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The *Drosophila* gene coding for the α subunit of a stimulatory G protein is preferentially expressed in the nervous system

(guanine nucleotide-binding protein/signal transduction/*in situ* hybridization)

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ABSTRACT **In mammals, the α subunit of the stimulatory guanine nucleotide-binding protein ($G_s\alpha$) functions to couple a variety of extracellular membrane receptors to adenylate cyclase. Activation of $G_s\alpha$ results in the stimulation of adenylate cyclase and an increase in the second messenger cAMP. A 1.7-kilobase cDNA has been identified and characterized from *Drosophila* that codes for a protein 71% identical to bovine $G_s\alpha$. The similarity is most striking in the regions thought to be responsible for the interactions with receptors and effectors, suggesting that the basic components of this signal-transduction pathway have been conserved through evolution. RNA blot hybridization and DNA sequence analysis suggest that a single transcript, expressed predominantly in the head, is present in *Drosophila*. *In situ* hybridization studies indicate that the *Drosophila* $G_s\alpha$ transcript is localized primarily in the cells of the central nervous system and in the eyes.**

Guanine nucleotide-binding (G) proteins couple the receptors for a wide variety of extracellular signals to a number of intracellular effector systems in the production of diverse biological effects. Upon interaction with an activated receptor, the G-protein α subunit (Gα) exchanges bound GDP for GTP. The activated α subunit dissociates from the receptor and β/γ subunits to interact with effector enzymes or ion channels (1–3). The intrinsic GTPase activity of the α subunit then hydrolyzes the bound GTP to GDP to return the complex to the unstimulated state. In this scheme, the α subunit is responsible for the specific interaction with both the receptor and the effector, while the β/γ subunits appear to inactivate the α subunit and may anchor the complex to the membrane (1–3). Thus, different G proteins have different α subunits but similar or identical β/γ subunits.

Classification of G proteins is based historically on the functional interaction of α subunits with specific effector proteins. For example, transducins are responsible for the activation of the cGMP phosphodiesterase in the retina. G_s and G_i stimulate and inhibit adenylate cyclase, respectively. G_o is an abundant G protein in the nervous system whose function has yet to be determined. Molecular cloning of cDNAs coding for the various α subunits from a number of mammalian sources (4–8) and yeast (9, 10) has demonstrated that distinct molecular forms exist within each functional class of α subunit (11–15). The functional significance of these alternative forms is unclear.

The study of G-protein function has been limited to conventional *in vitro* biochemistry (1–3) and the genetic manipulation of cultured cells (16–19). The association of G-protein alterations with a variety of pathological conditions (20–23), however, points to a critical role for these proteins and the transduction events they mediate in processes that require the complex interaction of many cells. The sophisticated genetics and ease of manipulation of *Drosophila* make it a particularly attractive system in which to study the role of G proteins in complex biological processes such as development and behavior (24). To this end, we have identified cDNAs for a number of Gα subunits expressed in the *Drosophila* nervous system. We report here the isolation and characterization of cDNAs that code for a $G_s\alpha$-like protein from *Drosophila* and show that the *Drosophila* homolog is expressed most abundantly in the nervous system. We believe that this is the first report of a $G_s\alpha$ protein in a nonmammalian organism.†

METHODS

Materials. An adult *Drosophila melanogaster* head cDNA library was kindly provided by P. Salvaterra (City of Hope Research Institute, Duarte, CA). The bovine $G_s\alpha$ (4) and rat $G_i\alpha$ (5) cDNAs were kindly provided by J. D. Robishaw and H. Itoh, respectively.

Isolation of cDNA Clones. The *Drosophila* head cDNA library was screened under low-stringency conditions with a 1.0-kilobase (kb) *Nco* I–*Mlu* I fragment from the bovine $G_s\alpha$ cDNA. This fragment consists exclusively of coding sequence and spans the entire coding region except for the 185 base pairs (bp) coding for the carboxyl terminus of the protein. Hybridizations were carried out in 25% (vol/vol) formamide/5× SSC/5× Denhardt's solution/50 mM sodium phosphate, pH 6.8/10% (wt/vol) dextran sulfate at 37°C (1× SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7; 1× Denhardt's solution is 0.02% Ficoll/0.02% bovine serum albumin/0.02% polyvinylpyrrolidone). Filters were washed in 0.5× SSC at 50°C.

