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Biochemistry

Gα12 and Gα13 subunits define a fourth class of G protein α subunits

(GTP-binding protein/signal transduction)

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ABSTRACT **Heterotrimeric guanine nucleotide-binding regulatory proteins (G proteins) are central to the signaling processes of multicellular organisms. We have explored the diversity of the G protein subunits in mammals and found evidence for a large family of genes that encode the α subunits. Amino acid sequence comparisons show that the different α subunits fall into at least three classes. These classes have been conserved in animals separated by considerable evolutionary distances; they are present in mammals, *Drosophila*, and nematodes. We have now obtained cDNA clones encoding two murine α subunits, Gα12 and Gα13, that define a fourth class. The translation products are predicted to have molecular masses of 44 kDa and to be insensitive to ADP-ribosylation by pertussis toxin. They share 67% amino acid sequence identity with each other and <45% identity with other α subunits. Their transcripts can be detected in every tissue examined, although the relative levels of the Gα13 message appear somewhat variable.**

Guanine nucleotide-binding regulatory proteins (G proteins) are heterotrimers composed of α, β, and γ subunits (for reviews see refs. 1–3). The α subunits belong to a much larger group of GTPases, including elongation factor Tu and Ras, which share similar structural elements (4, 5). In all of these GTPases, a cycle of guanine nucleotide exchange and hydrolysis enables the protein to exist in two distinct states. This cycle allows G proteins to transiently relay signals from cell-surface receptors to intracellular effectors. The receptors comprise a diverse family of proteins characterized by their transmembrane structure; they have seven membrane-spanning domains with highly conserved amino acid sequences. Upon interaction with the appropriate agonist, the receptor serves to accelerate the exchange of GDP for GTP on the G protein α subunit. This exchange is believed to be accompanied by dissociation of the α and β-γ subunits, allowing α (and in some cases β-γ) to interact with effectors. The intrinsic GTPase activity terminates the signal, returning the α subunit to its basal GDP-bound state.

More than 100 different receptors in mammals are thought to couple through G proteins to a variety of effectors (6). The diverse α subunits, which mediate receptor function, can be classified on the basis of their amino acid sequence similarity. Stimulatory (G_s) and inhibitory (G_i) G protein subtypes have been implicated in the regulation of adenylate cyclase and the gating of certain ion channels (7). In the highly specialized visual system, biochemical experiments have elucidated the role of transducin (G_{t1}) in regulating phosphodiesterase and subsequently in controlling the levels of cGMP during the photoreceptor cascade (8). These systems are the best characterized examples of G protein-mediated effector activation. However, many other effector systems have been shown to be regulated by G proteins.

Pertussis toxin has proven to be an important tool in the dissection of G protein-mediated pathways. Certain α subunits can be ADP-ribosylated by this toxin, thereby uncoupling the G protein from receptors. There are, however, some signaling processes that are refractory to toxin inhibition but seem to be mediated by G proteins. In particular, phospholipase C (PLC) is involved in both pertussis toxin-sensitive and -insensitive pathways (9). Recently, using molecular biological techniques we found a class of α subunits termed G_q that were predicted to be insensitive to pertussis toxin (10). Two groups independently purified the corresponding α subunits and have now demonstrated that these proteins can activate one of the PLC isotypes, PLC-β (11–13).

We are interested in understanding how G protein-mediated signal transduction has adapted to the complex signaling processes that define multicellular organisms. To this end, we have examined G protein diversity in the mouse. Using a method based on the PCR, we found evidence for extensive diversity among the G protein α subunits (14). A small screen uncovered five sequences termed Gα10–Gα14. Analysis of amino acid sequence conservation suggested that the known α subunits could be grouped into three distinct classes, G_s, G_i, and G_q (10). Gα11 and Gα14 belong to the G_q class. In this paper, we present the cDNA sequences of Gα12 and Gα13, which define the fourth class of α subunits.*

MATERIALS AND METHODS

PCR. cDNA was made from 5 μg of total RNA with random hexanucleotide primers using Moloney murine leukemia virus reverse transcriptase in a 50-μl reaction volume. Conditions were those supplied by the manufacturer (Bethesda Research Laboratories). The reactions were heated to 100°C for 5 min. The cDNA was then diluted to 400 μl with water containing DNase-free RNase A at 10 μg/ml and was incubated at 37°C for 30 min. Two microliters of the cDNA mixture was used in a 20-μl PCR reaction. The oligonucleotides used for PCR amplification of the cDNA were as follows: *oMP19*, CGGATCCAARTGGATHCAYTGYTT; *oMP20*, GGAATTCRTCYTTYTTRTTNAGRAA; *oMP21*, GGAATTCRTCYTTYTTRTTYAARAA; and *CT56*, CGGATCCARRTGGHTNSARTGYTT, in which R = A or G, Y = C or T, S = C or G, H = A, C, or T, and N = A, C, G, or T. PCR was performed for 35 cycles on a Perkin–Elmer/Cetus thermal cycler. During each cycle, samples were denatured for 0.5 min at 94°C, annealed for 0.5 min at 40°C,

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Abbreviations: PLC, phospholipase C; G protein, guanine nucleotide-binding regulatory protein; G_s and G_i, stimulatory and inhibitory G protein, respectively; G_q, class of G protein α subunits predicted to be insensitive to pertussis toxin.

