_1\gamma_2$; * $p < 0.0001$, ** $p < 0.001$.

† Significant differences in responses to $\beta\gamma$ dimers in comparison with native $\beta_1\gamma_1$; † $p < 0.0001$, †† $p < 0.001$.

§ Significant differences in responses to $\beta\gamma$ dimers containing γ subunits altered CAAX sequence to direct modification with different prenyl groups with those containing γ subunits with native prenyl groups; § $p < 0.0001$, §§ $p < 0.001$.

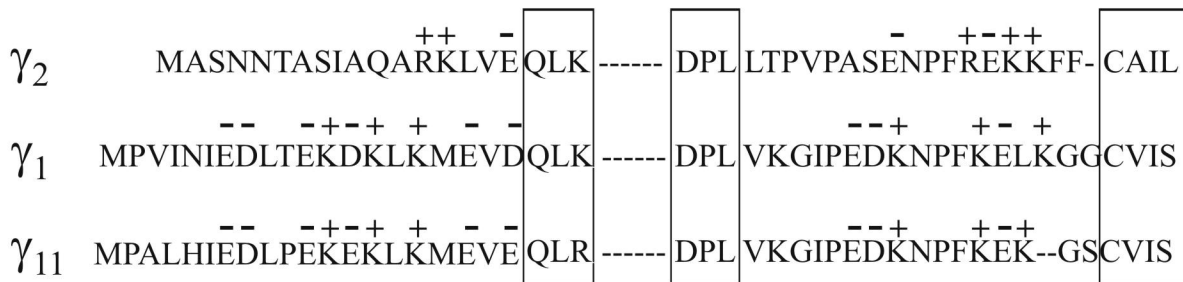
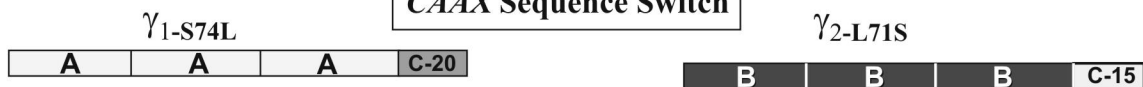
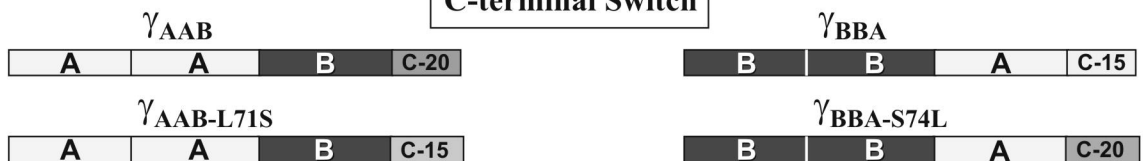
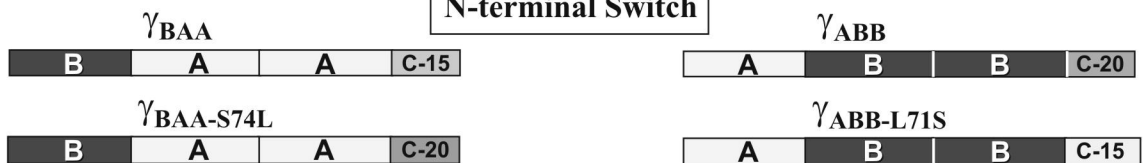
Figure 1**A****N-Terminus****C-Terminus****B****Native γ Subunits****CAAX Sequence Switch****C-terminal Switch****N-terminal Switch**

