Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 7973–7977, October 1990
Biochemistry

G protein diversity is increased by associations with a variety of γ subunits

(signal transduction/subunit constitution)

NARASIMHAN GAUTAM*, JOHN NORTHUP†, HAYA TAMIR†, AND MELVIN I. SIMON*

*Division of Biology, California Institute of Technology, Pasadena, CA 91125; and †Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06437

Contributed by Melvin I. Simon, July 26, 1990

ABSTRACT **Guanine nucleotide-binding regulatory proteins (G proteins) are heterotrimeric proteins that transduce extracellular signals into intracellular changes. Functionally different G proteins have been identified by their different α subunits. The β and γ subunits have been assumed to constitute a common pool shared among various G protein heterotrimers. Two γ subunits (γ_3 and γ_4) have been identified through molecular cloning; these are in addition to two subunits (γ_1 and γ_2) that were previously characterized. G proteins purified from a variety of mammalian tissues were examined with antisera specific to three of the γ subunits. The antisera react with different γ subunits associated with some of the purified G proteins but not all. This demonstrates that different G protein heterotrimers from different tissues carry structurally distinct members of the γ-subunit family. Diversity in the structure of the γ as well as the α and β subunits and preferential associations between members of subunit families increase structural and possibly functional diversity of G proteins.**

When hormones, neurotransmitters, or light stimulate appropriate receptors, the guanine nucleotide-binding protein (G protein) α subunit exchanges guanosine 5′-diphosphate (GDP) for GTP and modulates the function of intracellular effectors such as adenylate cyclase and ion-conducting channels (1). The vast array of receptors and effectors that are coupled through G proteins has suggested that there may be similar complexity among G proteins. The α subunits are structurally diverse (2, 3). However, the situation regarding the β and γ subunits is not clear. The β and γ subunits of G proteins are a tightly bound complex and have not been separated without denaturation. Although some differences in the properties of $\beta\gamma$ complexes from different G proteins have been noted (4, 5), biochemical analyses for the most part have not revealed functional differences. There are reports of activities associated with the $\beta\gamma$ complex, and genetic studies in yeast support the notion that the $\beta\gamma$ complex plays an active role (6, 7). The full extent of heterogeneity among the β and γ subunits is not known. If the β and γ subunits are as heterogeneous as the α subunits, associations between different β and γ subunits and their interaction with specific α subunits could result in distinct G proteins with characteristic properties. We are therefore examining the structure of the G protein γ subunits and their association with various G proteins.

The initial characterization of different G proteins suggested that their γ subunits were heterogeneous (8–10). These results did not distinguish between heterogeneity due to differences in the primary structure of proteins and that due to posttranslational modifications. Molecular cloning has helped identify two structurally distinct G protein γ subunits (11–14). Here we report the identification of two additional structurally distinct γ subunits, indicating that the γ subunits are encoded by an extensive gene family like the α subunits.‡ Antisera specific to these γ subunits have been used to examine the subunit constitution of various G proteins isolated from different mammalian tissues.

MATERIALS AND METHODS

Bovine retinas were from Hormel (Austin, MN), guanosine 5′-[β,γ]imidotriphosphate (p[NH]ppG) was from Sigma. Restriction enzymes were from New England Biolabs, *Taq* polymerase was from United States Biochemical. Oligonucleotides were synthesized at the microchemical facility at Caltech. Peptides were also synthesized and conjugated to keyhole limpet hemocyanin at the microchemical facility at Caltech by standard procedures.

cDNA Synthesis and Polymerase Chain Reaction (PCR). *Isolation of the γ_3 cDNA from bovine brain.* The template cDNA was synthesized and amplified by PCR essentially as described (13, 15). The primers for PCR had the following nucleotide sequences: sense, BG14, GT(G/T)GA(G/T) CA(A/G)CT(G/T)AA(A/G) AT; antisense, BG15, TC(C/A)-CT(C/A)AA(C/A)GG(G/A)TT(T/C)TC. These primers were specific to amino acids 16–21 and 58–63 with reference to the amino acid sequence of γ_2 (Fig. 1*B*). cDNAs that had been synthesized from bovine brain mRNA by using an oligo(dT) primer or the degenerate antisense oligonucleotide BG15 were used as templates for PCR. The PCR primers were expected to amplify cDNAs for members of the γ-subunit family including the previously characterized γ_2 cDNA (13). To prevent the PCR reaction from being saturated with the γ_2 cDNA, PCR primers were synthesized with only 2 bases in positions where the degeneracy was 4-fold. The resultant mismatches between the oligonucleotide primers and the nucleic acid sequence of the γ_2 cDNA were expected to favor the amplification of different members of the γ subunit family. PCR reaction mixes were denatured at 94°C for 5 min before adding the enzyme, followed by 30 cycles of 1-min denaturation at 94°C, 1-min annealing at 38°C, and 30-sec extension at 72°C. Amplified DNA was treated with Klenow fragment of DNA polymerase and T4 DNA polymerase in the presence of deoxynucleotide triphosphates. The DNA was then precipitated and phosphorylated with ATP and polynucleotide kinase. This reaction mixture was electrophoresed on 2.5–3% Nusieve (Seaplaque) agarose gels. The band of expected size was cut out and ligated to Bluescript (Stratagene). The subcloned fragment was sequenced with Sequenase (United States Biochemical).