*The sequences reported in this paper have been deposited in the GenBank data base [accession nos. M63659 (Gα12) and M63660 (Gα13)].

and extended for 0.5 min at 72°C. Each oligonucleotide was used in the PCR at 10 ng/μl. The buffer and *Taq* polymerase were supplied by Cetus.

PCR RNA Analysis. PCR was performed on cDNA prepared from total RNA, as described above. To control for possible contamination of the RNAs by chromosomal DNA, we treated each preparation of RNA as described above, except reverse transcriptase was not added to the cDNA synthesis mixture. These controls were then subjected to PCR alongside the other samples. The PCR products were electrophoresed through a 2% agarose gel, blotted to Hybond-N filter (Amersham), and hybridized according to the manufacturer's instructions. These blots were probed with radiolabeled oligonucleotides specific to Gα11 (CTCGCTTAGTGCCACC), Gα12 (CTCGCTCGAGGACACCATGAAC), and Gα13 (TTCACTTGAAGAGACAAGGAAA). The oligonucleotides were end-labeled with [γ-^{32}P]ATP, as described (15). Blots were washed three times at room temperature for 5 min in 0.90 M NaCl/0.90 M sodium citrate (6× SSC)/0.1% SDS followed by a 1-min wash in the same solution at the calculated melting temperature of each oligonucleotide.

Isolation of cDNAs. To obtain cDNAs encoding Gα12 and Gα13, a random-hexanucleotide-primed mouse brain cDNA library in λZAPII (Stratagene) was screened by using standard techniques. Six hundred thousand clones were plated at

Gα12

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                                                TCGGGGCGGCGGCGG -121
CGGACGCAGCCTGAGGGAGCGGCGGGCGGGGCGGCGCCTGGCCCGGCAGGGCCGCGCCGG -61
CTGAGGGGCGTCCATGGCGCGCGGGCCCCAGCGGGGCGCGGCCGCGGCCTGAGGGCGGCC -1

ATGTCCGGGGTGGTGCGGACCCTTAGCCGCTGCTTGCTGCCGGCCGAGGCCGGAGCCCGC 60
M  S  G  V  V  R  T  L  S  R  C  L  L  P  A  E  A  G  A  R   20

GAGCGCAGGGCGGGCGCGGCGCGCGACGCGGAGCGCGAGGCCCGACGGCGCAGCCGCGAC 120
E  R  R  A  G  A  A  R  D  A  E  R  E  A  R  R  R  S  R  D   40

ATCGACGCGCTGCTGGCCCGCGAGCGGCGCGCGGTGCGGCGGCTGGTCAAGATCCTGCTG 180
I  D  A  L  L  A  R  E  R  R  A  V  R  R  L  V  K  I  L  L   60

CTGGGCGCCGGCGAGAGCGGCAAGTCCACCTTCCTCAAGCAGATGCGCATCATCCACGGC 240
L  G  A  G  E  S  G  K  S  T  F  L  K  Q  M  R  I  I  H  G   80

CGGGAGTTCGACCAGAAGGCGCTGCTGGAGTTCCGCGACACCATCTTCGACAACATCCTT 300
R  E  F  D  Q  K  A  L  L  E  F  R  D  T  I  F  D  N  I  L   100

AAGGGTTCGAGGGTTCTTGTGGACGCTCGAGACAAGCTCGGCATTCCCTGGCAGCACTCT 360
K  G  S  R  V  L  V  D  A  R  D  K  L  G  I  P  W  Q  H  S   120

GAGAACGAGAAGCACGGGATGTTTCTGATGGCCTTCGAGAACAAGGCAGGGCTGCCTGTG 420
E  N  E  K  H  G  M  F  L  M  A  F  E  N  K  A  G  L  P  V   140

GAGCCTGCCACCTTCCAGCTCTACGTGCCAGCCCTGAGTGCCCTCTGGAGAGACTCGGGG 480
E  P  A  T  F  Q  L  Y  V  P  A  L  S  A  L  W  R  D  S  G   160

