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Cloning of a *Drosophila melanogaster* guanine nucleotide regulatory protein β-subunit gene and characterization of its expression during development

(signal transduction/DNA sequence)

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ABSTRACT **A *Drosophila melanogaster* gene encoding a protein with >80% sequence identity to the β subunits of mammalian guanine nucleotide-binding regulatory proteins (G proteins) has been cloned. The gene, which was mapped to 13F on the X chromosome by *in situ* hybridization, was cloned from a *Drosophila* genomic library by using a bovine transducin β-subunit cDNA probe. Genomic DNA blot hybridization analysis indicated that there is a single *Drosophila* G-protein β-subunit gene. Multiple transcripts were detected throughout development; in adult flies the mRNA is expressed at higher levels in heads than in bodies. The proposed coding region is uninterrupted by introns, but there is evidence for differential mRNA splicing in the 5′ nontranslated region.**

G proteins are a family of guanine nucleotide-binding proteins that couple transmembrane receptors to intracellular effector components of a variety of signal-transduction pathways (1). The best characterized members of this family include transducin, which couples rhodopsin to a cyclic-GMP phosphodiesterase in vertebrate retinas (2), and G_s and G_i, which function in the hormonally regulated stimulation and inhibition, respectively, of adenylate cyclase (1). G proteins are heterotrimers comprised of α (39–52 kDa), β (35–36 kDa), and γ (8–10 kDa) subunits. Members of this family share functional, structural, and common antigenic features. Interaction of the intact αβγ trimer with an activated receptor allows the α subunit to exchange GDP for GTP and dissociate from the βγ complex. An active α subunit then alters the activity of the effector. An intrinsic GTPase activity ultimately inactivates the α subunit. The α subunit then reassociates with βγ and can again be activated by the receptor.

The α subunit of each G protein is unique and characteristic, and it has been suggested that the diverse γ subunits (3) and the several β subunits (4) may also serve to confer specificity. Two different forms of mammalian β subunits of 35 kDa and 36 kDa have been purified (5, 6), and cDNA clones corresponding to each form have been isolated and sequenced (7–11). The two forms of the protein are expressed in many different types of tissue and are immunologically distinguishable (12). The 36-kDa form, β_1 (7), and the 35-kDa form, β_2 (4, 10), are encoded by separate genes.

The β and γ subunits form a tight complex whose function is to present α subunits to their corresponding receptors (5). However, βγ may also have other roles, including activation of K^+ channels in heart (13), inhibition of Ca^{2+}/calmodulin-stimulated adenylate cyclase activity in brain (14), and stimulation of phospholipase A_2 activity in retina (15).

The high degree of conservation of mammalian G proteins (16) and the biochemical evidence for the existence of G proteins in insects (17–19) and other invertebrates (20, 21) prompted us to search for homologous genes in *Drosophila melanogaster*. We chose *Drosophila*, a metazoan organism amenable to genetic manipulation, to better understand the functions of these ubiquitous proteins. Mutants with abnormalities in signal-transduction processes may result from mutations in G-protein genes. Potential phenotypes of G-protein mutations include membrane polarization (22) and sensory (23) and learning (24) deficiencies.

We report here the identification, by cross-species hybridization, and characterization of a *Drosophila* gene encoding a G-protein β-subunit (Gβ) homolog. The single *Drosophila* Gβ gene is expressed throughout development and the deduced amino acid sequence is 80–85% identical to that of mammalian Gβ proteins.†

MATERIALS AND METHODS

Standard Techniques. DNA purification, poly(A)$^+$ RNA selection, DNA and RNA blotting, and screening of recombinant phage libraries were done by standard procedures (25).

***Drosophila* RNA Preparation.** Flies were maintained and collected at various developmental stages according to established procedures (26). RNA was prepared from flies that had been frozen in liquid nitrogen and stored at −70°C according to Cathala *et al.* (27) with the following modifications. The solubilization buffer contained 1% $NaDodSO_4$ and pelleted RNA was solubilized in 3 M LiCl/1 M guanidinium isothiocyanate by shearing with an 18-gauge needle. After resuspension in solubilization buffer, RNA was extracted three times with 1 volume of phenol and twice with 0.5 volume of chloroform/1-butanol (4:1) and precipitated with 2 volumes of ethanol and 0.1 volume of 3 M NaOAc.

