Isolation of cDNA Clones Encoding Eight Different Human G Protein γ Subunits, Including Three Novel Forms Designated the γ4, γ10, and γ11 Subunits*

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With the growing awareness that the G protein β and γ subunits directly regulate the activities of various enzymes and ion channels, the importance of identifying and characterizing these subunits is underscored. In this paper, we report the isolation of cDNA clones encoding eight different human γ subunits, including three novel forms designated γ4, γ10, and γ11. The predicted protein sequence of γ4 shares the most identity (60–77%) with γ2, γ3, and γ7 and the least identity (38%) with γ11. The γ4 is modified by a geranylgeranyl group and is capable of interacting with both β1 and β2 but not with β3. The predicted protein sequence of γ11 shows only modest to low identity (33–53%) with the other known γ subunits, with most of the differences concentrated in the N-terminal region, suggesting γ11 may interact with a unique subclass of α. The γ10 modified by a geranylgeranyl group is capable of interacting with β1 and β2 but not with β3. Finally, the predicted protein sequence of γ11 shows the most identity to γ1 (76% identity) and the least identity to the other known γ (33–44%). Unlike most of the other known γ subunits, g11 is modified by a farnesyl group and is not capable of interacting with β2. The close resemblance of γ11 to γ1 raises intriguing questions regarding its function since the mRNA for γ11 is abundantly expressed in all tissues tested except for brain, whereas the mRNA for γ1 is expressed only in the retina where the protein functions in phototransduction.

Intracellular transmission of extracellular signals are most commonly mediated by a family of guanine nucleotide-binding proteins (G proteins) that couple with various receptors and effectors to produce appropriate cellular responses. The G proteins are heterotrimers, composed of α, β, and γ subunits. In response to binding of the appropriate ligand, the receptor stimulates the exchange of bound GDP for GTP on the α subunit, resulting in the dissociation of the α subunit from the β and γ subunits. The GTP-bound α subunit has been shown to directly regulate the activity of downstream effectors (1–3). Recently, it has been shown that the βγ subunits, which exist as a tightly associated complex in vivo (1), can also regulate the activity of a specific subset of downstream effectors, including adenyl cyclase subtypes II and IV, phospholipase A2, phospholipase C subtypes β1, 2, and 3, and K+ and Ca2+ channels (4–6). Thus, the G protein α and βγ subunits produce bifurcating signals that regulate effector function. Moreover, the βγ subunits can directly bind to receptors (7) and can increase agonist-dependent phosphorylation and desensitization of receptors by directly interacting and recruiting the β-adrenergic receptor kinases to the membrane (8–9). Thus, the βγ subunits play prominent roles in both effector regulation and receptor recognition. As the number of α, β, and γ subunits continues to grow, the task of unraveling the subunit composition and function of individual G proteins is becoming more complex.

Both the β and γ subunits belong to large multigene families. Complete cDNAs encoding five distinct mammalian β subunits (β1–β5) have been identified thus far (10). A rat heart cDNA identified recently may encode a sixth β subunit, which is 96% identical to the human β5 subunit (11). At the amino acid level, the β subunits are highly conserved. In contrast, the γ subunits are much more divergent, suggesting this may determine the functional specificity of the βγ subunit complex. Complete cDNAs representing five different γ subunits have been reported with the isolation of the γ1 subunit from bovine retina (12), the γ2, γ3, and γ7 subunits from bovine brain (13–16), and the γ11 subunit from bovine and rat liver (17). The existence of a putative γ4 subunit has also been reported with the isolation of a PCR2 fragment from mouse kidney and retina (15). In the present paper, we report the isolation and characterization of cDNA clones encoding the human homologs of the five known γ1, γ2, γ3, γ5, and γ7 subunits as well as three previously unknown γ4, γ10, and γ11 subunits. Comparison of the γ4, γ10, and γ11 subunits reveals some interesting amino acid homologies. Of particular interest, the γ11 subunit shows only a low level of homology (35–53%) with the other γ subunits, suggesting the existence of a new subclass of γ subunits. On the other hand, the γ11 subunit shows a high level of homology (76%) to the γ1 subunit. This close resemblance to the γ1 subunit raises important questions regarding the function of the γ11 subunit since the mRNA for γ11 is expressed in a wide variety of tissues, whereas the mRNA for γ1 is expressed only in the retina where

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1 The trend in the literature has been to name the γ subunits in the order in which they were cloned. Accordingly, the γ6 subunit (13) has been renamed the γ7 subunit.