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: G protein, guanine nucleotide-binding regulatory protein; PCR, polymerase chain reaction.
‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. M37182).

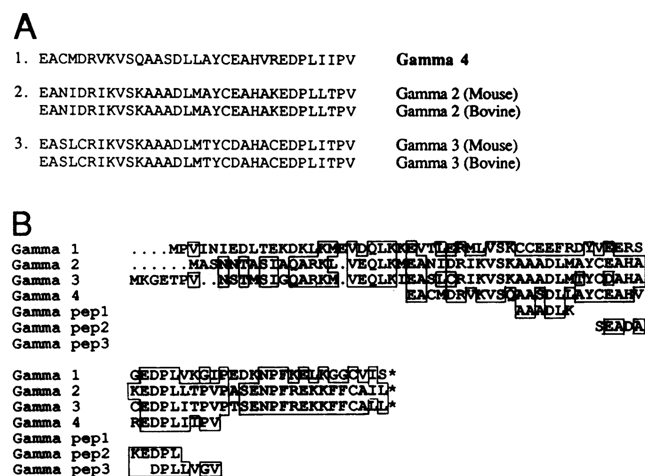

FIG. 1. Predicted amino acid sequences of mouse and bovine γ subunits. (*A*) Amino acids encoded by cDNAs amplified with PCR from mouse retina and kidney. (*B*) Comparison of the predicted amino acid sequences of all known γ subunits (γ_1 from refs. 11 and 12; γ_2 from refs. 13 and 14) along with the amino acid sequences of three different peptides (pep1 and -2 from ref. 13 and pep3 from ref. 14). Amino acid identities are boxed. Asterisks denote stop codons. Gaps introduced to align the sequences are denoted as dots.

Isolation of γ-Subunit cDNAs from Mouse Tissues. The cDNA templates used for isolating mouse-specific γ-subunit cDNAs were synthesized from purified mouse mRNA from adult mouse retina and kidney by using a set of hexamers with random sequences as primers. Total RNA was heated to 68°C for 5 min, chilled, and reverse transcribed with primers for 90 min at 37°C. One hundred-microliter reaction mixtures contained 2 μg of RNA and 1 μg of primer. One microliter of this reaction mixture was used as a template for PCR amplification. The PCR reaction mixture contained 200 ng of primers DG17 and DG18 in 20 μl. Amplification conditions were 1 min at 94°C, 30 sec at 72°C, and 30 sec at 46°C. The nucleotide sequences of the PCR primers were as follows: DG17, GTIGAICA(A/G)CTIAA(A/G)AT; DG18, GG(A/G)TT(T/C)TCI(G/C)(A/T)IG(C/T)IGG. [Inosine (I) was substituted at the third position of codons where the degeneracy was 4-fold.] The primers are specific to two regions of homology between γ_2 and γ_3 subunit. These regions correspond to amino acids 16–21 and 55–60 of γ_2 (Fig. 1*B*).

Isolation of cDNA Clones. A bovine brain cDNA library in the phage vector λgt10 was screened with the PCR-generated cDNA fragment by standard procedures. The PCR fragment inserted in Bluescript was amplified with primers specific to Bluescript and labeled with [α-^{32}P]ATP by primer extension. cDNA clones that hybridized to the probe were subcloned in Bluescript and sequenced.

RNA Blot Analysis. A nitrocellulose filter containing polyadenylylated RNA that was hybridized to γ_2-subunit cDNA (13) was stripped and hybridized to the PCR amplified fragment PCR 1415.13 (see *Results*) by standard procedures. The PCR fragment, inserted in Bluescript, was amplified with primers specific to Bluescript and labeled with [α-^{32}P]ATP by primer extension. Prehybridization at 65°C and hybridization at 41°C were performed in a solution containing 5× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate), 25 mM potassium phosphate (pH 7.6), 50% formamide, 10% dextran sulfate, 0.2% NaDodSO$_4$, and denatured salmon sperm DNA (100 μg/ml). The filter was washed sequentially at increasing levels of stringency. The final wash was at 65°C with 0.1× SSC and 0.1% NaDodSO$_4$ for 1 hr.