Nucleotide Sequencing. Restriction fragments were sequenced by the dideoxy method (25) with double-stranded templates (26) after subcloning into pBS(M13−) (Stratagene).

Northern and Southern Blots. Total RNA was prepared as described (27). Poly(A)$^+$ RNA was selected on oligo(dT)-cellulose (Collaborative Research), electrophoresed in 1.2% agarose/6% formaldehyde gels, and blotted to Nytran (Schleicher & Schuell). Hybridizations were done in 50% formamide/5% SDS/0.4 M sodium phosphate, pH 7.2/1 mM EDTA at 37°C. Washes were done in 0.1× SSC at 65°C. Southern blots were hybridized under low-stringency conditions as described above. High-stringency blots were hybridized in the same buffer containing 50% formamide/3× SSC and washed in 0.1× SSC at 65°C.

Chromosomal Localizations. *In situ* hybridizations to polytene chromosomes were done by using biotinylated probes as described (28) except that the probes were made with biotin-

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Abbreviations: G protein, guanine nucleotide-binding protein; Gα, G-protein α subunit.

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†The sequence reported in this paper has been deposited in the EMBL/GenBank data base (accession no. M23233).

21-dUTP (Clontech) and detected with a horseradish peroxidase system (Vector Laboratories).

Hybridizations to Tissue Sections. Hybridization to tissue sections was done with RNA probes generated *in vitro* by using either T3 or T7 promoters after the subcloning of the appropriate fragments into pBS(M13−). RNA transcripts were labeled using [α-[^{35}S]thio]UTP. RNA was prepared and hybridized to 8-μm tissue sections that had been fixed with 4% formaldehyde, then treated in 0.2 M HCl for 20 min, 2× SSC for 30 min at 60°C, proteinase K at 37°C for 15 min, and 4% formaldehyde for 20 min, and then acetylated and dried (29). After hybridization overnight at 50°C, slides were rinsed briefly in 4× SSC and then incubated for 15 min at 60°C in 50% formamide/0.3 M NaCl/30 mM Tris, pH 7.5/1 mM EDTA/10 mM dithiothreitol. This procedure greatly improved the signal-to-noise ratio. Slides were then washed in 4 liters of 2× SSC (room temperature, 30 min), 0.1× SSC (55°C, 10 min), and 0.1× SSC (room temperature, 10 min). Slides were dried and coated with photographic emulsion (Kodak NTB2).

RESULTS AND DISCUSSION

Approximately 5 × 10^5 recombinant phage from the *Drosophila* head cDNA library were screened with the 1.0-kb *Nco* I–*Mlu* I fragment from the bovine $G_s\alpha$ cDNA, and 13 positive phage clones were isolated. Restriction analysis placed these isolates into two groups. The major group consisted of 12 recombinants containing inserts of 1.4–1.7 kb. Southern blot analysis showed that this group hybridized strongly to the bovine $G_s\alpha$ cDNA and weakly to a rat $G_i\alpha$ cDNA (data not shown). The longest member of this group, λDGs12, was chosen for nucleotide sequence analysis (Fig. 1*a*).

The nucleotide sequence and deduced amino acid sequence of λDGs12 are shown in Fig. 1*b*. λDGs12 contains an open reading frame coding for a protein of 385 residues (45,003 Da). The assignment of the initiator ATG is based on the homology with the deduced amino acid sequences of other Gα subunits, most of which begin with the sequence Met-Gly-Cys (4–8). In addition, the nucleotide sequence around the proposed initiator ATG, CTGCGATGG, is a good match to the Kozak consensus sequence for translation initiation, CC$\overset{A}{G}$CCATG(G) (30), matching in seven of nine positions (underlined). However, this sequence does not match a consensus sequence, $\overset{C}{A}$AA$\overset{A}{C}$CATG, proposed for *Drosophila* initiation codons (31). The 5′ untranslated region contains two additional ATG codons, at positions −252 and −242, followed by 22-codon and 10-codon open reading frames, respectively. Only the ATG at −252 is in the same reading frame as the initiator ATG. This 5′ untranslated sequence is found in at least two additional, independently isolated cDNAs. The 3′ untranslated region of λDGs12 is 194 nucleotides long and terminates in a stretch of 5 adenine residues. Nucleotide sequencing of the 3′ untranslated region of two additional independent isolates demonstrated that these cDNAs terminated at the same site but with poly(A) tracts of 14–25 residues. A pair of overlapping consensus polyadenylylation signals (32) are located 12 and 16 bp upstream of these adenine residues.