2 The abbreviations used are: PCR, polymerase chain reaction; EST, expressed sequence tag; FPP, farnesyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate; UTR, untranslated region; bp, base pair(s); kb, kilobase(s).

3 The cloning and sequencing of two new γ subunits, which are distinct from any of the γ subunits described in this manuscript, were described while this manuscript was under review (45, 46).
it functions in phototransduction. In addition to presenting their cDNA and deduced amino acid sequences, we examine the tissue distribution of the γ4, γ10, and γ11 subunits and show their selective interactions with the β1, β2, and β3 subunits.

**EXPERIMENTAL PROCEDURES**

Isolation and Analysis of cDNA Clones Encoding Human G Protein γ Subunits—To obtain cDNAs encoding the human G protein γ subunits, a human cDNA data base consisting of approximately 300,000 expressed sequence tags (ESTs) was searched for homologous sequences to the known bovine, rat, and mouse γ subunits by BLASTN and TBLASTN sequence alignment algorithms (18). The EST method involves automated DNA sequence analysis of random cDNA clones (19, 20) from a variety of tissue- or cell-specific cDNA libraries. For each human gene, several ESTs were identified from multiple cDNA libraries. These cDNA libraries were constructed by cloning digested(DT)-primed cDNAs into the cloning vector pBluescript II SK(Stratagene). Nearly all of the identified ESTs originated from a full-length cDNA. For each human γ clone, a single cDNA was chosen, sequenced to completion, and used for further study.

Northern Blot Hybridization—A Northern blot containing 2 μg of poly(A)+ mRNA prepared from several human tissues (Clontech) was hybridized at 42°C in 50% formamide, 3×SSPE (20×SSPE = 3 M NaCl, 0.2 M sodium phosphate, 0.02 M EDTA, pH 7.4), 0.1% Ficoll, 0.1% sodium chloride, 0.1% polyvinylpyrrolidone, 0.1% bovine albumin serum, 2% SDS, and 10 μg/ml sheared salmon sperm DNA. Fragments of the γ4, γ10, and γ11 cDNAs were isolated by double digestion of the corresponding cDNA clones in pBluescript vector with EcoRI and XhoI restriction enzymes. Probes were generated from the purified fragments by random priming with the Klenow fragment of DNA polymerase I in the presence of [32P]dCTP (3,000 Ci/mmol, Amersham). After hybridization, high stringency washes were performed at 65°C in 0.1×SSC (1×SSC is 0.15 M sodium chloride, 0.015 M sodium citrate), 0.1% SDS. Blots were exposed for the indicated times at −80°C with an intensifying screen.

**TABLE I**

| Bov | Bov | Bov | Bov | Bov | Mse | Mse |
|-----|-----|-----|-----|-----|-----|-----|
| y4  | y5  | γ2  | γ3  | γ4  | γ4  | γ4  |
| 71  | 64  | 50  | 67  | 67  | 67  | 67  |
| 72  | 64  | 64  | 50  | 50  | 67  | 67  |
| 73  | 64  | 64  | 64  | 64  | 64  | 64  |
| 74  | 64  | 64  | 64  | 64  | 64  | 64  |
| 75  | 64  | 64  | 64  | 64  | 64  | 64  |
| 76  | 64  | 64  | 64  | 64  | 64  | 64  |

| y1  | y2  | y3  | y4  | y5  | y6  | y7  | y8  | y9  | y10 | y11 |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 71  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |
| 72  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |
| 73  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |
| 74  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |
| 75  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |
| 76  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  | 60  |

Identification of three novel γ subunits by identity comparison of the newly cloned human γ subunits with known γ subunits from other species Numbers represent percentage identity calculated with DNA Star using the Needleman-Wunsch method. For the γ4 subunit, the percentage identity is calculated based on the partial sequence of a PCR fragment from mouse. The sequences of two additional γ subunits, γ6 and γ10, have been identified by other investigators (45, 46).