NaDodSO$_4$ Gel and Immunoblot Analysis. Antibodies were raised in rabbits against synthetic peptides conjugated to keyhole limpet hemocyanin by Cocalico Biologicals, Philadelphia. Proteins separated on 15% polyacrylamide gels containing NaDodSO$_4$ were transferred to nitrocellulose overnight at 4°C by standard procedures. The amino acid sequences of these peptides were as follows: Pγ_1, MPVINIEDLTEKDKL; Pγ_2, MASNNTASIAQARKL; Pγ_3, MKGETPVNSTMSIG.

The antisera were examined with the respective peptides by dotting 1 μl of the peptides at a concentration of 12–16 μg/μl on nitrocellulose and by using standard procedures. The peptides were incubated in 0.025 M Tris·HCl, pH 8.3/0.192 M glycine/0.1% NaDodSO$_4$ before dotting on nitrocellulose. Proteins separated on 16.5% acrylamide gels in the presence of 6 M urea (16) or on 15% polyacrylamide gels containing NaDodSO$_4$ were immunoblotted according to standard procedures. Antibodies bound to antigen were visualized with either radioiodinated goat anti-rabbit IgG or goat anti-rabbit IgG conjugated to alkaline phosphatase (Promega).

Purification of G Proteins. Transducin was purified from dark-adapted bovine retinas according to published procedures (17). The $\beta\gamma$ complex of transducin was isolated from the α subunit by chromatography over Blue Sepharose (Pharmacia). The $\beta\gamma$ complex flowed through in the presence of a low salt buffer [100 mM Tris·HCl, pH 7.5/6 mM MgSO$_4$/1 mM EDTA/1 mM dithiothreitol/25% (vol/vol) glycerol], while the α subunit was bound to the resin. Protein that flowed through was concentrated and stored in 40% glycerol at −20°C. G proteins from different tissues and the $\beta\gamma$ complex from placenta enriched for the β_2 subunit (β subunit of 35 kDa) were isolated essentially according to published procedures (18–22). G$_i$ protein from placenta was isolated as a heterotrimeric protein. The $\beta\gamma$ complexes from placenta either contained both the β_1 (β subunit of 36 kDa) and β_2 subunits or contained the β_2 subunit alone as determined from immunoblots with antisera specific to the β_1 and β_2 subunits. The $\beta\gamma$ complex with the β_2 subunit alone was isolated by Ultrogel ACA-54 (LKB) chromatographic fractionation of the total $\beta\gamma$ complex from placenta.

RESULTS

Isolation of a Fragment of the γ_3-Subunit cDNA by PCR. The PCR was used to isolate a fragment of the cDNA for γ_3 subunit from bovine brain mRNA. The primers for PCR (BG14 and BG15) were specific to two different regions of homology between the amino acid sequences of γ_1 and γ_2 subunits. Single-stranded cDNA templates were synthesized from bovine brain mRNA with oligo(dT) or BG15 as primers for reverse transcription. Based on homology with γ_1 and γ_2, the PCR primers BG14 and BG15 were expected to amplify 143-base-long cDNAs. A cDNA fragment of ≈140 bases was amplified from template cDNA reverse transcribed with BG15. This fragment was isolated and subcloned into a plasmid, and the nucleotide sequence was determined.

Of 49 PCR-generated fragments that were sequenced, 8 were identical in the amplified region between the two primers. The nucleotide sequence of 1 of these fragments, PCR 1415.13, is highlighted in Fig. 2. The deduced amino acid sequence is 76% identical to the corresponding region of γ_2 subunit. None of the PCR-amplified fragments had a sequence identical to that of γ_2, showing that the strategy of using oligonucleotides with a skewed distribution of bases at the third positions of codons was effective.