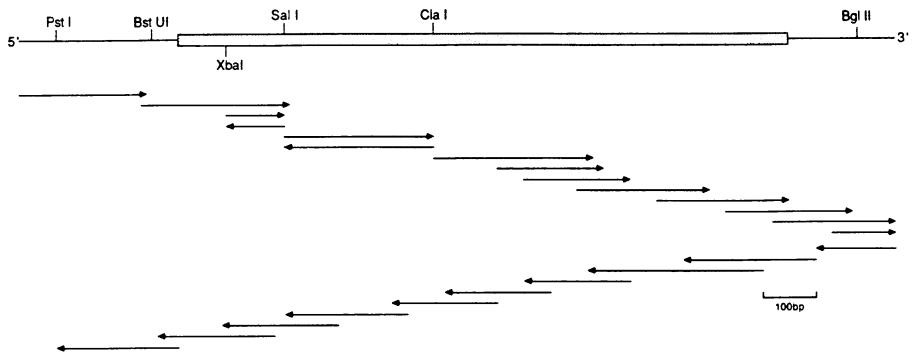
Fig. 2 shows a comparison of the deduced amino acid sequence of λDGs12 to those of Gα subunits representative of the various vertebrate G-protein classes (4–8). From this comparison it is evident that λDGs12 is a cDNA coding for a Gα protein. Taking into account only identical residues, the *Drosophila* protein has an amino acid homology of 71% to the long form of bovine $G_s\alpha$. The level of homology to $G_i\alpha$, $G_o\alpha$, and transducin is lower but still significant (41–44%). The similarity is highest in the four regions of homology that have been identified between the Gα subunits, the *ras* oncogene proteins, and bacterial elongation factor Tu (1–8). These highly conserved regions (A, C, E, and G) are thought to be responsible for guanine nucleotide binding and hydrolysis (33, 34). These regions are also highly conserved in this *Drosophila* Gα cDNA (Fig. 2). Region C is identical in the *Drosophila* and vertebrate G proteins. Regions A and G are identical to the corresponding regions of bovine $G_s\alpha$ and 73–78% identical to rat $G_i\alpha$ and bovine $G_o\alpha$. Region E is less conserved but is still 78% and 56% identical with $G_s\alpha$ and $G_i\alpha$.

In mammals, the alignment of the deduced amino acid sequence of the various Gα polypeptides has shown that $G_s\alpha$ is the most divergent member of the Gα family (1–8). As noted earlier, the protein coded for by λDGs12 has a much higher overall homology to $G_s\alpha$ (71%) than to any other of the Gα subunits (41–43%). In fact, the *Drosophila* protein is virtually identical to $G_s\alpha$ in many of the regions where $G_s\alpha$ diverges from the other Gα subunits. For example, residues 32–45 of the *Drosophila* protein are virtually identical to residues 29–42 of bovine $G_s\alpha$, matching in 14 of 15 positions. In contrast, only 2 of these residues match the corresponding regions of $G_i\alpha$ and $G_o\alpha$. The homology to $G_s\alpha$ is also striking in regions interspersed between the postulated guanine nucleotide contact sites, which are thought to carry out the various functions of the Gα chains (34). In these models of a composite Gα protein, interaction with effector protein has been assigned to the region between the first two guanine nucleotide-binding regions (A and C, Fig. 2). The protein encoded by λDGs12 and the smaller form of bovine $G_s\alpha$ are 65% identical in this region. The region of receptor interaction has been assigned to the carboxyl-terminal region. Recent studies using chimeric cDNA constructs have suggested that the carboxyl terminus is responsible as well for some portion of effector interaction (19). As shown in Fig. 2, the *Drosophila* protein and bovine $G_s\alpha$ are 92% identical over the last 64 amino acid residues. The *Drosophila* protein and other Gα proteins are only 27–30% identical over this region. We propose that λDGs12 codes for a *Drosophila* $G_s\alpha$ subunit and thus, that this protein interacts with the same classes of receptors and effectors in *Drosophila* as mammalian $G_s\alpha$. This possibility can be tested directly by assessing the ability of *Drosophila* $G_s\alpha$ to functionally complement the lack of endogenous $G_s\alpha$ in murine S49 cyc^- cells (35).