Results

Isolation and Classification of cDNA Clones Encoding Human G Protein γ Subunits—In the course of a large scale DNA sequencing project dedicated to the identification and characterization of ESTs from the human genome, approximately 300,000 cDNA clones were partially sequenced from a variety of tissue- or cell-specific cDNA libraries. Comparison of this data base with the protein sequence data base revealed at least 49 ESTs, originating from eight distinct human genes, that showed significant identity to bovine, rat, and mouse cDNA clones encoding the γ subunits. Complete sequencing of a representative cDNA clone for each of the eight groups allowed a comparison with the known γ subunits from other species (Table I). Based on their striking identity at the amino acid level (97–100%), we determined that five of the eight cDNA clones represent the human homologs of the γ1, γ2, γ3, γ4, and γ11 subunits previously cloned from other species. Further, we concluded that the remaining three of the eight cDNA clones represent novel γ subunits based on the findings that 1) these cDNA clones encode proteins that show a significantly lower degree of identity to known γ subunits at the amino acid level (30–97% identity) and 2) the amino acid differences that were present were distributed throughout the proteins, indicating that they did not arise by alternative splicing of known γ subunits. Since the predicted amino acid sequence of one of the three cDNA clones showed marked identity (97%) to a PCR fragment of a putative mouse γ4 subunit (15), we believe that this cDNA clone represents the human homolog of the mouse γ4 subunit, which has never been cloned. Accordingly, these cDNA clones were designated the γ4, γ10, and γ11 subunits.1,3
cDNAs Encoding Eight Different Human G Protein Subunits

**Fig. 1. Nucleotide and predicted amino acid sequence of the human γ4 subunit.** The first ATG codon of the open reading frame starts at position 99. Amino acids are denoted by single-letter codes. cDNA reported here was isolated from the adrenal gland tumor library. The cDNA includes 98 and 365 bp of 5'- and 3'-untranslated (UTR) sequences, respectively (Fig. 1). The first ATG codon at position 99 has the characteristics of a translation initiation codon with the expected purines at positions +3 and +4 (23). A second ATG codon at position 111 lacks the expected purines, making it less likely to be the initiation codon. A typical polyadenylation signal (AATAAA) was not found, but a poly(A) tail toward the 3'-end revealed significant homology (Fig. 4). The 3'-UTR contains a polyadenylation signal (AATAAA) underscored with solid lines.

**Fig. 2. Nucleotide and predicted amino acid sequence of the human γ10 subunit.** The open reading frame starts at position 24. Amino acids are denoted by single-letter codes. A potential polyadenylation signal (AATAAAA) is underscored with solid lines.

**Fig. 3. Nucleotide and predicted amino acid sequence of the human γ11 subunit.** The open reading frame starts at position 108. Amino acids are denoted by single-letter codes. A potential polyadenylation signal (AATAAAA) is underscored with solid lines.

Comparison of γ Subunits—Comparison of the predicted protein sequences of the newly identified γ4, γ10, and γ11 subunits to the human homologs of the γ2, γ3, γ10, and γ11 subunits revealed significant homology (Fig. 4). For the γ4 subunit, the homology ranged from a low of 38% for the γ11 subunit to a high of 77% for the γ2 subunit. For the γ10 subunit, the homology ranged from a low of 35% for the γ5 subunit to a high of only 53% for the γ2, γ3, and γ11 subunits. This relatively low level of homology suggests the γ10 subunit may represent a new sub-class that is only distantly related to the other γ subunits. Finally, for the γ11 subunit, the homology ranged from a low of 33 to 44% for the γ2, γ3, and γ11 subunits to a high of 76% for the γ11 subunit. This close resemblance to the γ11 subunit is particularly interesting since the γ11 subunit is the most divergent of the γ subunits identified thus far.

