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Biochemistry

G protein diversity: A distinct class of α subunits is present in vertebrates and invertebrates

(GTP-binding protein/signal transduction/*Drosophila*)

MICHAEL STRATHMANN AND MELVIN I. SIMON

Division of Biology 147-75, California Institute of Technology, Pasadena, CA 91125

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ABSTRACT Heterotrimeric guanine nucleotide-binding proteins (G proteins) are integral to the signal transduction pathways that mediate the cell's response to many hormones, neuromodulators, and a variety of other ligands. While many signaling processes are guanine nucleotide dependent, the precise coupling between a variety of receptors, G proteins, and effectors remains obscure. We found that the family of genes that encode the α subunits of heterotrimeric G proteins is much larger than had previously been supposed. These novel alpha subunits could account for some of the diverse activities attributed to G proteins. We have now obtained cDNA clones encoding two murine α subunits, $G\alpha_q$ and $G\alpha 11$, that are 88% identical. They lack the site that is ordinarily modified by pertussis toxin and their sequences vary from the canonical Gly-Ala-Gly-Glu-Ser (GAGES) amino acid sequence found in most other G protein α subunits. Multiple mRNAs as large as 7.5 kilobases hybridize to $G\alpha_q$ specific probes and are expressed at various levels in many different tissues. $G\alpha 11$ is encoded by a single 4.0-kilobase message which is expressed ubiquitously. Amino acid sequence comparisons suggest that $G\alpha_q$ and $G\alpha 11$ represent a third class of α subunits. A member of this class was found in *Drosophila melanogaster*. This α subunit, $DG\alpha_q$, is 76% identical to $G\alpha_q$. The presence of the G_q class in both vertebrates and invertebrates points to a role that is central to signal transduction in multicellular organisms. We suggest that these α subunits may be involved in pertussis toxin-insensitive pathways coupled to phospholipase C.

The G proteins are a family of guanine nucleotide-binding proteins that relay signals from cell surface receptors to intracellular effectors. Members of this family are heterotrimers composed of α, β, and γ subunits. The α subunit is believed to confer receptor and effector specificity on the heterotrimer. When the G protein is activated by interaction with receptor, the α subunit exchanges bound GDP for GTP. The intrinsic GTPase activity of the α subunit restores it to the basal state in which GDP is bound. This form of signal transduction is basic to the mechanisms that cells use in responding to hormones, neurotransmitters, and a variety of other ligands (for reviews see refs. 1–3). The process is highly conserved in evolution. Indeed, G proteins are central to intercellular communication among even simple eukaryotes. For example, G proteins are involved in the yeast mating-type pathway (4, 5), and several G protein α subunits are differentially expressed during development in the slime mold *Dictyostelium discoideum* (6, 7).

We are interested in how G protein-mediated signal transduction has adapted to the diverse signaling requirements of complex multicellular organisms. Reconstitution studies and the use of pertussis and cholera toxins to modify specific G protein α subunits have demonstrated the involvement of the $G\alpha_s$ and $G\alpha_i$ subtypes in gating of specific ion channels (8, 9) and in the regulation of adenylyl cyclase in a variety of organisms (10). In the highly specialized visual system in mammals, biochemical experiments have led to the elucidation of the role of $G\alpha_{t1}$ (rod transducin) in regulating phosphodiesterase and subsequently in controlling the levels of cyclic GMP (11). There are, however, many processes that are refractory to toxin inhibition but nonetheless appear to be mediated by guanine nucleotide-binding proteins (12, 13). To understand the extent of involvement of the G protein system and the nature of the specificity required for function, we have examined the diversity of the G protein family in complex organisms.

Recently, we developed an approach involving the polymerase chain reaction (PCR, ref. 14) to detect novel sequences that share highly conserved domains common to all G protein α subunits. We found evidence for extensive diversity in the mammalian G protein α subunit family (15). A small screen uncovered five novel sequences termed $G\alpha 10$ through $G\alpha 14$. In this paper we present the cDNA sequences of two α subunits that define another class of G proteins. This class, termed G_q, is distinguished by amino acid sequence homology and includes $G\alpha_q$, $G\alpha 11$, and $G\alpha 14$. We argue that the G_q class appeared early in evolution; it is found in both vertebrates and invertebrates. We present the sequence of a member of the G_q class in *Drosophila*.*