Isolation of the γ_3-Subunit cDNA from a Bovine Brain cDNA Library. The homology between the amino acid sequence encoded by the fragment PCR 1415.13 and the amino acid sequences of the known γ subunits suggested that it was part of a cDNA coding for a different G protein γ subunit. To isolate the entire cDNA for this protein ≈5 × 10^5 phage plaques of a bovine brain cDNA library in λgt10 were

```
  1 AACTCCCTGCAAGGTGGGGGCACCCATCTCAGGCAGAAGGTTTTGGTGCACCCCACTGAT 60

 61 CAGATTCCCCAGGACTGTCGCTGCCTGTGGCCTCAGGATGAAAGGGGAGACCCCTGTGAA 120
                                           M  K  G  E  T  P  V  N

121 CAGCACTATGAGTATTGGGCAAGCCCGCAAGATGGTGGAACAGCTTAAGATTGAAGCCAG 180
     S  T  M  S  I  G  Q  A  R  K  M  V  E  Q  L  K  I  E  A  S

181 CTTGTGCCGGATAAAGGTGTCCAAGGCAGCAGCAGACCTGATGACTTACTGTGATGCCCA 240
     L  C  R  I  K  V  S  K  A  A  A  D  L  M  T  Y  C  D  A  H

241 CGCCTGTGAGGATCCCCTCATCACCCCTGTGCCCACTTCGGAGAACCCCTTCCGGGAGAA 300
     A  C  E  D  P  L  I  T  P  V  P  T  S  E  N  P  F  R  E  K

301 GAAATTCTTCTGTGCTCTCCTCTGAGCTGCCCTGTTCCTCTTCACAACTCTTCACCTTTC 360
     K  F  F  C  A  L  L  *

361 CCTCTCCCGGGCCCTTCCTTATTTATGTCAGTAACTGTCGTGAG 404
```

FIG. 2. Nucleic acid sequence of the γ_3 cDNA. The amino acid sequence encoded by this cDNA is shown below. The portion of the cDNA amplified by PCR is underlined.

screened, and the PCR-generated fragment PCR 1415.13 was used as a probe. Five clones that hybridized to the probe at high stringency were picked for further analysis. The cDNA insert from one of these clones, λCG7, was ≈700 base pairs long and its nucleotide sequence is shown in Fig. 2. The sequence corresponding to PCR 1415.13 was found in the cDNA from nucleotides 155–296. In one particular reading frame, this cDNA encodes a 75-amino-acid-long protein that is 73% identical to the amino acid sequence of γ_2 and 36% identical to the amino acid sequence of γ_1 (Fig. 1*B*). The ATG at nucleotide 98 has been assumed to be the initiating codon based on the homology of the predicted protein to that of γ_1 and γ_2. The 5′ and 3′ noncoding regions of the γ_3 cDNA did not share significant homology with the corresponding regions of either γ_1 or γ_2. In comparison to γ_1 or γ_2, the differences in the nucleic acid sequence of γ_3 are distributed over the whole cDNA, suggesting that the mRNA corresponding to this cDNA is not the product of splicing reactions involving the mRNAs for the two known γ subunits.

Isolation of cDNAs for Members of the γ Subunit Family from Mouse Tissues. To determine the degree of species diversity among the amino acid sequences of different γ subunits and to further probe the extent of diversity in this family, the same approach based on PCR was used on cDNA templates from mouse kidney and retina. Oligonucleotides that were specific to amino acid sequences within two blocks of homology among the different γ subunits were synthesized. Fragments of cDNAs were isolated by PCR as described above. The amino acid sequences encoded by these cDNAs are shown in Fig. 1*A*. A cDNA that encodes an additional γ subunit (γ_4) was isolated from both tissues. The amino acid sequence encoded by this cDNA showed 34% identity with the corresponding region of γ_1, 75% identity with the corresponding region of γ_2, and 67% identity with the corresponding region of γ_3. A comparison of the primary structures of the different γ subunits is shown in Fig. 1*B*. In addition, nucleic acid sequences encoding the γ_2 and γ_3 homologs from mouse were obtained. γ_2 cDNA was from kidney and γ_3 cDNA was from both kidney and retina. The amino acid sequences encoded by these cDNAs were identical to the corresponding regions of γ_2 and γ_3 isolated from bovine brain (Fig. 1*A*).