Most of the homology among the γ subunits was concentrated in several discrete regions (Fig. 4). The N-terminal region of the γ4, γ10, and γ11 subunits is the most divergent at the amino acid level (region I), consistent with the newly defined role of this region in determining the specificity of the interaction between the γ and α subunits (25). An internal region of 14 amino acids, which has been implicated in determining the specificity of the interaction between the γ and β subunits (26), is conserved to varying degrees between the γ4, γ10, and γ11 subunits (region II). Finally, the C-terminal region containing the CAAX sequence (C = cysteine; A = aliphatic; X = leucine, serine, or methionine), which has been shown to direct prenylation and carboxyl-methylation of these proteins (27, 28), is conserved in the γ4, γ10, and γ11 subunits (region III). Three other regions that are highly conserved in mammalian and Drosophila γ subunits (29) are also conserved in the γ4, γ10, and γ11 subunits (regions A, B, C). Although their roles have not yet been established, these regions may be important in generating similar conformations of the γ subunits.

Prenylation of the γ4, γ10, and γ11 Subunits—Prenylation is a post-translational modification that involves the addition of a C15 farnesyl or a C20 geranylgeranyl group to proteins terminating in a CAAX sequence. To determine the type of prenyl group added to these proteins, cDNAs for the γ4, γ10, and γ11 subunits were transcribed and translated in the TNT-coupled rabbit reticulocyte system, which has been shown to possess the necessary enzymes for utilizing FPP or GGPP as precursors for the prenylation reaction (28). As shown in Fig. 5A, translation in the presence of [35S]methionine gave protein products...
that are highly conserved in all expressed at high levels in heart, placenta, lung, skeletal muscle, kidney, and pancreas, at lower levels in liver, and at undetectable levels in brain. Thus, in contrast to both the \( \gamma_4 \) and \( \gamma_{10} \) subunits, the two mRNA species encoding the \( \gamma_{11} \) subunit are not expressed at detectable levels in brain but are expressed in several other tissues. The lack of expression of the \( \gamma_{11} \) subunit in the brain is surprising since all of the other \( \gamma \) subunits identified thus far have been shown to be expressed in brain with the exception of the \( \gamma_3 \) subunit, which is expressed only in the retina. In view of their striking amino acid homology, it will be interesting to determine the basis for the lack of expression of the \( \gamma_3 \) and \( \gamma_{11} \) subunits in the brain.

Selective Interaction of the \( \gamma_4, \gamma_{10}, \) and \( \gamma_{11} \) Subunits with Various \( \beta \) Subunits—To determine which combinations of \( \beta \) subunits and newly identified \( \gamma \) subunits are capable of forming functional dimers, we used a previously developed tryptic digestion method (30). This method is based on the finding that in vitro translated \( \beta \) monomers are cleaved at numerous sites by trypsin, whereas in vitro translated \( \beta \) dimers are cleaved at a single site, resulting in the appearance of a 26-kDa fragment of the \( \beta \) subunit that is resistant to further digestion by trypsin. Thus, the appearance of a stable 26-kDa protected fragment can be used as a marker of \( \beta \) dimerization. Included as a positive control for this series of studies was the \( \gamma_3 \) subunit, which has been shown to form dimers with both the \( \beta_2 \) and \( \beta_3 \) subunits, but not with the \( \beta_1 \) subunit, by this (30) and other corroborating (31–33) methods.

As shown in Fig. 7, the in vitro translated \( \beta_1, \beta_2, \) and \( \beta_3 \) monomers were almost completely digested by trypsin (panels A–C). In contrast, in vitro translated mixtures of the \( \beta_1 \) subunit and either the \( \gamma_2, \gamma_4, \gamma_{10}, \) or \( \gamma_{11} \) subunit yielded a 26-kDa protected fragment when digested by trypsin under identical conditions (panel A). Likewise, in vitro translated mixtures of the \( \beta_2 \) subunit and either the \( \gamma_2, \gamma_4, \) or \( \gamma_{10} \) subunit produced a 26-kDa protected fragment when digested by trypsin (panel B). However, when an in vitro translated mixture of the \( \beta_3 \) subunit and the \( \gamma_{11} \) subunit was digested by trypsin, no such fragment was generated (panel B), even though the levels of the \( \beta_1 \gamma_{11} \) subunits and the \( \beta_2 \gamma_{11} \) subunits in the in vitro translated mixtures were comparable. Taken together, these results indicate that the \( \beta_1 \) subunit is able to form dimers with the \( \gamma_4, \gamma_{10}, \) and \( \gamma_{11} \) subunits, whereas the \( \beta_2 \) subunit is able to form dimers with the \( \gamma_4 \) and \( \gamma_{10} \) subunits but not with the \( \gamma_{11} \) subunit. The inability of the \( \beta_3 \) subunit to form a dimer with the \( \gamma_{11} \) subunit is particularly interesting since the \( \gamma_{11} \) subunit most closely resembles the \( \gamma_3 \) subunit at the amino acid level, and the \( \gamma_{11} \) subunit is unable to form a dimer with the \( \beta_2 \) subunit (30).