Protein studies have shown that vertebrate $G_s\alpha$ is structurally heterogeneous, existing as at least two species with apparent molecular weights of 45,000 and 52,000 in SDS/polyacrylamide gels (1–3). Molecular cloning studies have demonstrated the existence of multiple vertebrate $G_s\alpha$ mRNAs, produced by a single gene, that code for proteins varying in both the number and sequence of residues in the region corresponding to amino acids 72–88 of the largest form of $G_s\alpha$ (11, 12). λDGs12 is missing amino acids 72–86 of the long form of bovine $G_s\alpha$ (Fig. 2). Thus, λDGs12 corresponds most closely to the smaller forms of $G_s\alpha$ found in mammalian tissues. To determine whether we had isolated cDNAs coding for forms of *Drosophila* $G_s\alpha$ that also vary in this region, this region of the remaining 11 $G_s\alpha$ isolates was sequenced by using a primer based on the nucleotide sequence of λDGs12. All 11 were found to be identical to λDGs12 in this region. It is possible, however, that variant forms of *Drosophila* $G_s\alpha$ exist but are expressed at low abundance, in different tissues, or at specific developmental stages and thus were missed in the initial screening. Alternatively, *Drosophila* $G_s\alpha$ subunits may be subject to variation in different regions of the protein.

The *Drosophila* $G_s\alpha$ appears to be a cholera toxin substrate but not a pertussis toxin substrate. The sequence around Arg-189 is essentially identical to that of bovine $G_s\alpha$ around Arg-201, the residue modified by cholera toxin (36). Pertussis toxin modifies susceptible G proteins at a cysteine residue

a



b

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                                -299-CTCGCAACCGAGTCGTGCACCTATAGTCTTGGCCCGAAGTTGCTCCCATGAATAAAAATGT
                                                   -280                -260                -240

TCGCCTGCTGCAGAGATCCACTGAAGCGCTGAGCCAACGCCAACGGTGGTGCCACTGAGATCGGAGATCGGAGATCGCAGACCGGAGACCGACGAGCACACAAGGGATTGTGGTTTGAT
         -220                -200                -180                -160                -140                -120

CCGAGGAACCCAAGCACTCAGCTATACTGGAGCAGAACGGAGAAAGCACAAGGGGTGCCGAGTCGCCCGACGCGAACCAGGAAAACCGAGCGACGTGTTGGTGGACCCCGTGGGCTGCG
          -100                 -80                 -60                 -40                 -20

1                                       10                                      20                                      30
MET Gly Cys Phe Gly Ser Pro Thr Ser Lys Gln Ser Asp Val Asn Ser Glu Asp Ser Lys Ser Gln Lys Arg Arg Ser Asp Ala Ile Ser
ATG GGT TGC TTT GGG TCG CCC ACC TCC AAG CAG TCG GAC GTG AAC TCG GAG GAC TCG AAG AGC CAG AAG CGC CGG AGC GAT GCA ATA TCT
1                    20                        40                        60                        80

                                        40                                      50                                      60
Arg Gln Leu Gln Lys Asp Lys Gln Leu Tyr Arg Ala Thr His Arg Leu Leu Leu Leu Gly Ala Gly Glu Ser Gly Lys Ser Thr Ile Val
AGA CAG TTG CAG AAG GAC AAA CAG CTC TAC AGG GCC ACA CAC AGG CTG CTC CTC CTG GGG GCG GGC GAG TCC GGC AAA TCA ACC ATA GTC
          100                      120                        140                        160                      180

                                        70                                      80                                      90
Lys Gln MET Arg Ile Leu His Val Asp Gly Phe Ser Asp Ser Glu Lys Lys Gln Lys Ile Asp Asp Ile Lys Lys Asn Ile Arg Asp Ala
AAG CAA ATG CGA ATA TTG CAT GTC GAC GGA TTT TCT GAC TCG GAA AAG AAA CAG AAA ATT GAT GAT ATT AAA AAG AAT ATT CGA GAC GCT
                     200                       220                       240                       260