Expression of the γ_3 mRNA. Hybridization of radioactively labeled PCR 1415.13 fragment at high stringency to poly(A)$^+$ RNA from various bovine tissues showed that the transcript specific to this cDNA is expressed at detectable levels only in brain (Fig. 3) and at lower levels in testis (data not shown). Based on the amounts of RNA from different tissues that were loaded on each lane the expression of the γ_3 transcript is ≈4-fold less in testis than in brain. The size of the transcript

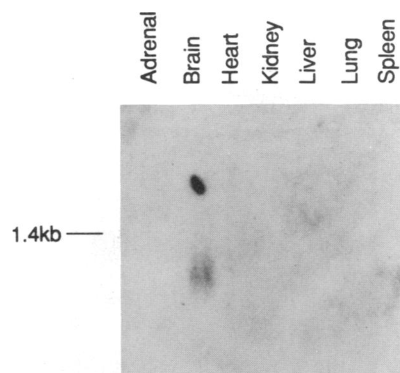

FIG. 3. Expression of RNA specific to γ_3. The RNA blot probed with γ_2 (9) was stripped and analyzed by standard procedures. RNA from the following bovine tissues (μg of RNA per lane are indicated in parentheses after tissue) hybridized to fragment 1415.13: adrenal (1), brain (1), heart (2), kidney (2), liver (2), lung (2), spleen (3). The size of the transcript from brain was estimated from RNA molecular size markers ranging from 9.5 to 0.24 kb. Only the portion of the autoradiogram including the transcript is shown. This probe also hybridized to a transcript of ≈1.4 kb from testis.

in brain is ≈1 kilobase (kb). The γ_3 transcript size is ≈1.4 kb in testis and appears as a smear, which could indicate the presence of more than one transcript. It is possible that these differences are due to tissue-specific processing of the γ_3 mRNA.

Examination of the γ Subunits of Different Purified G Proteins with Specific Antisera. Peptides specific to a portion of the NH_2-terminal amino acid sequence of each γ subunit were synthesized as follows: the first 15 amino acids of γ_1 (Pγ_1), the first 15 amino acids of γ_2 (Pγ_2), and the first 14 amino acids of γ_3 (Pγ_3). These peptides were conjugated to keyhole limpet hemocyanin through a cysteine at the COOH terminus and antibodies were raised in rabbits. The amino acid sequence of Pγ_1 did not share significant homology with γ_2 or γ_3. The amino acid sequence of Pγ_2 was 50% identical to a stretch of amino acids in γ_3. The amino acid sequence of Pγ_3 was 29% identical with the corresponding sequence of γ_2. Despite these homologies, the antisera raised against the γ_2 and Pγ_3 reacted only with the corresponding peptides, showing that their reactivity was specific to the γ_2 and γ_3 proteins.

The G proteins, G_i (from several different mammalian tissues), a mixture of G_i and G_o (from brain), transducin (from rod photoreceptors), and the $\beta\gamma$ complex from placenta enriched for the β_2 subunit, were purified. γ_1, γ_2, and γ_3 antisera were used to examine the γ subunits of these proteins by immunoblotting. Fig. 4*Aa* shows that the γ subunits of these G proteins have different mobilities. In addition, the G proteins from liver and brain contain two γ subunits. The results of immunoblotting these proteins are shown in Fig. 4*A b–d*. γ_1 antiserum reacts with the γ subunit of transducin alone (Fig. 4*Ab*). γ_2 antiserum reacts with one of the γ subunits of the G protein from liver and a γ subunit with the same mobility from brain (Fig. 4*Ac*). It reacts weakly with a placental $\beta\gamma$ complex that contains both the β_1 and β_2 subunits (Fig. 4*Bb*) but not with the placental $\beta\gamma$ complex that was purified so that it contained only the β_2 subunit (Fig. 4*Ac*). γ_3 antiserum reacts exclusively with a γ subunit from brain (Fig. 4*Bc*). These results show that G proteins purified from different mammalian tissues are structurally distinct with reference to the γ subunit. Also, $\beta\gamma$ complexes from the same tissue as in the case of placenta contain different γ subunits depending on the constitution of their β subunits. These results also imply that the γ subunits of G_i from platelets and placenta, one of the γ subunits from brain and liver as well as the γ subunit in the $\beta_2\gamma$ complex from placenta, all of which did not react with any of the antisera,