Finally, no protected fragment was generated when in vitro translated mixtures of the \( \beta_3 \) subunit and the \( \gamma_2, \gamma_4, \gamma_{10}, \) or \( \gamma_{11} \) subunit were digested by trypsin (panel C). However, this result is more difficult to interpret since no positive control exists for the \( \beta_3 \) subunit at the present time. Thus, it is not certain whether this result indicates that the \( \beta_3 \) subunit is not able to form dimers with any of the known \( \gamma \) subunits or whether the \( \beta_3 \) subunit is able to form dimers with some of the known \( \gamma \) subunits but that tryptic digestion of the resulting \( \beta_3 \gamma \) dimers does not generate a protected fragment of \( \beta_3 \). In this regard, the \( \beta_3 \) subunit has been reported to contain one or more potential tryptic digestion sites (lysine 177) in the 26-kDa fragment than the \( \beta_1 \) or \( \beta_2 \) subunits (30). Thus, if trypsin cleaves at lysine 177 in addition to arginine 129, then a 26-kDa fragment of the \( \beta_3 \) subunit may not be observed. To rule out this possibility, in vitro translated mixtures of the \( \beta_3 \) subunit and the \( \gamma_2, \gamma_4, \gamma_{10}, \) or \( \gamma_{11} \) subunits were digested by Arg-C, a protease that cleaves...
these results indicate that the \( \gamma_4, \gamma_{10}, \) and \( \gamma_11 \) subunits have the ability to selectively associate with particular \( \beta \) subunits, consistent with previous results on the \( \gamma_4, \gamma_5, \gamma_9, \) and \( \gamma_7 \) subunits \( (30-33) \). In this regard, an examination of the amino acids that are common to the \( \gamma_4 \) and \( \gamma_11 \) subunits, but are not common to the \( \gamma_5, \gamma_4, \) and \( \gamma_{10} \) subunits, may shed further light on the regions of the \( \gamma \) subunit that are important for forming dimers with the \( \beta_2 \) subunit.

### DISCUSSION

Diversity and Distribution of \( \gamma \) Subunits—With the recent description of additional roles of the G protein \( \beta\gamma \) dimers in signal transduction \( (4-9) \), the importance of identifying and characterizing these proteins is underscored. Since the \( \gamma \) subunits are thought to determine the functional specificity of the \( \beta\gamma \) dimers, most of our attention has focused on these proteins. With the cloning of the \( \gamma_4, \gamma_{10}, \) and \( \gamma_11 \) subunits in the present paper, the \( \gamma \) subunit family consists of a minimum of 10 members \( (12-17) \). Analysis of the amino acid sequence conservation suggests that the \( \gamma \) subunit family can be divided into four distinct subclasses, one containing the \( \gamma_4 \) and \( \gamma_11 \) subunits, a second containing the \( \gamma_2, \gamma_9, \gamma_4, \) and \( \gamma_7 \) subunits, a third containing the \( \gamma_5 \) subunit, and a fourth containing the \( \gamma_11 \) subunit. Although the subclasses exhibit \( \approx 50\% \) homology to each other, the division of the \( \gamma \) subunit family into these subclasses is based not only on amino acid homology but also to some extent on functional similarities. Thus, within a subclass, members display similar post-translational modifications and similar abilities to interact with the \( \beta \) and \( \alpha \) subunits of the G proteins. For example, the \( \gamma_1 \) and \( \gamma_11 \) subunits, which comprise one subclass, are modified by a farnesyl group, do not interact with the \( \beta_2 \) subunit, and at least in the case of the \( \gamma_11 \) subunit, do not interact with the \( \alpha_5 \) subunit \( (25) \). In contrast, the \( \gamma_2, \gamma_9, \gamma_4, \) and \( \gamma_7 \) subunits, which comprise another subclass, are modified by a geranylgeranyl group, interact with the \( \beta_2 \) subunit, and at least in the case of the \( \gamma_2, \gamma_9, \gamma_7 \) subunits, interact with the \( \alpha_5 \) subunit \( (34) \). It is likely that the total number of different \( \gamma \) subunits has not yet been uncovered and that even more members of this family will be identified in the future.