                                        100                                     110                                     120
Ile Leu Thr Ile Thr Gly Ala MET Ser Thr Leu Asn Pro Pro Val Ala Leu Glu Lys Lys Glu Asn Glu Pro Arg Val Glu Tyr Ile Gln
ATC TTG ACT ATT ACA GGA GCC ATG AGC ACA CTT AAT CCA CCT GTA GCT TTA GAA AAG AAG GAA AAT GAA CCC AGA GTG GAG TAC ATT CAG
          280                      300                       320                       340                      360

                                        130                                     140                                     150
Asp Tyr Ala Ser Ser Pro Asp Phe Asn Tyr Pro Pro Glu Phe Tyr Glu His Thr Glu Glu Leu Trp Lys Asp Lys Gly Val Leu Gln Thr
GAT TAT GCA TCT AGT CCG GAC TTT AAT TAT CCT CCT GAA TTT TAT GAA CAT ACA GAA GAA CTA TGG AAA GAC AAG GGC GTT CTT CAA ACC
                     380                       400                       420                       440

                                        160                                     170                                     180
Tyr Glu Arg Ser Asn Glu Tyr Gln Leu Ile Asp Cys Ala Lys Tyr Phe Leu Asp Arg Val Ser Thr Ile Lys Asn Pro Asn Tyr Thr Pro
TAT GAG AGG TCG AAT GAG TAT CAA TTA ATC GAT TGT GCG AAA TAC TTC CTG GAC CGA GTG AGC ACA ATC AAG AAT CCA AAC TAC ACC CCT
          460                      480                       500                       520                      540

                                *   190                                     200                                     210
Asn Glu Gln Asp Ile Leu Arg Cys Arg Val Leu Thr Ser Gly Ile Phe Glu Thr Arg Phe Gln Val Asp Lys Val Asn Phe His MET Phe
AAT GAG CAG GAT ATT CTT CGG TGC CGT GTT TTG ACT TCT GGA ATA TTT GAA ACA AGA TTT CAA GTG GAC AAA GTA AAC TTT CAC ATG TTC
                     560                       580                       600                       620

                                        220                                     230                                     240
Asp Val Gly Gly Gln Arg Asp Glu Arg Arg Lys Trp Ile Gln Cys Phe Asn Asp Val Thr Ala Ile Ile Phe Val Thr Ala Cys Ser Ser
GAT GTC GGT GGC CAG CGG GAC GAG CGT AGG AAA TGG ATT CAG TGT TTC AAT GAT GTA ACT GCT ATC ATA TTC GTA ACT GCG TGC TCA AGT
          640                      660                       680                       700                      720

                                        250                                     260                                     270
Tyr Asn MET Val Leu Arg Glu Asp Pro Thr Gln Asn Arg Leu Arg Glu Ser Leu Asp Leu Phe Lys Ser Ile Trp Asn Asn Arg Trp Leu
TAT AAC ATG GTT TTG CGG GAA GAT CCC ACC CAG AAC CGA CTT CGA GAA TCT TTG GAT TTG TTC AAG AGT ATT TGG AAC AAC AGA TGG CTT
                     740                       760                       780                       800

                                        280                                     290                                     300
Arg Thr Ile Ser Ile Ile Leu Phe Leu Asn Lys Gln Asp Leu Leu Ala Glu Lys Ile Lys Ala Gly Lys Ser Lys Leu Ser Glu Tyr Phe
CGC ACG ATT TCT ATT ATA CTA TTT TTA AAT AAG CAA GAT TTG TTA GCA GAG AAA ATT AAG GCT GGA AAA AGT AAA TTG TCG GAA TAT TTC
          820                      840                       860                       880                      900

                                        310                                     320                                     330
Ser Glu Phe Asn Lys Tyr Gln Thr Pro Ile Asp Thr Gly Asp Ala Ile MET Glu Ser Asn Asp Asp Pro Glu Val Ile Arg Ala Lys Tyr
TCC GAG TTT AAC AAA TAC CAA ACG CCA ATC GAC ACA GGT GAC GCA ATA ATG GAA TCC AAT GAC GAC CCA GAA GTA ATA CGA GCA AAA TAT
                     920                       940                       960                       980