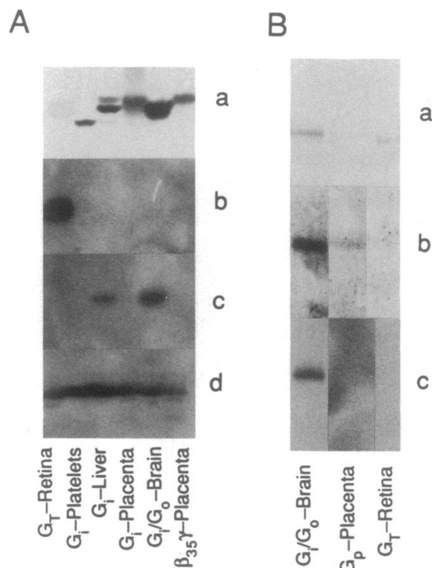

FIG. 4. Purified G proteins from a variety of mammalian tissues examined with antisera specific to different γ subunits. Only portions of the gel or immunoblots with the γ subunits are shown. This figure includes data from independent gels. All antisera were used at 1:100 dilution. Preimmune sera corresponding to different antisera did not react with the γ subunits. Preimmune sera and antisera reacted with the β subunits under the conditions used. (*A*) G protein γ subunits were resolved by electrophoresis in the presence of urea (16). The different lanes contain the following (from left to right): 5.4 μg of G_t (transducin) from bovine retina, 3.9 μg of human platelet G_i, 5.2 μg of rabbit liver G_i, 5.6 μg of human placental G_i, 5.6 μg of bovine brain G_o/G_i, 1.7 μg of human placental $\beta_{35}\gamma$ complex [contains only the β_2 subunit (21, 22)]. (*a*) Silver-stained gel. Replicate nitrocellulose blots were probed with γ_1 antiserum (*b*), γ_2 antiserum (*c*), and antiserum against the β subunit (*d*) to show that all of them contain equal amounts of β subunit. (*B*) G protein γ subunits were separated on 15% $NaDodSO_4$ gels. The different lanes contain the following (from left to right): 8 μg of G_i/G_o from bovine brain, $\approx$1 μg of $\beta\gamma$ complex from human placenta that contains both the β_1 and β_2 subunits in *a* and 2.2 μg in *b* and *c*, 1 μg of $\beta\gamma$ complex from G_t from bovine retina. (*a*) Coomassie blue-stained γ subunits; (*b*) γ_2 antiserum; (*c*) γ_3 antiserum.

are structurally distinct from the characterized γ subunits. It is formally possible that the mobility differences between different γ subunits are due to posttranslational modifications that prevent antibody reaction. However, this would imply that the modification is at the NH_2 terminus of these γ subunits, for which there is no evidence thus far. Modifications are known to occur at the COOH terminus as discussed below. Therefore, a more probable reason for the differences in the mobility of different γ subunits is their distinctive primary structures.

DISCUSSION

The primary structure of the γ_3 protein has several interesting features. It is very similar to γ_2 over most of the length of the protein but is highly divergent toward the NH_2 terminus. There is only 40% amino acid identity between the first 15 amino acids of γ_2 and γ_3; the remaining portion of these proteins are 82% identical. This pattern is similar to the amino acid sequence homology observed between γ_1 and γ_2 where the first 15 amino acids of γ_2 show no homology at all to the corresponding γ_1 sequence. The NH_2-terminal 15 amino acids of γ_3 also have little homology with those of γ_1. The NH_2-terminal 20 amino acids of γ_2 share significant homology with a hypothetical effector binding domain of the mammalian Ki-*ras*, N-*ras* and Ha-*ras* oncogene products (13). The NH_2-terminal sequence of the different γ subunits could therefore constitute a domain that confers functional specificity on each member of this family. Curiously, the amino acid sequence NXT (X, any amino acid) is conserved in the NH_2-terminal amino acid sequences of the γ_3 and the γ_2 proteins. This sequence is known to be a signal for N-linked glycosylation of proteins (23). There is no evidence thus far that any of the G protein subunits are glycosylated. The predicted primary structure of the γ_3 protein possesses the characteristic COOH-terminal sequence CaaX (a, aliphatic amino acid) that is present in all the γ subunits and ras proteins. The cysteine in this sequence is known to be farnesylated in the case of ras proteins, and there is evidence that the γ subunit from yeast is modified in this manner (24, 25). In addition, a γ subunit from brain has been shown to be methyl esterified on a COOH-terminal cysteine residue (26). More recently, it has been shown that two different γ subunits are modified at this site by geranylgeranylation and carboxyl methylation (27, 33). The γ_3 sequence possesses a stretch of basic residues at amino acid positions 66, 68, and 69 upstream of the CaaX tail that are conserved in γ_2 and in a class of ras-related proteins (24, 28). The γ subunits of G proteins could therefore be posttranslationally modified by mechanisms similar to those that are involved in processing proteins that belong to the ras family.