Among members of the \( \gamma \) subunit family, there are marked differences in the tissue distribution. Some members, such as the \( \gamma_4, \gamma_5, \gamma_9, \) and \( \gamma_7 \) subunits, are restricted to one or a few tissues, whereas others, such as the \( \gamma_4, \gamma_5, \gamma_{10}, \) and \( \gamma_11 \) subunits, are expressed in a wide variety of tissues \( (16) \). Furthermore, in most cell types within a tissue, only a certain subset of \( \gamma \) subunits is present \( (35, 36) \). It is likely that such differences in distribution may be important in limiting the number of combinatorial associations of the \( \alpha, \beta, \) and \( \gamma \) subunits into functionally distinct G proteins. In this regard, differences in the subcellular localization of various \( \gamma \) subunits have also been

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4 M. Rahmatullah and J. D. Robishaw, unpublished results.
reported (37). It is likely that particular \(\alpha\) and \(\beta\) subunits will be found to share these patterns of subcellular localization in future studies.

Significance of Prenylation of \(\gamma\) Subunits—Two different prenyl groups are added to proteins terminating in CAAX sequences, with the residue in the \(-X\) position playing a major role in determining which type of prenyl group is added (38). In the case of \(\gamma_1\) subunit in which the amino acid in the \(-X\) position is a serine, the protein is modified by a C15 farnesyl group (28). On the other hand, in the case of the \(\gamma_2\), \(\gamma_3\), and \(\gamma_4\) subunits, in which the amino acid in the \(-X\) position is leucine, these proteins are modified by a C20 geranylgeranyl group (27). Consistent with these earlier results, we show in the present study that the \(\gamma_4\) and \(\gamma_{10}\) subunits, both containing a leucine in the \(X\) position, are modified by a geranylgeranyl group, whereas the \(\gamma_{11}\) subunit, containing a serine in the \(X\) position, is modified by a farnesyl group.

The functional significance of adding a geranylgeranyl versus a farnesyl group has not been addressed for this family of proteins. However, the idea that different types of prenyl groups impart distinct functional properties is suggested by analysis of retinal and brain \(\beta\gamma\) subunits. In particular, striking differences between the retinal and brain \(\beta\gamma\) subunits have been reported in terms of membrane association (39), interaction with G protein \(\alpha\) subunits (25), receptors (40), receptor kinases (9), and effectors (32). Since the retinal and brain \(\beta\gamma\) subunits share a common \(\beta_1\) subunit, these differences would appear to be due to their unique \(\gamma\) subunits. In this regard, the retinal \(\beta\gamma\) subunits contain a farnesylated \(\gamma_1\) subunit, whereas the brain \(\beta\gamma\) subunits are composed mainly of a mixture of the geranylgeranlylated \(\gamma_2\), \(\gamma_3\), and \(\gamma_4\) subunits. Consistent with the idea that farnesyl groups are less hydrophobic than geranylgeranyl groups, the retinal \(\gamma_1\) subunits can be readily eluted from membranes at low ionic strength (39), whereas the brain \(\gamma_2\), \(\gamma_3\), and \(\gamma_4\) subunits require detergents to be eluted from membranes. However, it is not yet known whether the difference in membrane association is due to a difference in the primary structures of the \(\gamma\) subunits, the nature of the prenyl group added to the proteins, or some combination of both. In this regard, it will be interesting to examine the \(\gamma_{11}\) subunit.

Since the \(\gamma_{11}\) subunit shares several unique structural features of the \(\gamma_1\) subunit that are not observed in any other \(\gamma\) subunit, including modification by a farnesyl group, we predict that the \(\gamma_1\) and \(\gamma_{11}\) subunits may represent a unique subclass of the \(\gamma\) subunit family that interacts reversibly with the membrane.