                                        340                                     350                                     360
Phe Ile Arg Asp Glu Phe Leu Arg Ile Ser Thr Ala Ser Gly Asp Gly Lys His Tyr Cys Tyr Pro His Phe Thr Cys Ala Val Asp Thr
TTC ATA CGA GAC GAG TTT CTG CGT ATA TCT ACC GCT AGC GGA GAC GGA AAA CAC TAC TGC TAT CCA CAT TTC ACA TGC GCC GTT GAC ACA
         1000                     1020                      1040                      1060                     1080

                                        370                                     380                                     390
Glu Asn Ile Lys Arg Val Phe Asn Asp Cys Arg Asp Ile Ile Gln Arg MET His Leu Arg Gln Tyr Glu Leu Leu  •
GAA AAC ATT AAA CGT GTG TTT AAT GAT TGC AGA GAC ATT ATT CAA AGG ATG CAC CTT CGT CAA TAT GAA TTG TTA TAG GTTATCCCCATCGCC
                    1100                      1120                      1140                      1160

GTAATGCAAGTAAATAAAAATATTAATGACTTACTGTTTTAAATATAATTATCAAATGTAATTTGTATTTAAATAGTCCCTTGATTAAAACAAAAAATTCGCTGCATTTAGATCTGGTA
    1180                1200                1220                1240                1260                1280