MATERIALS AND METHODS

PCR. PCR was performed as described previously (15). cDNA was made from poly(A)$^+$ RNA with random hexanucleotide primers by using reverse transcriptase from Moloney murine leukemia virus. Conditions were those described by the supplier (BRL). The oligonucleotides used for PCR amplification of the cDNA were as follows:

oMP19, CGGATCCAARTGGATHCAYTGYTT;
oMP20, GGAATTCRTCYTTYTTRTTNAGRAA;
oMP21, GGAATTCRTCYTTYTTRTTYAARAA;
GQ112, CTGAAGGAGTACAAYCTGGT;
GQ3, GACACGAATGACAGGAGTGCT; and
G113, CCTCAAGCCACATTGAGTCA,

in which R = A or G, Y = C or T, H = A, C, or T, and N = A, C, G, or T. PCR was performed on a Perkin Elmer Cetus thermal cycler. During each cycle, samples were denatured for 1.0 min at 94°C, extended for 1.0 min at 72°C, and annealed for 0.5 min at the following temperatures: oMP19 + oMP20 + oMP21, 42°C; GQ112 + GQ3, 53°C; and GQ112 + G113, 53°C. Each oligonucleotide was used in the PCR at 10 ng/μl. Thirty-five cycles were performed on approximately 5

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Abbreviation: PCR, polymerase chain reaction.
*The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M55412 for $G\alpha_q$ and M55411 for $G\alpha 11$.

ng of cDNA in a 50-μl reaction volume. The buffer and *Taq* polymerase were supplied by Cetus.

Northern Analysis. Total RNA and poly(A)$^+$ RNA were run on 1% agarose gels and transferred to GeneScreen (DuPont) as described (15). Probes specific to $G\alpha_q$ or $G\alpha 11$ 3′ untranslated regions were made by PCR amplifying the cDNA clones Gq7 and G23 (Fig. 1) with oligonucleotide pairs GQ112 + GQ3 ($G\alpha_q$) and GQ112 + G113 ($G\alpha 11$). The amplified products were electrophoresed on low melting point agarose gels (Seaplaque, FMC), excised, and labeled by random priming as described (15). The RNA blots were hybridized as described (15).

PCR Northern. PCR was performed on cDNA prepared with reverse transcriptase (supplied by BRL) from total RNA as described above. The degenerate oligonucleotides oMP19, oMP20, and oMP21 were used for 35 cycles of amplification. The PCR products were electrophoresed through a 2% agarose gel, blotted to GeneScreen, and hybridized according to the manufacturer's instructions. These blots were probed with radiolabeled oligonucleotides specific to $G\alpha_q$ (ATTCGCTAAGCGCTACTAGA) and $G\alpha 11$ (CTCGCTTAGTGCCACC). The oligonucleotides were end-labeled with [γ-^{32}P]ATP as described (ref. 16, p. 10.59). Blots were washed at room temperature twice for 5 min in 0.90 M NaCl/0.90 M sodium citrate (6× SSC)/0.1% SDS, twice for 5 min in 6× SSC, and finally they were washed for 1 min in 6× SSC at the melting temperature of each oligonucleotide.

Isolation of cDNAs. The G69 cDNA clone (Fig. 1*A*) was isolated from an oligo(dT)-primed mouse brain library in the λ cloning vector mλJSM8 (unpublished results); 10^5 clones were screened at a density of 10^4 plaques per 150-mm plate. Nitrocellulose filters (Millipore) were prepared by standard techniques (ref. 16, p. 12.30). Appropriate restriction enzymes were used to excise the coding regions of cDNA clones encoding rat $G\alpha_s$, $G\alpha_{i1}$, $G\alpha_{i2}$, $G\alpha_{i3}$, and $G\alpha_o$; bovine $G\alpha_{t1}$ and $G\alpha_{t2}$; and human $G\alpha_z$. These cDNAs were com-