cDNAs encoding four structurally distinct γ subunits have now been identified. The differences in the mobilities of γ subunits of various G proteins (Fig. 4*Aa*) and the isolation of proteolytic fragments from brain G proteins with amino acid sequences that are distinct but homologous to γ subunits (see refs. 13 and 14) (Fig. 1*B*) suggest that unidentified members of the γ-subunit family still exist. Furthermore, the portion of the amino acid sequences of γ_2 and γ_3 obtained from mouse tissue is identical to that of their bovine homologs (Fig. 2*A*). The differences in the amino acid sequences between different γ subunits are thus conserved across species. A similar conservation of amino acid sequences across species has been noticed in the α- and β-subunit families (2, 29). A reason for this conservation could be that the distinct structures of different γ subunits play a critical role in the function of the proteins. This would also imply that the different γ subunits have distinct functions. The γ subunits like the α subunits of G proteins are thus an extensive family of proteins with diverse structures. The expression of these subunits is also similar to the α subunits. The transcript specific to γ_3 is expressed primarily in mammalian brain (Fig. 3). The mRNA for γ_1 is expressed only in retina (R. Miake-Lye and M.I.S., unpublished data) and γ_2 is expressed in almost all tissues that were examined, although at widely varying levels (13). The pattern of tissue-specific expression of different γ subunits thus falls into three classes: (*i*) ubiquitous, γ_2; (*ii*) specific to a particular tissue, γ_3; (*iii*) cell-type specific, γ_1.

The antibodies used in these studies are against peptides whose amino acid sequences were derived from nucleic acid sequences of cDNAs. Their ability to recognize γ subunits of purified G proteins indicates that the γ_1, γ_2, and γ_3 cDNAs encode proteins that are part of heterotrimeric G proteins. The presence of different γ subunits in G proteins from various mammalian tissues is consistent with the expression of their RNAs in these tissues. γ_2 is present in G proteins from brain and liver where its RNA is also detected (13); γ_1 is associated with the G protein from rod photoreceptors and its RNA is detectable only in retina; γ_3 is found in the G protein from brain and its RNA is detectable only in this tissue (and at lower levels in testis). It is thus possible that the specificity of association between different γ subunits and G proteins from various mammalian tissues demonstrated here is a result of restricted expression of specific members of α-, β-, and γ-subunit family members. Alternatively, structurally distinct γ subunits could distinguish between different α and β subunits. The β subunits are now known to be heterogeneous

(30). Specific associations between different β and γ subunits are therefore possible. There is evidence to support this notion. The G protein, transducin, from rod photoreceptors has only one form of the β subunit (β_1) (31) and only one form of the γ subunit (γ_1) (Fig. 4). γ_2 is present only in $\beta\gamma$ complexes from placenta that contain both the β_1 and β_2 subunits but not in those that contain the β_2 subunit alone (Fig. 4).

Some experiments have shown differences in the properties of $\beta\gamma$ complexes associated with G proteins from different tissues (4, 5), while others have indicated that they are interchangeable. Strikingly conflicting results have also been obtained with regard to the activation of the potassium channel in heart by $\beta\gamma$ complexes (6, 32). A reason for these results may be the heterogeneity of the β and γ subunits that we have encountered. Purification of G proteins is always based on the identification of a specific α subunit in the heterotrimer. The α subunit of G_i from liver reacted only with the antiserum specific to α_{i3} and not with antisera specific to α_{i1}, α_{i2}, and the α subunits of the G proteins G_o and G_z. It is possible that this G protein from liver contains recently identified α subunits (3); however, the presence of more than one γ subunit in a G protein purified in this manner indicates that different mixtures of $\beta\gamma$ complexes could be associated with purified G proteins. If, as seems likely, structurally distinct $\beta\gamma$ complexes have different functions, the functional diversity of G proteins would be increased enormously through combinatorial associations between members of the three subunit families. In addition, restricting the associations between these subunits in different cells would provide a mechanism to regulate the interactions between specific receptors and effectors.

We thank Carol Lee for assistance with sequencing; Ryn Miake-Lye and Tom Wilkie for materials; and Tom Amatruda, Bob Bourret, Michael Strathmann, and Tom Wilkie for discussions. This work was supported by National Institutes of Health Grant GM 34236. J.N. is an established investigator of the American Heart Association and is supported by National Institutes of Health Grant G40154.