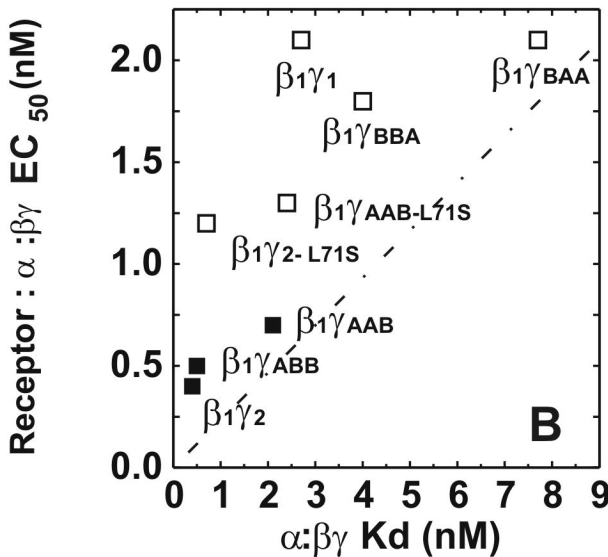
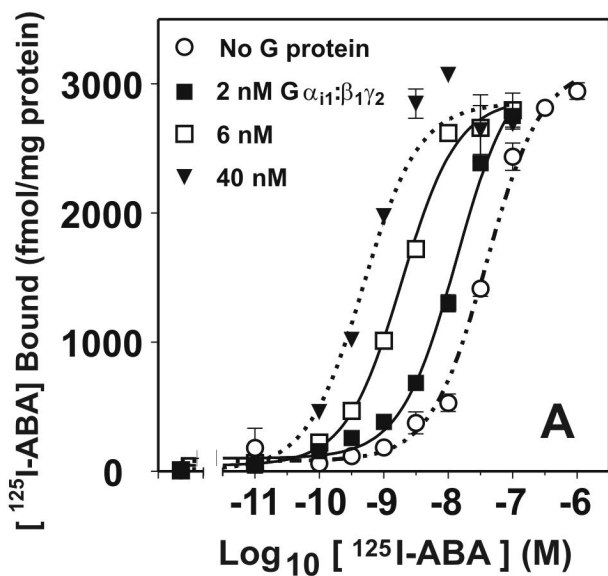
Figure 2**Adenosine A1 Receptor**