ATCAGGGAAGCCTTCAGCCGCAGAAGCGAGTTCCAGCTGGGTGAATCAGTGAAGTACTTC 540
I  R  E  A  F  S  R  R  S  E  F  Q  L  G  E  S  V  K  Y  F   180

CTGGATAACTTGGACCGGATTGGCCAGCTGAACTACTTCCCCAGTAAGCAAGACATCCTG 600
L  D  N  L  D  R  I  G  Q  L  N  Y  F  P  S  K  Q  D  I  L   200

CTGGCTAGAAAGGCCACCAAGGGAATCGTGGAACATGACTTCGTTATAAAGAAAATCCCA 660
L  A  R  K  A  T  K  G  I  V  E  H  D  F  V  I  K  K  I  P   220

TTTAAGATGGTGGATGTGGGCGGCCAGAGGTCACAGCGCCAGAAGTGGTTCCAGTGCTTC 720
F  K  M  V  D  V  G  G  Q  R  S  Q  R  Q  K  W  F  Q  C  F   240

GACGGCATCACATCTATCCTGTTCATGGTGTCCTCGAGCGAGTATGACCAGGTCCTCATG 780
D  G  I  T  S  I  L  F  M  V  S  S  S  E  Y  D  Q  V  L  M   260

GAGGACAGGCGCACCAACCGGCTGGTGGAGTCCATGAACATCTTCGAGACCATCGTCAAC 840
E  D  R  R  T  N  R  L  V  E  S  M  N  I  F  E  T  I  V  N   280

AACAAGCTCTTCTTCAACGTCTCCATCATCCTCTTCCTCAACAAGATGGACCTCCTGGTG 900
N  K  L  F  F  N  V  S  I  I  L  F  L  N  K  M  D  L  L  V   300

GAGAAGGTGAAGTCTGTGAGCATTAAGAAGCACTTCCCAGATTTCAAGGGCGACCCGCAC 960
E  K  V  K  S  V  S  I  K  K  H  F  P  D  F  K  G  D  P  H   320

CGGCTGGAGGACGTCCAGCGCTACCTGGTGCAGTGCTTCGACAGGAAGCGCAGGAACCGC 1020
R  L  E  D  V  Q  R  Y  L  V  Q  C  F  D  R  K  R  R  N  R   340

AGCAAGCCCCTGTTCCACCACTTCACCACCGCCATAGACACCGAGAACATCCGCTTCGTG 1080
S  K  P  L  F  H  H  F  T  T  A  I  D  T  E  N  I  R  F  V   360

TTTCATGCTGTGAAGGACACGATCCTGCAGGAGAACCTGAAAGACATCATGCTGCAGTGA 1140
F  H  A  V  K  D  T  I  L  Q  E  N  L  K  D  I  M  L  Q      379

GAGACAGCCCCTCCCTCTGCTGTCCTCTCGGCCTCTGGGGCTGTGGCCGCCCACGTGGTT 1200
CTGGTGTGTCTGCCGCCTGCATGGACCTGCAGTGGGCATCTCGAATCCACACCATCTAAT 1260
ACCTGGCTCAAGAGTGCTGGACTACCAGCCACACCAGCGAGCTCCGGGCAAGAGGACGCG 1320
GGAGTGTCAGTGATAGCTACTGAATCGGGGACTGTGAGACTACTGAATCCACACCATCAC 1380
TCTGCTGTTGATCTGTAAAACCTGGGGACACACGTTTCGCCTCTCTACAGAAACTCTTCC 1440
TTGTTGACACAGTTAACCAAGGTTCAGGTGTACGCAGACACACGCGTGCTCTCATTAATG 1500
ACAGAAACCCAACCCCAGCTGGCCTTGCAGCCGGCTCTCCCCTCTGCCGTGCAGGTTCTC 1560
ACTGAGTCACACTAACTCGGGAGCTTGGCCTCACACTAGGCTGTAAAGAGCATGCGATGC 1620
TGCTAAGCTCACCGTGTCCAAGCCCAGGCCTCAGTGCCACCTCCCAGAGTGAACACACAG 1680
CCTTAGCATCGTGCCAGCACCCTCCGAGTCTCGCAAAGGCGCAGGCGGGGTCAGGTGTCC 1740
ACACT                                                        1745
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Gα13

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                                                     TCCCTCCC -121
CGGGCGCGCTCGGGCCGCCGCTGCGCTCCCCGCCCTCCAGCCGCCTTGCCGGAGCCGCCC -61
GCTGCCGGAGGAGGAGGTGGAGGAGCCGCAGGGGCCCGCCGAGGCGGCGGCGGCGGCAAG -1

ATGGCGGACTTCCTGCCGTCGCGCTCCGTGCTGTCCGTGTGCTTCCCGGGCTGCGTGCTG 60
M  A  D  F  L  P  S  R  S  V  L  S  V  C  F  P  G  C  V  L   20