TGACCTTTCACAAAACTCAACATTAAAGAATAATAAATAAACATGTTAATAACAAAAA-1350
    1300                1320                1340
```

FIG. 1. (*a*) Restriction map and sequencing strategy of λDGs12. The coding region (nucleotides 1–1155) is indicated by the bar. Arrows indicate the extent and direction of sequencing. (*b*) Nucleotide sequence and deduced amino acid sequence of λDGs12. Nucleotide 1 is the first nucleotide of the ATG translation start codon. The potential site of ADP-ribosylation by cholera toxin is indicated by an asterisk. The translation termination codon is indicated by a dot. Potential polyadenylylation signals are underlined.

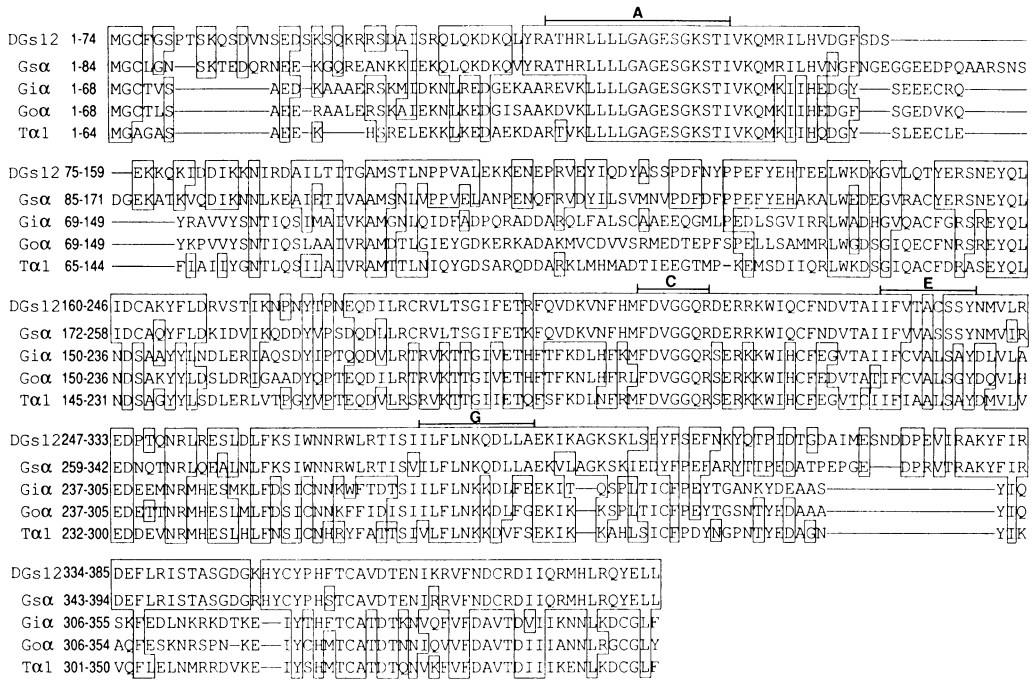

FIG. 2. Alignment of the deduced amino acid sequence (standard one-letter symbols) of the *Drosophila* Gα subunit with those of bovine $G_s\alpha$, $G_o\alpha$, and transducin (Tα1) and rat $G_i\alpha$. Only residues identical to those of the *Drosophila* subunit have been boxed. The postulated guanine nucleotide-binding regions (A, C, E, G) are indicated.

found in the conserved sequence Cys-Gly-Leu-(Phe/Tyr) at the carboxyl terminus (36, 37). The *Drosophila* $G_s\alpha$ subunit ends in the sequence Tyr-Glu-Leu-Leu (Fig. 1*b*).

Southern blot analysis of *Drosophila* genomic DNA with λDGs12 as a probe was consistent with the presence of a single gene for $G_s\alpha$ (Fig. 3). A single major band of hybridization was seen after digestion of genomic DNA with *Bam*HI, *Eco*RI, *Hin*dIII, or *Pst* I. The additional low molecular weight band seen with *Pst* I is consistent with the presence of a *Pst* I site in λDGs12. The minor high molecular weight band seen in the *Bam*HI, *Eco*RI, and *Pst* I digests may have resulted from hybridization to related sequences in the *Drosophila* genome or to partial digestion products. These additional bands may also have resulted from the presence of small portions of $G_s\alpha$ exons in fragments containing mostly intron sequences. A low molecular weight band was seen with *Eco*RI digests after long autoradiographic exposures (data not shown). No additional bands were seen at low stringency (Fig. 3). *In situ* hybridization of the biotinylated 1.7-kb fragment of λDGs12 to polytene chromosomes localized the *Drosophila* $G_s\alpha$ gene to position 60A of the genome (data not shown). No mutations have been identified that map to this region of the second chromosome.

In Northern blot analysis of poly(A)$^+$ RNA isolated from whole adult flies, heads, and bodies, λDGs12 hybridized to a single transcript of ≈1.9 kb found predominantly in heads (Fig. 4). No additional transcripts were detected after longer exposures (Fig. 4). When the poly(A) tail is taken into account, it seems likely that λDGs12 is close to full length.

To examine the distribution of $G_s\alpha$ mRNAs in *Drosophila*, ^{35}S-labeled RNAs were made *in vitro* from a fragment corresponding to the coding sequence of λDGs12 and hybridized to tissue sections. As seen in dark-field (Fig. 5*a*) and phase-contrast (Fig. 5*b*) microscopic images, the strongest hybridization of antisense RNA was to the cortex (arrows) of the brain, which contains the neuronal cell bodies. Little or no hybridization was detected in the central neuropil, which contains neurites and synapses but no cell bodies. An intermediate level of hybridization was present in the eyes. The cortex of the optic lobes (small arrows) had consistently lower levels of hybridization than the midbrain cortex (big arrows). In addition, the cortex of the ventral ganglion showed