A

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                    CGCGCCGGCGGGGCTGCAGCGAGGCACTTCGGAAGA -1
ATGACTCTGGAGTCCATCATGGCGTGCTGCCTGAGCGAGGAGGCCAAGGAAGCCCGGAGG 59
M  T  L  E  S  I  M  A  C  C  L  S  E  E  A  K  E  A  R  R  20
ATCAACGACGAGATCGAGCGGCACGTGCGCAGGGACAAGCGCGACGCCCGCCGGGAGCTC 119
I  N  D  E  I  E  R  H  V  R  R  D  K  R  D  A  R  R  E  L  40
AAGCTGCTGCTGCTGGGGACAGGGGAGAGTGGCAAGAGCACCTTCATCAAGCAGATGAGG 179
K  L  L  L  L  G  T  G  E  S  G  K  S  T  F  I  K  Q  M  R  60
ATCATCCACGGGTCGGGCTACTCTGACGAAGACAAGCGCGGCTTCACCAAGCTGGTGTAT 239
I  I  H  G  S  G  Y  S  D  E  D  K  R  G  F  T  K  L  V  Y  80
CAGAACATCTTCACGGCCATGCAGGCCATGATCAGAGCGATGGACACGCTCAAGATCCCA 299
Q  N  I  F  T  A  M  Q  A  M  I  R  A  M  D  T  L  K  I  P  100
TACAAGTATGAACACAATAAGGCTCATGCACAATTGGTTCGAGAGGTTGATGTGGAGAAG 359
Y  K  Y  E  H  N  K  A  H  A  Q  L  V  R  E  V  D  V  E  K  120
GTGTCTGCTTTTGAGAATCCATATGTAGATGCAATAAAGAGCTTGTGGAATGATCCTGGA 419
V  S  A  F  E  N  P  Y  V  D  A  I  K  S  L  W  N  D  P  G  140
ATCCAGGAGTGCTACGACAGACGACGGGAATATCAGTTATCTGACTCTACCAAATACTAT 479
I  Q  E  C  Y  D  R  R  R  E  Y  Q  L  S  D  S  T  K  Y  Y  160
CTGAATGACTTGGACCGTGTAGCCGACCCTTCCTATCTGCCTACACAACAAGACGTGCTT 539
L  N  D  L  D  R  V  A  D  P  S  Y  L  P  T  Q  Q  D  V  L  180
AGAGTTCGAGTCCCCACTACAGGGATCATCGAATACCCCTTTGACTTACAAAGTGTCATT 599
R  V  R  V  P  T  T  G  I  I  E  Y  P  F  D  L  Q  S  V  I  200
TTCAGAATGGTCGATGTAGGGGGCCAAAGGTCAGAGAGAAGAAAATGGATACACTGCTTT 659
F  R  M  V  D  V  G  G  Q  R  S  E  R  R  K  W  I  H  C  F  220
GAAAATGTCACCTCCATCATGTTTCTAGTAGCGCTTAGCGAATATGATCAAGTTCTTGTG 719
E  N  V  T  S  I  M  F  L  V  A  L  S  E  Y  D  Q  V  L  V  240
GAGTCAGACAATGAGAACCGCATGGAGGAGAGCAAAGCACTCTTTAGAACAATTATCACC 779
E  S  D  N  E  N  R  M  E  E  S  K  A  L  F  R  T  I  I  T  260
TACCCCTGGTTCCAGAACTCCTCTGTGATTCTGTTCTTAAACAAGAAAGATCTTCTAGAG 839
Y  P  W  F  Q  N  S  S  V  I  L  F  L  N  K  K  D  L  L  E  280
GAGAAAATCATGTATTCCCACCTAGTCGACTACTTCCCAGAATATGATGGACCCCAGAGA 899
E  K  I  M  Y  S  H  L  V  D  Y  F  P  E  Y  D  G  P  Q  R  300
GATGCCCAGGCAGCTCGAGAATTCATCCTGAAAATGTTCGTGGACCTGAACCCCGACAGT 959
D  A  Q  A  A  R  E  F  I  L  K  M  F  V  D  L  N  P  D  S  320
GACAAAATCATCTACTCCCACTTCACGTGCGCCACAGATACCGAGAACATCCGCTTCGTC 1019
D  K  I  I  Y  S  H  F  T  C  A  T  D  T  E  N  I  R  F  V  340
TTTGCAGCCGTCAAGGACACCATCCTGCAGCTGAACCTGAAGGAGTACAATCTGGTCTAA 1079
F  A  A  V  K  D  T  I  L  Q  L  N  L  K  E  Y  N  L  V  *  360
CCGTGCCTCCCAGAAACTGGTTCTTCCCTCCCTGTGGGTTGTTGAAGATAAACAAGAGGG 1139
ACTGTATTTCTGCGGAAAACAATTTGCATAATACTAATTTATTGCCGTCCTGGACTCTGT 1199
GAGCGTGTCCACAGAGCTGTAGTAAATATTATGATTTTATTTAAACTATTCAGAGGAAAC 1259
AGGATGCTGAAGTACAGTCCCAGCACATTTCCTCTTTTTTTTTTTTTTAGGCAAACCTTG 1319
ATGTATTTTAAATTTTCAGTCATTCACTCACAGTATAAAAGCACTCCTGTCATTCGTGTC 1379
CTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCATTCC               1423
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B