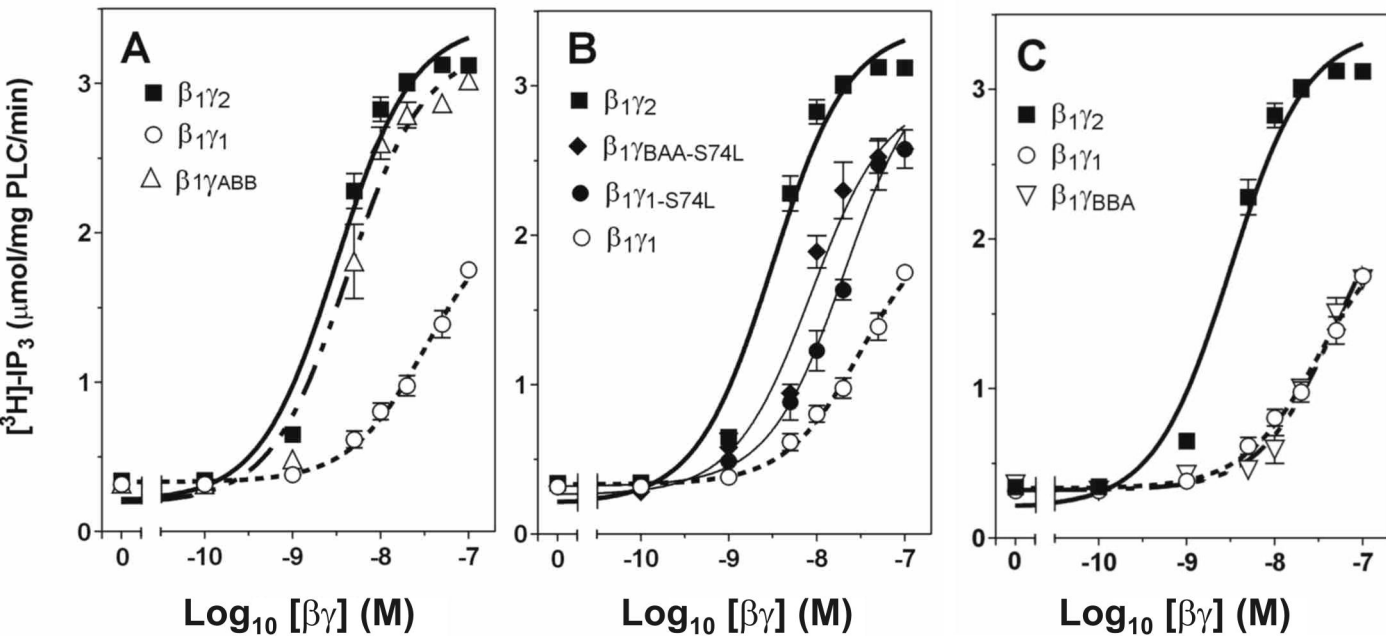
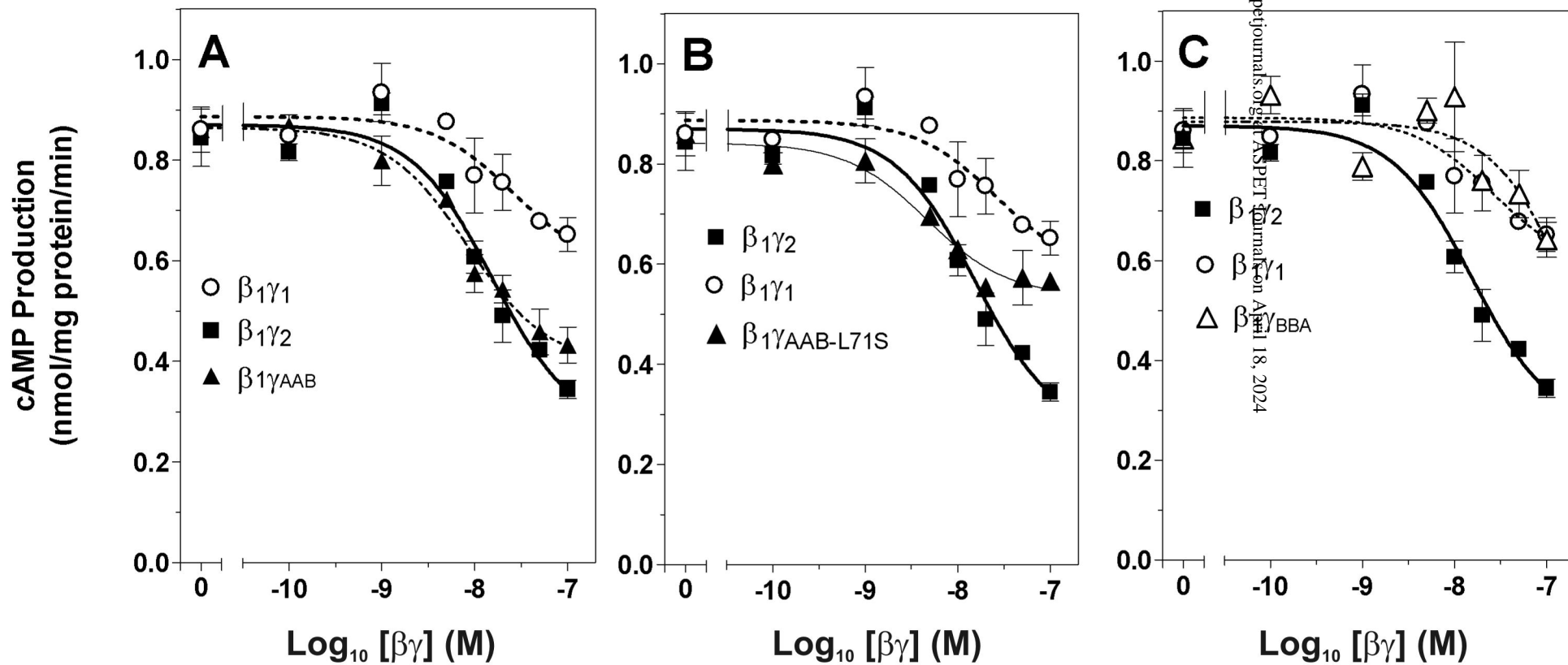
Figure 3PLC- β 

Figure 4

Type I Cyclase



from molpharm.aspetjournals.org on April 18, 2024

Figure 5

Type II Cyclase