hybridization to these probes (data not shown). Little hybridization above background levels was detected in other tissues such as muscles, gut, fat bodies (Fig. 5), and ovaries. Similar results were obtained with a fragment containing only 5′ untranslated sequences (data not shown). No differential pattern of hybridization was observed with sense-strand RNA probes. Thus, $G_s\alpha$ message is most abundant in the central nervous system, with less in the eyes. Its restriction to the cell bodies of neurons is consistent with this being the site of transcription and translation. The signal in the eye could not be localized to a particular cell type, although the photoreceptor cells themselves would be likely candidates.

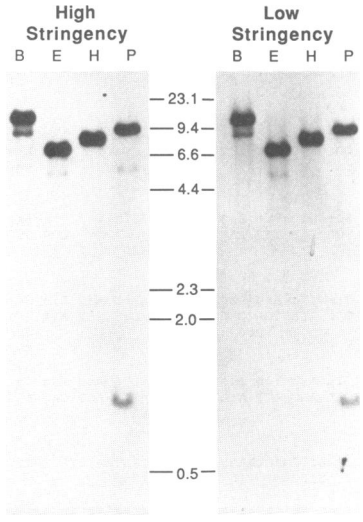

FIG. 3. Southern blot analysis. *Drosophila* DNA was cut with *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), or *Pst* I (P) and analyzed by hybridization to the 1.7-kb *Eco*RI fragment of λDGs12 under high- or low-stringency conditions. Five micrograms of DNA was run in each lane. *Hin*dIII fragments of λ DNA were used as size (kb) markers.

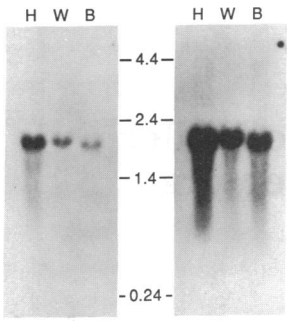

FIG. 4. Northern blot analysis of λDGs12. Poly(A)$^+$ RNA was isolated from heads (H), whole flies (W), and bodies (B). Twenty micrograms of RNA was loaded in each lane. The 1.7-kb *Eco*RI fragment of λDGs12 was used as probe. Short and long exposures of the same blot are shown. Positions of molecular size standards (0.24- to 9.5-kb RNA "ladder," Bethesda Research Laboratories) are indicated.

Its apparent presence in eyes raises the possibility that this Gα could participate in phototransduction to couple the absorption of light to phospholipase C, the effector that functions in this system (38). However, the relatively low level of expression in eyes (Fig. 5) and the high sequence homology to vertebrate $G_s\alpha$ suggest that this is unlikely.

The isolation of cDNA clones for *Drosophila* $G_s\alpha$ has demonstrated the strong conservation of primary sequence with the vertebrate $G_s\alpha$ subunit. *In situ* hybridization to tissue sections has localized its transcript primarily to cells of the nervous system. Recently, cDNA clones coding for *Drosophila* $G_i\alpha$-like (ref. 39; F.Q. and M.A.F., unpublished data) and $G_o\alpha$-like proteins (N. Thambi, F.Q., W.J.W., and M.A.F., unpublished data) have been isolated in addition to the G-protein β-subunit gene (40). A detailed genetic analysis of the function of these proteins is now possible and is expected to provide basic insights into the role of G proteins in complex, multicellular processes. Genetic manipulation of $G_s\alpha$ in *Drosophila* should be particularly interesting in this regard because alterations in the regulation of adenylate cyclase by the *rutabaga* gene and phosphodiesterase by the *dunce* gene lead to developmental and learning defects, apparently by altering cAMP levels (41–43).

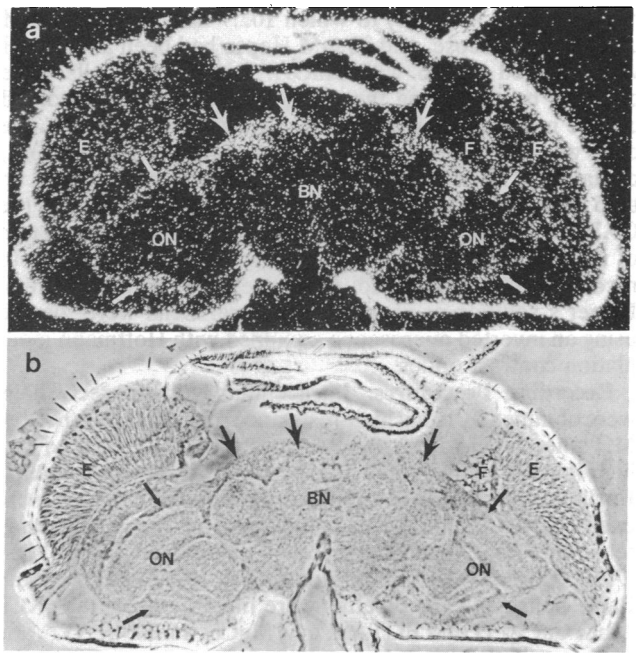

FIG. 5. *In situ* localization of $G_s\alpha$ transcript in a horizontal section of a fly head. (*a*) Dark-field image. (*b*) Phase-contrast image of the same section. Note hybridization in the cortex, which contains the cell bodies (arrows), and the eyes but not in the central neuropil. There is a nonspecific signal over the cuticle (bright line surrounding the head). E, eye; ON, optic lobe neuropil; F, fat body; BN, midbrain neuropil; little arrows, optic lobe cortex; big arrows, midbrain cortex.

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