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  TTCGGGGAACCGGGAAGAGGTAGGGCCGGGCCGGCGGCAGCCGGAGGACCGCGACG   -1
ATGACTCTGGAGTCCATGATGGCGTGTTGCCTGAGCGACGAGGTGAAGGAGTCGAAGCGC 59
M  T  L  E  S  M  M  A  C  C  L  S  D  E  V  K  E  S  K  R  20
ATCAACGCGGAGATCGAGAAACAGCTGCGGCGGGACAAGCGCGACGCCCGGCGCGAGCTC 119
I  N  A  E  I  E  K  Q  L  R  R  D  K  R  D  A  R  R  E  L  40
AAGCTGCTGCTACTTGGCACTGGCGAGAGCGGGAAGAGTACCTTCATCAAGCAGATGCGC 179
K  L  L  L  L  G  T  G  E  S  G  K  S  T  F  I  K  Q  M  R  60
ATCATCCACGGGGCCGGCTACTCGGAGGAGGACAAGCGCGGCTTCACCAAGTTGGTGTAC 239
I  I  H  G  A  G  Y  S  E  E  D  K  R  G  F  T  K  L  V  Y  80
CAGAACATCTTTACCGCCATGCAGGCCATGGTGCGCGCCATGGAGACGCTCAAGATCCTC 299
Q  N  I  F  T  A  M  Q  A  M  V  R  A  M  E  T  L  K  I  L  100
TACAAGTATGAGCAGAACAAGGCCAATGCACTCCTGATCCGGGAGGTCGATGTGGAGAAG 359
Y  K  Y  E  Q  N  K  A  N  A  L  L  I  R  E  V  D  V  E  K  120
GTCACAACTTTTGAGCACCAGTATGTGAATGCCATCAAGACGCTGTGGAGTGACCCTGGT 419
V  T  T  F  E  H  Q  Y  V  N  A  I  K  T  L  W  S  D  P  G  140
GTCCAGGAGTGTTACGATCGCAGGCGGGAGTTCCAGCTATCTGACTCGGCTAAGTACTAC 479
V  Q  E  C  Y  D  R  R  R  E  F  Q  L  S  D  S  A  K  Y  Y  160
TTGACGGACGTGGACCGCATCGCCACAGTAGGCTACCTGCCCACCCAGCAGGATGTGCTG 539
L  T  D  V  D  R  I  A  T  V  G  Y  L  P  T  Q  Q  D  V  L  180
CGGGTACGCGTGCCCACCACTGGCATCATCGAGTACCCGTTTGACCTGGAGAACATCATC 599
R  V  R  V  P  T  T  G  I  I  E  Y  P  F  D  L  E  N  I  I  200
TTCAGGATGGTGGATGTGGGCGGGCAGAGGTCAGAGCGCAGGAAGTGGATCCATTGCTTT 659
F  R  M  V  D  V  G  G  Q  R  S  E  R  R  K  W  I  H  C  F  220
GAGAACGTGACTTCCATCATGTTCTTGGTGGCACTAAGCGAGTATGACCAAGTCCTGGTG 719
E  N  V  T  S  I  M  F  L  V  A  L  S  E  Y  D  Q  V  L  V  240
GAGTCAGACAATGAGAACCGCATGGAGGAGAGCAAGGCCCTGTTCCGCACAATCATCACC 779
E  S  D  N  E  N  R  M  E  E  S  K  A  L  F  R  T  I  I  T  260
TACCCCTGGTTCCAGAACTCGTCTGTCATTCTCTTCCTCAACAAGAAGGACCTTCTAGAA 839
Y  P  W  F  Q  N  S  S  V  I  L  F  L  N  K  K  D  L  L  E  280
GACAAGATCCTGCACTCACACTTGGTCGATTACTTCCCTGAGTTTGATGGGCCACAGAGG 899
D  K  I  L  H  S  H  L  V  D  Y  F  P  E  F  D  G  P  Q  R  300
GATGCACAGGCCGCACGCGAGTTCATTCTGAAGATGTTTGTGGACCTGAACCCTGACAGC 959
D  A  Q  A  A  R  E  F  I  L  K  M  F  V  D  L  N  P  D  S  320
GACAAAATCATCTACTCCCACTTCACATGTGCCACCGACACCGAGAACATCCGCTTTGTG 1019
D  K  I  I  Y  S  H  F  T  C  A  T  D  T  E  N  I  R  F  V  340
TTCGCAGCTGTGAAGGACACCATCCTGCAGCTGAACCTGAAGGAGTACAACCTGGTGTGA 1079
F  A  A  V  K  D  T  I  L  Q  L  N  L  K  E  Y  N  L  V  *  359
CCGCGGCATGGCTGCCACCAGGGCCAGCTTCCTCCGCATCCGGTCACCCAAACCGAGGGA 1139
CCTGGGCACACGGGGGCTGGGGCACATCTCTCTTCCCGCTCACTTCCTGTCCCTGTCCCT 1199
CTTCCTCAAGGGCAGAGTGGCCTCATTTCGGCCTTGACTCAATGTGGCTTGAGGTTTTTT 1259
TTTTTCTTAGAAAACAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA             1307
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C

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Gαq   MTLESIMACCLSEEAK..(16)
Gα11  MTLESMMACCLSDEVK..
Gαi1       MGCTLSAEDK..
Gαo        MGCTLSAEER..
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D