ACGAACGGCGAGGCCGAGCAGCAGCGCAAGTCCAAGGAGATCGACAAATGCCTGTCGCGG 120
T  N  G  E  A  E  Q  Q  R  K  S  K  E  I  D  K  C  L  S  R   40

GAGAAGACCTACGTGAAGCGGCTGGTGAAGATCCTGCTGCTGGGCGCGGGCGAGAGCGGC 180
E  K  T  Y  V  K  R  L  V  K  I  L  L  L  G  A  G  E  S  G   60

AAGTCCACCTTCCTGAAGCAGATGCGGATCATCCACGGCCAGGACTTCGACCAGCGCGCG 240
K  S  T  F  L  K  Q  M  R  I  I  H  G  Q  D  F  D  Q  R  A   80

CGCGAGGAGTTCCGCCCCACCATCTACAGCAACGTGATCAAAGGTATGAGGGTGCTGGTA 300
R  E  E  F  R  P  T  I  Y  S  N  V  I  K  G  M  R  V  L  V   100

GATGCCCGAGAGAAGCTTCATATTCCCTGGGGAGATAACAAAAACCAGCTCCATGGAGAC 360
D  A  R  E  K  L  H  I  P  W  G  D  N  K  N  Q  L  H  G  D   120

AAGTTGATGGCATTTGATACCCGCGCCCCCATGGCTGCCCAGGGGATGGTGGAGACTCGA 420
K  L  M  A  F  D  T  R  A  P  M  A  A  Q  G  M  V  E  T  R   140

GTGTTCCTGCAGTATCTTCCTGCTATCAGAGCCTTATGGGAGGACAGTGGTATACAGAAT 480
V  F  L  Q  Y  L  P  A  I  R  A  L  W  E  D  S  G  I  Q  N   160

GCCTACGATCGGCGCCGGGAATTCCAGCTGGGTGAGTCTGTAAAGTATTTCTTGGATAAC 540
A  Y  D  R  R  R  E  F  Q  L  G  E  S  V  K  Y  F  L  D  N   180

TTGGATAAACTTGGAGTACCGGATTACATTCCATCACAGCAAGATATCCTGCTTGCCAGA 600
L  D  K  L  G  V  P  D  Y  I  P  S  Q  Q  D  I  L  L  A  R   200

AGGCCCACCAAAGGCATCCATGAGTACGACTTTGAAATTAAAAATGTTCCTTTCAAAATG 660
R  P  T  K  G  I  H  E  Y  D  F  E  I  K  N  V  P  F  K  M   220

GTTGATGTAGGTGGCCAGAGATCAGAACGGAAACGCTGGTTTGAATGCTTTGACAGTGTG 720
V  D  V  G  G  Q  R  S  E  R  K  R  W  F  E  C  F  D  S  V   240

ACGTCGATACTTTTCCTTGTCTCTTCAAGTGAATTTGACCAGGTGCTTATGGAGGACCGC 780
T  S  I  L  F  L  V  S  S  S  E  F  D  Q  V  L  M  E  D  R   260

CAGACCAATCGCCTTACAGAATCTCTGAACATTTTTGAAACAATTGTCAACAATCGGGTT 840
Q  T  N  R  L  T  E  S  L  N  I  F  E  T  I  V  N  N  R  V   280

TTCAGCAACGTCTCCATAATCCTCTTCTTAAACAAGACAGACTTGCTCGAGGAGAAAGTG 900
F  S  N  V  S  I  I  L  F  L  N  K  T  D  L  L  E  E  K  V   300

CAAGTTGTTAGCATCAAAGACTATTTCCTAGAATTTGAAGGGGACCCCCACTGCTTAAGA 960
Q  V  V  S  I  K  D  Y  F  L  E  F  E  G  D  P  H  C  L  R   320

GACGTCCAAAAGTTTCTGGTGGAATGCTTCCGGGGGAAACGCCGGGACCAGCAGCAGAGG 1020
D  V  Q  K  F  L  V  E  C  F  R  G  K  R  R  D  Q  Q  Q  R   340

CCGTTGTACCACCACTTCACCACCGCGATCAACACAGAGAACATCCGCCTCGTGTTCCGG 1080
P  L  Y  H  H  F  T  T  A  I  N  T  E  N  I  R  L  V  F  R   360

GACGTGAAGGACACGATCCTTCATGACAACCTGAAGCAGCTCATGCTGCAGTGATGACAA 1140
D  V  K  D  T  I  L  H  D  N  L  K  Q  L  M  L  Q            377