1. Ross, E. M. (1989) *Neuron* **3,** 141–152.
2. Lochrie, M. A. & Simon, M. I. (1988) *Biochemistry* **27,** 4957–4962.
3. Strathmann, M., Wilkie, T. & Simon, M. I. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 7407–7409.
4. Cerione, R. A., Gierschick, K.-H. P., Stanizewski, C., Benovic, J. L., Codina, J., Somers, R., Birnbaumer, L., Spiegel, A., Lefkowitz, R. J. & Caron, M. G. (1987) *Biochemistry* **26,** 1485–1491.
5. Casey, P. J., Graziano, M. P. & Gilman, A. G. (1989) *Biochemistry* **28,** 611–616.
6. Kim, D., Lewis, D. L., Graziadei, L., Neer, E. J., Bar-Sagi, D. & Clapham, D. E. (1989) *Nature (London)* **337,** 557–560.
7. Whiteway, M., Hougan, L., Dignard, D., Thomas, D. Y., Bell, G. C., Saari, L., Grant, F. J., Ohara, P. & Mackay, V. L. (1989) *Cell* **56,** 467–477.
8. Gierschik, P., Codina, J., Simons, C., Birnbaumer, L. & Spiegel, A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 727–731.
9. Hildebrandt, J. D., Codina, J., Rosenthal, W., Birnbaumer, L., Neer, E. J., Yamazaki, A. & Bitensky, M. W. (1985) *J. Biol. Chem.* **260,** 14867–14872.
10. Roof, D. J., Applebury, M. L. & Sternweis, P. C. (1985) *J. Biol. Chem.* **260,** 16242–16249.
11. Hurley, J. B., Fong, H. K. W., Teplow, D. B., Dreyer, W. J. & Simon, M. I. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 6948–6952.
12. Yatsunami, K., Pandya, B. V., Oprian, D. D. & Khorana, H. G. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1936–1940.
13. Gautam, N., Baetscher, M., Aebersold, R. & Simon, M. I. (1989) *Science* **244,** 971–974.
14. Robishaw, J. D., Kalman, V. K., Moomaw, C. R. & Slaughter, C. A. (1989) *J. Biol. Chem.* **264,** 15758–15761.
15. Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. & Arnheim, N. (1985) *Science* **230,** 1350–1354.
16. Schagger, H. & Von Jagow, G. (1987) *Anal. Biochem.* **166,** 368–379.
17. Fung, B. K. K., Hurley, J. B. & Stryer, L. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 152–156.
18. Sternweis, P. C., Northup, J. K., Smigel, M. D. & Gilman, A. G. (1981) *J. Biol. Chem.* **256,** 11517–11526.
19. Bokoch, G. M., Katada, T., Northup, J. K., Ui, M. & Gilman, A. G. (1984) *J. Biol. Chem.* **259,** 3560–3567.
20. Sternweis, P. C. & Robishaw, J. D. (1984) *J. Biol. Chem.* **259,** 13806–13813.
21. Evans, T., Brown, M. L., Fraser, E. D. & Northup, J. K. (1986) *J. Biol. Chem.* **261,** 7052–7059.
22. Evans, T., Fawzi, A., Fraser, E. D., Brown, M. L. & Northup, J. K. (1987) *J. Biol. Chem.* **262,** 176–181.
23. Hubbard, S. C. & Ivatt, R. J. (1981) *Annu. Rev. Biochem.* **50,** 555–583.
24. Hancock, J. F., Magee, A. I., Childs, J. E. & Marshall, C. J. (1989) *Cell* **57,** 1167–1177.
25. Schafer, W. R., Kim, R., Sterne, R., Thorner, J., Kim, S.-H. & Rine, J. (1989) *Science* **245,** 379–385.
26. Fung, B. K. K., Yamane, H. K., Ota, I. M. & Clark, S. (1990) *FEBS Lett.* **260,** 313–317.
27. Yamane, H. K., Farnsworth, C. C., Xie, H., Howald, W., Fung, B. K. K., Clarke, S., Gelb, M. H. & Glomset, J. A. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5868–5872.
28. Taparowski, E., Shimizu, K., Goldfarb, M. & Wigler, M. (1983) *Cell* **34,** 581–586.
29. Gautam, N. & Simon, M. I. (1990) in *ADP Ribosylation and G Proteins: Insights into Signal Transduction*, eds. Vaughn, M. & Moss, J. (Am. Soc. Microbiol., Washington), pp. 371–380.
30. Levine, M. A., Smallwood, P. M., Moen, P. T., Helman, L. J. & Ahn, T. G. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 2329–2333.
31. Amatruda, T., Gautam, N., Fong, H. K. W., Northup, J. K. & Simon, M. I. (1988) *J. Biol. Chem.* **263,** 5008–5011.
32. Codina, J., Yatani, A., Grenet, D., Brown, A. M. & Birnbaumer, L. (1987) *Science* **236,** 442–445.
33. Mumby, S. M., Casey, P. J., Gilman, A. G., Gutowski, S. & Sternweis, P. C. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5873–5877.