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Gαq/Gα11..LLLLGTGESGKSTFIKQ..(58)
Gαz       ..LLLLGTSNSGKSTIVKQ..
other Gα..LLLLGAGESGKSTIVKQ..
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E

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Gαq/Gα11..QLNLKEYNLV (359)
Gαi1      ..KNNLKDCGLF
Gαz       ..QNNLKYIGLC
Gαs       ..RMHLRQYELL
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FIG. 1. Sequence of $G\alpha_q$ and $G\alpha 11$. The sequence of $G\alpha_q$ (*A*) was obtained from two clones, Gq3 and Gq7. These clones were isolated from a random-primed mouse brain cDNA library by using G69 as a probe. These clones contain the following nucleotides: Gq3, −36 to 966; Gq7, 144 to 1423; and G69, 120 to 629. The $G\alpha 11$ sequence (*B*) is contained in two clones, G23 and G11-5′-9. G23, which extends from nucleotide 490 to nucleotide 1307, was isolated from an oligo(dT)-primed mouse brain cDNA library by using the $G\alpha 11$ PCR fragment (15) as a probe. G11-5′-9 was isolated from the random-primed library mentioned above. This clone extends from nucleotide −56 to nucleotide 957. The N termini (*C*), GTP-binding domains (*D*, ref. 2), and the C termini (*E*) of several α subunits are compared. The position of the last amino acid in each domain of $G\alpha_q$ is given in parenthesis.

bined in equimolar ratios and labeled by random priming as described (15). This probe was hybridized to the filters at 50°C in 0.90 M NaCl/6 mM EDTA/60 mM NaH_2PO_4 (6× SSPE)/0.1% SDS/5× Denhardt's solution containing denatured salmon sperm DNA at 100 μg/ml (1× Denhardt'solution = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrrolidone). The filters were washed in 6× SSC/0.1% SDS three times for 5 min at room temperature and once at 50°C for 15 min. To obtain the entire sequence of $G\alpha_q$ and Gα11, a random hexanucleotide or oligo(dT)-primed mouse brain cDNA library in mλJSM8 or λZAPII (Stratagene) was screened by using standard techniques. $DG\alpha_q$ was cloned from a *Drosophila melanogaster* head-specific library (see *Results*) kindly provided by B. Hamilton (Biology Division, California Institute of Technology, Pasadena, CA).

RESULTS

Nucleotide Sequence of $G\alpha_q$ and Gα11. The clone G69, which encodes part of $G\alpha_q$, was isolated from a mouse brain cDNA library by low-stringency hybridization. It cross-hybridized to a probe consisting of a mixture of cDNAs encoding all the previously known G protein α subunits (see *Materials and Methods*). Further screening yielded overlapping cDNA clones containing the entire $G\alpha_q$ coding sequence (Fig. 1*A*). The cloned PCR product corresponding to Gα11, which included 180 base pairs of sequence (15), was used as a probe to screen a mouse brain cDNA library. Several clones were purified and sequenced. Fig. 1*B* shows the sequence of Gα11 derived from overlapping cDNA fragments. The deduced amino acid sequence of Gα11 is 88% identical to that of $G\alpha_q$. Almost all of the amino acid differences between $G\alpha_q$ and Gα11 are concentrated in the N-terminal half of the protein. Of 42 amino acid differences, 38 are found in the N-terminal region composed of amino acids 1–200, while there are only 4 amino acid changes in a stretch of polypeptide encompassed by amino acids 201–359.

When $G\alpha_q$ and Gα11 are compared with the other α subunits, a number of noteworthy differences emerge. Fig. 1*C* shows a comparison of the N-terminal sequences of $G\alpha_q$ and Gα11 with those found for other α subunits. The methionine predicted by homology to be the first codon in $G\alpha_q$ and Gα11 is preceded by other methionines in frame. The six additional amino acids found in these two α subunits are highly conserved, suggesting that they are functionally significant. The nucleotide sequences of the two cDNAs diverge upstream of these codons, indicating that there may be no further extension of this reading frame. Also in this 5′ region, the Gα11 cDNA contains a stop codon in frame with the downstream coding sequence

The N termini of some α subunits are *N*-myristoylated on a glycine at the second position (Gly-2) (17). On the basis of their deduced amino acid sequences, $G\alpha_q$ and Gα11 are not substrates for myristoylation (Fig. 1*C*); this may affect their membrane association properties. However, $G\alpha_s$ is not myristoylated, yet this α subunit is membrane associated. Perhaps other forms of post-translational modification will prove to be responsible for anchoring these hydrophilic proteins to the membrane.