GACTCGCTGTTTTAATACCTTGTTGTGATTTTGATTGTTTTCTGTTTGTCTTGTTTTTCT 1200
CTTTAAATGGCAGTTTACAACAGCAGTTAGAAGAATCTCAGTTGTGTGTGTCTAACTTCT 1260
TGGAGAACTTAGTTCATCTGTGGCCTTTAGTTTGTGGCTGACAGCTGCTGAATTAACCCA 1320
TTGCCAATATCTGCTAGAATTTGGGCTTTGATTGGTTTCACTGTGGCTTACCTTCAAGTC 1380
CAATTATAACTTCCTGGCAACCTTACACTAGGTGTTTTTCAGGCCTTGAATTCTCATAAC 1440
TGTCCTGCAGTTCTAAGAATAGGCAGTATCTTTAAGAACTTGTAGGGATGAGAGTATGAA 1500
AATTCAGCTCTAAAGAGAGAGAGTGCCTTACCCAGCAATGACATGCCTCCCTATGTGAGC 1560
CCTGGTCTGGAAGGGCTGTTAGAGAAAAGTTTTAGGGAGGAGTCTCGGCCCACAGAGGCT 1620
TGGCCCCCTACACACACAGCAGTAGTTTGTTTTCCCTCAATTTCTCTAATTTCTGTAGGG 1680
CAGCAGTCTCAGCCCACAGAGGCTTGGCCCCCTACACACACAGCAGTAGTTTGTTTTTCT 1740
CAATCTCTTTACTTTCTGAAGGTAATGTCTTACTGCTTAAGTTTACAAGATGGATCTCAG 1800
CCTGATGCTGATGCGGTCACAAATGGTACATGTGCTATGTTGATCTGAGATGAGCAGTGA 1860
CACGCAGTTTCCTTTGCCATACATGCTGTCACTAACTTAATGTGCCATGTTACAACATCT 1920
GGAGAGGGCAAGCACCCTGGTGACGGACATCACCATAGCAGCCCCAGAGTGTGACTTTCT 1980
GGTCCTGTCTCCTTGGACTTTGTGCCTTGCTGGAGAGAACCACTGTTGGGTCTTCTTCCT 2040
TTTGCCCCACTGTCTCAACCTCAGCAGCTCTGAACTGTGGTGCTCTCGGGCTTCTCTTGT 2100
CCCCGAGAGTTTGGTGGGCAGCATTTCCCTGTGAACTGTTCAAAAAGGCATCCTTTCAGT 2160
ACCCTGTCCCTGGTTAGTGTCTCTCTGTGACAGAGTTTGTATGTGTTGTGTGTGTCATCG 2220
TGTGTGAGACACAGCTTCATTGAGGTACATTCAAATGTCTTAAGGATGCATCTAAGGATA 2280
AGCAATTTTTTTTT                                               2294
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FIG. 1. Nucleotide sequence and predicted amino acid sequence of Gα12 and Gα13. The complete sequences of two cDNA clones encoding Gα12 and Gα13 (rG12-5 and 3-G13, respectively) are shown. These clones were isolated from a random-primed mouse brain cDNA library. Sequence was obtained from two other clones (rG12-1 and rG12-2), which begin at bases 5 and −100, respectively, of the Gα12 sequence. Two hundred bases from the 5′ ends of both clones were sequenced and found identical to rG12-5.

a density of 3×10^5 plaques per 150-mm Petri dish (16). Nylon filters (Hybond-N) were prepared and hybridized according to the manufacturer's instructions. Probes were made from the Gα12 and Gα13 PCR fragments isolated previously. These fragments were labeled by using the Multiprime DNA labeling system (Amersham).

Sequence Analysis. rG12-5 and 3-G13 cDNA clones (Fig. 1) were subcloned into plasmid pMOB (17). To obtain sequence priming sites spaced throughout the clones, the γδ transposon was randomly inserted into the cDNAs, and a subset of these insertions spaced every 300–400 bases along the length in each clone was isolated by previously described methods (17). Supercoiled plasmid DNA was sequenced by using the Sequenase kit marketed by United States Biochemical.

RESULTS

Sequence of Gα12 and Gα13. Gα12 and Gα13 were initially isolated as PCR products. Mouse brain cDNA was amplified with a set of degenerate oligonucleotide primers corresponding to amino acid domains conserved in all α subunits (14). The resulting mixture of PCR products was characterized by sequencing individual clones.