Table II

| \(\beta_1\) | \(\beta_2\) | \(\beta_3\) | \(\gamma_1\) | \(\gamma_2\) | \(\gamma_3\) | \(\gamma_4\) | \(\gamma_5\) | \(\gamma_6\) | \(\gamma_{10}\) | \(\gamma_{11}\) |
|----|----|----|----|----|----|----|----|----|----|----|
| +  | +  | +  | +  | +  | +  | +  | +  | +  | +  | +  |
| +  | -  | -  | +  | +  | +  | +  | +  | +  | +  | +  |
| -  | +  | ND | ND | -  | -  | -  | -  | -  | -  | -  |

Dimerization of \(\gamma\) Subunits—The formation of distinct \(\beta\gamma\) dimers as the result of selective interactions of the 6 \(\beta\) and 10 \(\gamma\) subunits identified thus far is likely to contribute to the specificity of G protein-mediated signaling pathways. In the present study, we show the formation of distinct \(\beta\gamma\) dimers as the result of selective interactions of the \(\gamma_4\), \(\gamma_{10}\), and \(\gamma_{11}\) subunits with the \(\beta_1\), \(\beta_2\), and \(\beta_3\) subunits. To put these results in context, we show a summary of the known \(\beta\gamma\) interactions in Table II. Thus, similar to the \(\gamma_2\), \(\gamma_3\), \(\gamma_4\), and \(\gamma_7\) subunits (30–33), the \(\gamma_4\) and \(\gamma_{10}\) subunits are able to interact with the \(\beta_1\) and \(\beta_2\) subunits but not with the \(\beta_3\) subunit. On the other hand, the \(\gamma_{11}\) subunit is more similar to the \(\gamma_1\) subunit in that they interact with the \(\beta_1\) subunit but not with the \(\beta_2\) or \(\beta_3\) subunits (30, 31). Intriguingly, a short region of the \(\gamma_1\) subunit, which has been shown recently to discriminate between the \(\beta_1\) and \(\beta_2\) subunits (26), is found to be highly conserved in the \(\gamma_{11}\) subunit (region II in Fig. 4). Future studies will focus on those amino acids in this region that are unique to the \(\gamma_1\) and \(\gamma_{11}\) subunits with respect to their ability to selectively associate with the \(\beta_1\) and \(\beta_2\) subunits. One such candidate is cysteine 36 in the \(\gamma_1\) and \(\gamma_{11}\) subunits. Cross-linking studies have shown that cysteine 36 in the \(\gamma_1\) subunit is in close physical proximity to cysteine 25 in the \(\beta_1\) subunit (41). Although recent reports have suggested that the region surrounding cysteine 25 in the \(\beta_1\) and \(\beta_2\) subunits does not confer the selectivity of interaction with the \(\gamma_1\) subunit (31, 42), these results do not rule out the possibility that multiple domains of the \(\beta\) subunit may be necessary to confer selectivity. Thus, it is tempting to speculate that cysteine 36 and/or adjoining residues in the \(\gamma_1\) and \(\gamma_{11}\) subunits may confer the selectivity of interaction with the \(\beta_1\) and \(\beta_2\) subunits. However, since the \(\gamma_1\), \(\gamma_2\), \(\gamma_3\), \(\gamma_4\), \(\gamma_{10}\), or \(\gamma_{11}\) subunits do not appear to interact with the \(\beta_3\) subunit, it is clear that the region surrounding cysteine 36 is not the only important region of the \(\gamma\) subunit in determining the selectivity of \(\beta\) interaction. Identification of other regions in the \(\gamma\) subunits that confer selective interactions will be aided by the isolation of a \(\gamma\) subunit that interacts with the \(\beta_3\) subunit. In this regard, the Lee and colleagues (39) have purified a \(\beta\gamma\) subunit complex from the cone cells of retina that is composed of the \(\beta_3\) subunit and an unidentified \(\gamma\) subunit. Although the identity of this novel \(\gamma\) subunit is not yet known, it does not appear to be the \(\gamma_4\) subunit since the results of the present study suggest that the \(\gamma_4\) subunit is not capable of forming a dimer with the \(\beta_2\) subunit, as predicted by Kleuss and colleagues (43).

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