Fig. 1*D* compares the amino acid sequences in the region of the "GAGE-box." This domain is highly conserved among all α subunits and has been implicated in GTP binding. Mutations in this region affect the GTPase activity of the α subunit (18, 19). $G\alpha_z$ [also named $G\alpha_x$ (20, 21)] differs in this region from the other α subunits. The slow rates of guanine nucleotide exchange and GTP hydrolysis exhibited by $G\alpha_z$ may be due in part to these sequence changes (22). $G\alpha_q$ and Gα11 show differences in this region as well (Fig. 1*D*). Consequently, these α subunits may display unusual kinetic properties when the proteins are characterized (see *Discussion*).

The C-terminal region of the α subunit is of considerable interest. The cysteine residue that lies four amino acids from the end of most mammalian α subunits can be ADP-ribosylated by pertussis toxin, thereby inactivating the G protein. Among the known α subunits, only $G\alpha_s$, $G\alpha_{olf}$, and $G\alpha_z$ lack this cysteine. These proteins are refractory to modification by pertussis toxin (2, 22). Fig. 1*E* shows that $G\alpha_q$ and Gα11 also lack the cysteine residue at this position. Thus it is likely that the proteins corresponding to these cDNA clones will be insensitive to pertussis toxin modification.

Distribution of $G\alpha_q$ and Gα11. To determine the relative size of the mRNAs corresponding to $G\alpha_q$ and Gα11 and their distributions, Northern hybridization was performed with RNA purified from a variety of mouse tissues. Both $G\alpha_q$ and Gα11 are ubiquitously expressed (Fig. 2). Using a probe specific to the 3′ untranslated region of each cDNA clone, we found that $G\alpha_q$ has multiple messages, whereas Gα11 shows a single band of approximately 4 kb. The three largest $G\alpha_q$ transcripts (approximately 5, 6, and 7.5 kb) were evident (Fig. 2*A*) in other RNA preparations, suggesting that they are not the products of a single degraded message (data not shown). The very large size of the $G\alpha_q$ message and the variation in signal strength in some of the tissues led us to use another method to probe for the presence of $G\alpha_q$ and Gα11 specific RNA. This technique, termed PCR Northern analysis (T. M. Wilkie and M.I.S., unpublished), utilizes oligonucleotide primers designed to amplify a pool of α subunits by PCR. Specific sequences in the pool are detected by oligonucleotide hybridization. The Gα11 message (Fig. 2*C*) was found to be expressed in all tissues that were examined. The $G\alpha_q$ message (Fig. 2*C*) was also found in all tissues, although the relative levels appeared to be lower in RNA samples derived from intestine and testes and higher in brain and lung.

Analysis of the Relationships Among the G Protein α Subunits. Itoh *et al.* (23) have described the relationships between the α subunits on the basis of amino acid identity. These comparisons suggest evolutionary relationships that may exist among the G proteins. An expansion of this analysis

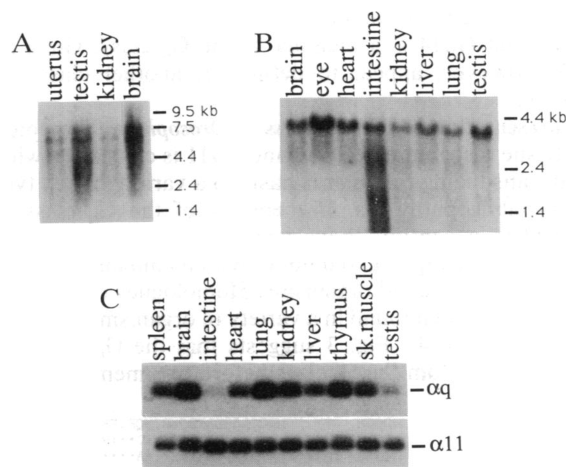

FIG. 2. Northern and PCR Northern analysis of $G\alpha_q$ and Gα11. Probes specific to $G\alpha_q$ (*A*) and Gα11 (*B*) were hybridized to poly(A)$^+$ RNA (*A*) and total RNA (*B*) from various mouse tissues. Loads were 20 μg of total RNA per lane and 4 μg of poly(A)$^+$ RNA per lane; however, ethidium bromide staining revealed that the amount of ribosomal RNA contamination varied among the different tissues. kb, Kilobases. PCR Northern analysis (*C*) was performed on various mouse tissues by using the degenerate oligonucleotides oMP19, oMP20, and oMP21. The amplified products were hybridized with radiolabeled oligonucleotides specific to $G\alpha_q$ and Gα11.