To obtain full-length cDNAs encoding Gα12 and Gα13, ≈6 × 10^5 clones from a random-primed mouse brain cDNA library were screened at high stringency by using the individual PCR products as probes. Twenty-five positive clones were obtained with the Gα12 probe, whereas four were found using the Gα13 probe. To determine the extent of the 5′ sequence present in these Gα12 clones, PCR was done on the primary positive clones with oligonucleotide primers specific to the cloning vector and to the known Gα12 sequence (16). Those clones yielding the largest amplified fragments were purified. The complete sequences of two cDNAs encoding Gα12 and Gα13 are shown in Fig. 1. The predicted translation products are 379 and 377 amino acids in length, respectively. Both proteins are predicted to have a molecular mass of 44 kDa. A small open reading frame (12 amino acids) is located just upstream of the Gα12 coding region; however, the first methionine lies in a poor context for translation initiation (18).

The amino termini of Gα12 and Gα13 are quite distinctive when compared with other mammalian α subunits (Fig. 2). A number of proteins, including $G\alpha_i$ and $G\alpha_o$, are known to be N-myristoylated on a glycine at the second position (Gly-2, ref. 19). This modification is essential for anchoring the *src* oncogene product to the membrane (20). A comparison of the known N-myristoylated proteins yields the consensus sequence Met-Gly-Xaa-Xaa-Xaa-Ser (2). Thus Gα12 and Gα13 are not predicted to be modified in this way. Perhaps these two α subunits undergo a different form of posttranslational modification.

The crystal structures of ras and elongation factor Tu, as well as genetic studies of the effects of specific mutations on GTPase function, have revealed several domains in these proteins critical for guanine nucleotide interactions (5). Analogous regions in G protein α subunits are readily identifiable (2). These regions are indicated in Fig. 2 as **A**, **C**, **G**, and **I** from Halliday's nomenclature (21). The function of the I region is not well characterized in the α subunits; however, it is very highly conserved. All previously known mammalian α subunits contain the sequence Thr-Cys-Ala-Thr-Asp-Thr, except $G\alpha_s$ and $G\alpha_{olf}$, which differ at a single position (Fig. 2). A mutation (Thr-144 to Ile) in the analogous region in ras reduces GTP affinity by 25-fold (22). The fact that both Gα12 and Gα13 differ markedly from the consensus sequence in this region suggests that they may interact with guanine nucleotides in a manner distinct from some of the more well characterized α subunits.

The carboxyl terminus of several α subunits can be ADP-ribosylated by pertussis toxin. The modification occurs at a cysteine four residues from the end of the protein. Those mammalian α subunits that lack this cysteine, $G\alpha_s$, $G\alpha_{olf}$, $G\alpha_z$, $G\alpha_q$, and Gα11, are all refractory to modification by pertussis toxin (2, 12, 23). The predicted amino acid sequences of Gα12 and Gα13 also lack this cysteine. Consequently, the translation products are likely to be insensitive to pertussis toxin.

Distribution of Gα12 and Gα13 Messages. RNA analysis indicates that the Gα12 message is ≈4 kilobases (kb), whereas the transcript encoding Gα13 is >6 kb (14). As a sensitive assay for the distribution of these transcripts in a wide variety of tissues, we used PCR RNA analysis (T. M. Wilkie and M.I.S., unpublished work). With this technique, multiple α subunit sequences are amplified by PCR from cDNA by using degenerate oligonucleotide primers. These primers correspond to amino acid domains that are conserved in all α subunits. An individual sequence is detected by Southern hybridization of the amplified pool with a labeled oligonucleotide specific for that sequence. The method provides a sensitive means of detecting the presence and relative abundance of a specific α subunit sequence (Fig. 3). Both Gα12 and Gα13 can be detected in every tissue examined. Gα12 is expressed at relatively constant levels in most

```
                                                                                   A
Gα12  MSGVVRTLSRCLLPAEAGARERRAGAARDAEREARRRSRDIDALLARERRAVRRLVKILLLGAGESGKSTFLKQMRIIHGREFDQK-ALLEFRDTIFDNILKGSRVLVDA
Gα13            MADFLPSRSVLSVCFPGCVLTNG•A•QQ•K•KE••KC•S••KTY•K••••••••••••••••••••••••••QD•••R-•RE•••P••YS•VI••M••••••
Gαs                        MGCLGNSKTEDQRNE•KAQ•EANKK•EKQ•QKDKQVY•ATHR•••••••••••••IV•••••L•VNG•NGESEKATKVQD•KN•LKEAIETI•A•
Gαi1                                MGCTLSAEDKA•VE••KM••RN•REDGEKAA•E••L•••••••••••••IV•••K•••EAGYSEE-ECKQYKAVVYS•TIQSIIAIIR•
Gαq                       MTLESIMACCLSEEAK••••INDE•ERHVR•DK•DA••EL•L••••T•••••••••I••••••••SGYSDE-DKRG•TKLVYQ••FTAMQAMIR•

Gα12  RDKLGIP--WQHSENEKHGMFLMAFENKAGLP----VEPATFQL•VPALSALWRDSGIREAFSRRSEFQLGESVKYFLDNLDRIGQLNYFPSKQDILLARKATKGIVEHD
Gα13  •E••H••---•GDNK•QL••DK••••DTR•PMAAQGM••TRV•LQ•L••IR•••E••••QN•YD••R•••••••••••••••••KL•VPD•I••Q•••••••RP••••H•Y•
Gαs   MSN•VP•VELANP••QFRVDYILSVM•VPNFDFPP-------EF•EH•-K•••E•E•V•ACYE•SN•Y••IDCAQ••••K••V•K•AD•V••D••L•RC•VL•S••F•TK
Gαi1  MGR•K•DFGDAARADDARQL•VL•GAAEE•F--------M•AE•-AGVIKR••K•••VQAC•N•SR•Y••ND•AA•Y•ND••••A•P••I•TQ••V•RT•VK•T••••TH
Gαq   M•T•K••YKYE•NKAHAQLVREVDV•KVSAFE----------NP••D•IKS••N•P••Q•CYD••R•Y••SD•T••Y•ND•••VADPS•L•TQ••V•RV•VP•T••I•YP

                C                                                                     G
Gα12  FVIKKIPFKMVDVGGQRSQRQKWFQCFDGITSILFMVSSSEYDQVLMEDRRTNRLVESMNIFETIVNNKLFFNVSIILFLNKMDLLVEKVK--SVSIKKHFPDF------
Gα13  •E••NV•••••••••••••E•KR••E•••SV•••••L•••••F••••••••Q••••T••L•••••••••RV•S•••••••••••T•••E•••Q--V••••DY•LE•------
Gαs   •QVD•VN•H•F••••••DE•R••I•••NDV•A•I•V•A••S•NM•IR••NQ••••Q•AL•L•KS•W••RWLRTI•V••••••Q•••A•••LAGKSK•EDY••E•ARYTTP
Gαi1  •TF•DLH•••F••••••••E•K••IH••E•V•A•I•C•AL•D••L••A••EEM••MH•••KL•DS•C•••W•TDT••••••••K••FE••I•--KSPLTICY•EY------
Gαq   •DLQSVI•R••••••••••E•R••IH••ENV•••M•L•AL••••••••V•SDNE••ME••KAL•R••ITYPW•Q•S•V••••••K•••E••IM--YSHLVDY••EY------

                                             I
Gα12  ------KGDPHRLEDVQRYLVQCFDR--KRRN-RSKPLFHHFTTAIDTENIRFVFHAVKDTILQENLKDIMLQ
Gα13  ------E••••C•R•••KF••E••RG--•••DQQQR••Y•••••••N•••••L••RD••••••HD•••QL•••
Gαs   EDATPEP•EDP•VTRAKYFIRDE•L•ISTASGDGRHYCYP•••C•V•••••••R••NDCR•I•QRMH•RQYE•L
Gαi1  ------A•S-NTY•EAAA•IQCQ•EDLN••KDTKEI--YT•••C•T••K•VQ•••D••T•V•IKN••••CG•F
Gαq   ------D•PQRDAQAAREFILKM•VDLNPDSD-KII--YS•••C•T••••••••••A•••••••••L•••EYN•V
```

FIG. 2. Amino acid comparison of Gα12 and Gα13, $G\alpha_s$, $G\alpha_{i1}$, and $G\alpha_q$. Dots represent identities with the Gα12 sequence. Boldface letters above the sequences identify conserved sequences.

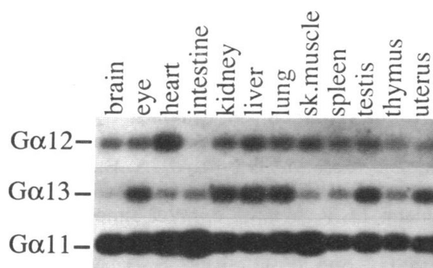

FIG. 3. PCR RNA analysis of Gα12, Gα13, and Gα11. RNA from various mouse tissues was reverse transcribed and PCR-amplified by using the degenerate oligonucleotides oMP19, oMP20, oMP21, and CT56. Amplified products were hybridized with radiolabeled oligonucleotides specific to Gα12, Gα13, and Gα11.

tissues, although it is noticeably lower in intestine. Gα13 appears to be expressed at somewhat higher levels in eye, kidney, liver, lung, and testis. Gα11 has been shown by RNA hybridization to be expressed at fairly constant levels in a variety of tissues (10); this pattern is reproduced by the PCR RNA analysis. Controls to ascertain the absence of contaminating chromosomal DNA were negative (data not shown, see *Materials and Methods*). It should be noted that PCR RNA analysis can minimize the effect of partially degraded RNA preparations, which upon RNA analysis will indicate artificially low expression levels. This effect is particularly pronounced with large messages and may explain the lower relative expression level originally observed for Gα13 in liver (14).