Gαs
Gαolf
Gs
Gαi2
Gαi3
Gαi1
GαoA
GαoB
Gi
Gαt1
Gαt2
Gαz
Gα14
Gα11
Gq
Gαq
40 50 60 70 80 90 100
Amino Acid Identity (%)

FIG. 3. Relationships among mammalian G protein α subunits. The α subunits are grouped by amino acid identity (2). Branch junctions approximate values calculated for each pair of sequences. $G\alpha_oA$ and $G\alpha_oB$ represent slice variants of the $G\alpha_o$ gene (24, 25). A broken line extends from Gα14 because the entire sequence of this α subunit is not available.

reveals three classes of α subunits: G_s, G_i, and G_q (Fig. 3). Within a class, α subunits display not only primary sequence relationships but also, to some extent, functional similarities. Thus in reconstitution studies some α subunits show significant levels of "crosstalk." For example, $G\alpha_{olf}$ can substitute for $G\alpha_s$ in coupling the β-adrenergic receptor to adenylyl cyclase (26). Also, the three $G\alpha_i$ subtypes open atrial potassium channels (27). The G_i class includes all the known α subunits that are susceptible to pertussis toxin modification.

$G\alpha_q$ and Gα11 form a separate class (Fig. 3). They show less than 50% amino acid sequence identity with any of the other α subunits. It is likely that $G\alpha_q$ and Gα11 will display significant crosstalk, since they differ at only four residues over the C-terminal 144 amino acids. This C-terminal domain is thought to contain the structural elements required for specific interactions with effector and receptor (28). Comparison of these amino acid sequences with the partial sequences uncovered in the PCR screen described earlier (15) reveals that Gα14 is a member of the G_q class. Gα12 and Gα13, however, appear to define yet another class of α subunits.

Representation of the G_q Class in *Drosophila*. The ubiquitous tissue distribution of $G\alpha_q$ and Gα11 is consistent with a signal transducing role that is basic to a variety of cell types and signaling pathways. If members of the G_q class are involved in a central "housekeeping" pathway, then this class should be represented not only in mammals but also in other distantly related organisms. Homologues of $G\alpha_s$, $G\alpha_i$, and $G\alpha_o$ have been found in a variety of organisms, including *Drosophila* (29–34). Fig. 3 suggests that the G_q class may have diverged from the G_i class before two members of the G_i class, G_o and $G\alpha_i$, diverged from each other. Thus it seemed possible that *Drosophila* would have a $G\alpha_q$ homologue. These arguments encouraged us to screen *D. melanogaster* α subunits for a member of the G_q class. We used the same PCR technique described previously (15) with mixed degenerate oligonucleotides corresponding to two highly conserved amino acid hexamer sequences found in all of the G proteins thus far studied. We screened clones derived from amplification of RNA isolated from 0- to 24-hr embryos of *Drosophila* and from total *Drosophila* adult RNA. We initially obtained a PCR product from both RNA sources which when sequenced differed from the murine $G\alpha_q$ at 3 of the deduced 51 amino acids. This PCR product was used to probe a *Drosophila* head cDNA library, and a clone was isolated and sequenced. The deduced amino acid sequence of the *Drosophila* $G\alpha_q$ homologue ($DG\alpha_q$) is compared with $G\alpha_q$ and Gα11 in Fig. 4. $DG\alpha_q$ is 76% identical (88% similar) to $G\alpha_q$. This α subunit lacks the putative N-terminal six additional amino acids; however, it has the characteristic changes in the sequence of the GTP-binding domain (GAGE box, Fig. 1*D*). In addition, $DG\alpha_q$ is predicted to be insensitive to pertussis toxin on the basis of its C-terminal amino acid sequence.

DISCUSSION

The analysis of cDNA clones encoding $G\alpha_q$ and Gα11 demonstrates the existence of ubiquitously expressed highly homologous G protein α subunits. During the characterization of these cDNA clones, Pang and Sternweiss used affinity chromotography with immobilized $\beta\gamma$ subunits (35) to purify novel α subunits (36). They found α subunits with an apparent molecular mass of 42 kDa. The amino acid sequences of tryptic fragments from these proteins were identical to the deduced amino acid sequence of $G\alpha_q$. One of the peptides also showed a sequence identical to that of Gα11. Furthermore, Pang and Sternweiss (36) found that the 42-kDa proteins have the properties that we would ascribe to $G\alpha_q$ and Gα11; they are not substrates for pertussis toxin modification, and they have unusual nucleotide binding kinetics. Using peptide antisera, they showed that the proteins are abundant in brain and lung extracts and present at lower levels in a variety of tissues.