Relationships Among the α Subunits. The previously known mammalian α subunits can be grouped by amino acid identity into three classes: G_s, G_i, and G_q (10). Members of one class, in general, are <50% identical to members of the other classes. These relationships represent not only primary sequence homologies but also, to some extent, functional similarities. Thus, within a class members often display significant levels of "crosstalk" in reconstitution experiments. For example, both $G\alpha_s$ and $G\alpha_{olf}$ can couple β-adrenergic receptors to adenylate cyclase (24). Also, the three types of $G\alpha_i$ proteins, both purified and recombinant forms, can open atrial potassium channels (25).

Gα12 and Gα13 define a fourth class of α subunits (Fig. 4). They are <45% identical to members of the other three classes, but they share 67% amino acid identity with each other.

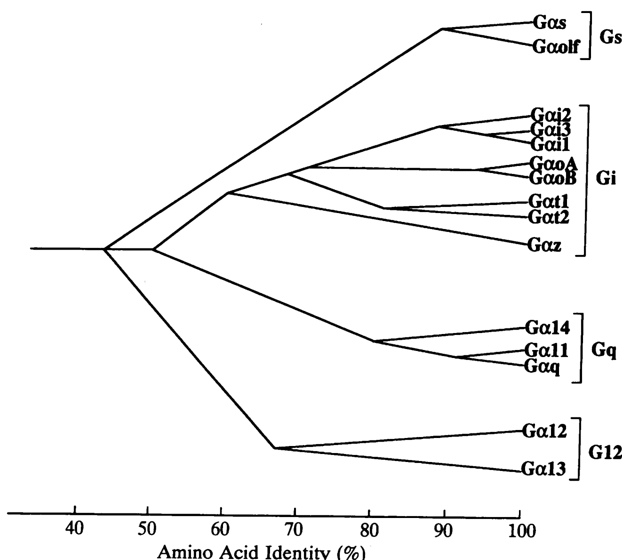

FIG. 4. Relationships among mammalian G protein α subunits. α subunits are grouped by amino acid identity (2, 10, 26, 27). Branch junctions approximate values calculated for each pair of sequences.

DISCUSSION

Cellular responses elicited by numerous different ligands can be affected by pertussis toxin. The ability to inhibit a particular pathway with this toxin has been widely used to implicate a G protein mediator. Only the G_i class includes α subunits known to be substrates for ADP-ribosylation by pertussis toxin. In fact, most genes encoding α subunits yield products that appear insensitive to this modification. The ubiquitously expressed Gα12 and Gα13 cDNAs reveal a fourth class of α subunits that are predicted to encode pertussis toxin-resistant proteins.

PLC-catalyzed release of inositol triphosphate and diacylglycerol was inferred to involve a pertussis toxin-resistant G protein that has been called "G_p" (9). Purified activated α subunits corresponding to the $G\alpha_q$ and/or Gα11 clones have been shown to stimulate PLC-β activity and to have some of the properties previously ascribed to G_p (13). However, there are a variety of PLC isoforms; many cells contain multiple related but distinct PLC gene products (28). Perhaps Gα12 and Gα13 couple to these other PLC isozymes or to other phospholipases that are activated through G-protein-mediated pathways.

The relationships among the α subunits depicted in Fig. 3 developed early in the evolution of the animal kingdom. Representatives of the G_s, G_i, and G_q classes have been found in *Drosophila* and nematodes (refs. 10, 29–36, U.-J. Kim, and M.I.S., unpublished work). It seems likely that the G12 class will be found in these organisms as well. In fact, Parks and Wieschaus (37) recently showed that the *Drosophila* gene concertina (*cta*) encodes a G protein α subunit. *cta* is clearly more related to Gα12 and Gα13 (56% amino acid sequence identity) than to the other α subunits (35–44% identity). Mutations in *cta* affect gastrulation and have a maternal effect; mothers homozygous for these mutations will survive, but their offspring do not develop properly. If the functions of specific α subunits are conserved in evolution, then we may expect a member of the G12 class, perhaps as yet unidentified, to influence early developmental processes in more complex organisms.

The application of molecular biological techniques to the study of G proteins has proven to be a powerful tool in the analysis of signal transduction in complex multicellular organisms. The notion that the diverse signaling requirements of mammals need only a small set of G proteins is being replaced by the perception of greater complexity. In addition to multiple classes of α subunits, extensive diversity of β and γ subunits has also been found (38–41). If combinatorial associations of the three subunits can occur in a functionally relevant manner, then the potential for G protein diversity is enormous.

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