There are many examples in the literature of effects of GTP and GTP analogs on signal transducing processes that are difficult to account for on the basis of the previously characterized G protein α subunits. Perhaps the most prominent among these is the observation that in many systems inositol trisphosphate and diacylglycerol release mediated by G protein activation of phospholipase C is pertussis toxin resistant (37). $G\alpha_z$ is thought to be a candidate for the G protein that might mediate pertussis toxin-insensitive activation of phospholipase C. However, $G\alpha_z$ expression appears to be restricted to neural tissues and platelets and it does not appear to be present in all tissues that show pertussis toxin-insensitive activation of phospholipase C (38). On the other hand, $G\alpha_q$ and Gα11 are ubiquitously expressed. Hence they

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DGqα        MECCLSEEAKEQKRINQEIEKQLRRDKRDARRELKLLLLGTGESGKSTFIKQMRIIHGSGYSDEDKRGYIKLVFQNIFMAMQSM  84
Gqα   MTLESI•A•••••••••AR•••D•••RHV•••••••••••••••••••••••••••••••••••••••••••••FT•••Y••••T•••A•  90
Gα11  MTLESM•A••••D•V••S••••A•••••••••••••••••••••••••••••••••••••••••••A•••E•••••FT•••Y••••T•••A•  90

      IKAMDMLKISYGQGEHSELADLVMSIDYETVTTFEDPYLNAIKTLWDDAGIQECYDRRREYQLTDSAKYYLSDLARIEQADYLPTEQDIL  174
      •R•••T•••P•KYEHNKAH•Q••REV•V•K•SA••N••VD•••S••N•P••••••••••••••S••T••••N••D•VADPS••••Q••V•  180
      VR••ET•••L•KYEQNKAN•L•IREV•V•K•••••HQ•V•••••••S•P•V•••••••••F••S•••••••T•VD••ATVG••••Q••V•  180

      RARVPTTGILEYPFDLDGIVFRMVDVGGQRSERRKWIHCFENVTSIIFLVALSEYDQILFESDNENRMEESKALFRTIITYPWFQNSSVI  264
      •V•••••••I••••••QSVI••••••••••••••••••••••••••M••••••••••V•V••••••••••••••••••••••••••••••  270
      •V•••••••I••••••EN•I••••••••••••••••••••••••••M••••••••••V•V••••••••••••••••••••••••••••••  270

      LFLNKKDLLEEKIMYSHLVDYFPEYDGPKQDHAAAKQFVLKKYLACNPDPERQCYSHFTTATDTENIKLVFCAVKDTIMQNALKEFNLG  353
      ••••••••••••••••••••••••••••QR•AQ••RE•I••MFVDL•••SDKII•••••C•••••••RF••A••••••L•LN•••Y••V  359
      ••••••••••D••LH•••••••••F•••QR•AQ••RE•I••MFVDL•••SDKII•••••C•••••••RF••A••••••L•LN•••Y••V  359
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FIG. 4. Amino acid comparison of the *Drosophila* $G\alpha_q$ homologue ($DG\alpha_q$) with $G\alpha_q$ and Gα11. Dots represent identities with the $DG\alpha_q$ sequence.

are good candidates for this role. Furthermore, there are a variety of isoforms of phospholipase C; many cells contain multiple related but distinct phospholipase C gene products (39). Members of the G_q class could be involved in the activation of specific phospholipase C isozymes. Speculation about the possible interaction of the G_q class with phospholipase C is strengthened by the recent finding that $DG\alpha_q$ message is localized mainly to the *Drosophila* eye and ocellus structures (40). Earlier work indicated that regulation of phospholipase C in invertebrate eyes is central to the phototransduction cascade (41). There are a variety of other roles that are possible for the α subunits described here—e.g., ion channel activation and phospholipase A2 activation.

cDNA clones with deduced amino acid sequences that are identical to $G\alpha 11$ have been found in RNA preparations from human tissue (T. T. Amatruda and M.I.S., unpublished results). The partial amino acid sequences found by Pang and Sternweiss (36) suggest that both $G\alpha_q$ and $G\alpha 11$ exist in rat brain extracts, and experiments with specific probes in our laboratory have detected $G\alpha_q$ and $G\alpha 11$ messages in a variety of tissues and cloned cell lines. These results suggest that both α subunits are expressed together in at least some cells.

The diversity of the G protein family continues to grow. As more α subunits are cloned, more classes will emerge. Within each class, highly homologous members are likely to display apparent crosstalk in reconstitution experiments. Indeed, it is difficult to discriminate between $G\alpha_{i1}$ and $G\alpha_{i3}$ by *in vitro* assays (27, 42). $G\alpha_q$ and $G\alpha 11$ may behave in similar fashion, since they are 97% identical over the domains apparently responsible for receptor and effector specificity. However, the strict evolutionary conservation of amino acid sequence differences that identify these α subunits argues that they are not redundant (2). The distinctions may become apparent as we begin to use more sophisticated assays of G protein function that include different combinations of $\beta\gamma$ dimers. There is evidence to suggest that all $\beta\gamma$ subunits are not equivalent (43, 44). Perhaps the diversity afforded by combinatorial associations of α, β, and γ subunits allows a cell to "fine tune" G protein function to the specific requirements of a particular signaling response.

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