Plasmids. The bovine transducin cDNA clone pTβ112-5 contains the 1.35-kilobase (kb) *Eco*RI insert from M13Tβ112-5 (7) cloned into the *Eco*RI site of the pBR322 derivative pBX. The *Drosophila* genomic clone λCH42A3 contains a 13-kb insert, which includes the Gβ coding region, replacing the internal *Eco*RI fragments of phage vector Charon 4. Tβ1J is a pUC19 subclone of λCH42A3 containing the 2.6-kb *Eco*RI fragment that hybridizes with the bovine insert from pTβ112-5. pTβC6R, pTβC3F, and pTβC5A are *Drosophila* cDNA clones with inserts of 2.4, 1.5, and 0.8 kb, respectively, cloned into the *Eco*RI site of pBS (Stratagene, La Jolla, CA). pTβC6D contains a 0.2-kb *Eco*RI fragment, which was separated from the 3′ end of TβC6R during the process of subcloning. pGRP49 (28) contains the *D. melanogaster* gene encoding ribosomal protein 49.

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Abbreviations: G protein, guanine nucleotide-binding protein; Gβ, G-protein β subunit.

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†The sequence reported in this paper is being deposited in the EMBL/GenBank data base (IntelliGenetics, Mountain View, CA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J04083).

Phage Library Screening. Plaque "lifts" from a Canton-S genomic library in Charon 4 (29) were probed with nick-translated pTβ112-5 in 5× Denhardt's solution/6× SSC/50% (vol/vol) formamide/0.1% $NaDodSO_4$/1 mM EDTA containing 100 μg of salmon sperm DNA per ml at 47°C. [Denhardt's solution is 0.02% bovine serum albumin/0.02% polyvinylpyrrolidone/0.02% Ficoll (M_r 400,000); SSC is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0.] The filters were then washed sequentially in 0.1% $NaDodSO_4$/2× SSC at 47°C, 0.1% $NaDodSO_4$/0.1× SSC at 47°C, and 0.1% $NaDodSO_4$/0.1× SSC at 70°C. An adult male Oregon-R cDNA library in λgt10 (30) was probed with synthetic oligonucleotides that had been end-labeled with ^{32}P by phage T4 polynucleotide kinase and purified on NENSorb-20 cartridges (New England Nuclear). Hybridization was in 6× SSC/10× Denhardt's solution/1% $NaDodSO_4$ containing 100 μg of salmon sperm DNA per ml at 42°C for 16 hr. Filters were washed in 6× SSC/0.1% $NaDodSO_4$ at 42°C.

DNA Sequencing. DNA sequence was determined by the dideoxy chain-termination method (31), with both the Pharmacia and the Sequenase (United States Biochemical, Cleveland) sequencing kits. DNA sequencing was performed with either single-stranded templates in M13mp vectors (32) or double-stranded plasmid templates (33). Oligonucleotide primers were synthesized with an Applied Biosystems (Foster City, CA) model 380B automated DNA synthesizer and purified by ethanol precipitation before use.

***In Situ* Hybridization to Polytene Chromosomes.** *In situ* hybridization to *D. melanogaster* salivary chromosomes was performed with nick-translated, biotinylated probes (34).

RESULTS

Cloning and Sequencing of a *Drosophila* Gβ Gene. A plasmid containing an insert corresponding to the coding region of a bovine transducin β-subunit cDNA clone (7) was used to probe a *D. melanogaster* Canton-S genomic recombinant DNA library (29). Approximately 20 genome equivalents were screened, and 24 positive clones were detected in the primary screen. Twelve of the positive clones were rescreened with the same probe, and 7 of these gave a positive hybridization signal. All of the positive clones contained a 2.6-kb *Eco*RI fragment that hybridized with the bovine probe. The DNA sequence of a 0.5-kb *Taq* I subclone of the 2.6-kb *Eco*RI fragment was determined, and comparison of the deduced amino acid sequence with the published bovine transducin β-subunit sequence (7) demonstrated that it is the *Drosophila* homolog of the bovine gene. Two 18-base synthetic oligonucleotides containing DNA sequence from the coding region of the genomic clone were used to probe a *D. melanogaster* Oregon-R adult male cDNA library (30). The oligonucleotide sequences used as probes are identified in the legend to Fig. 3. Of ≈1.5 × 10^5 clones screened, 6 were positive; these contained inserts of either 0.8, 1.5, or 2.4 kb that hybridized to the bovine transducin β probe.

A map of the *Drosophila* Gβ gene based on restriction digests and DNA sequencing of the genomic and cDNA clones is shown in Fig. 1. All of the DNA sequence that hybridizes to the bovine transducin β cDNA is contained on the 2.6-kb *Eco*RI fragment. The *Drosophila* cDNA clone TβC6R also hybridizes with the 2.1-kb *Eco*RI fragment that is 5′ to the 2.6-kb *Eco*RI fragment on the genomic map. The 3′ end of TβC6R does not correspond to Tβ1J and does not identify additional bands in Southern blots of λCH42A3.

Genomic DNA Blotting. A single band corresponding to the Gβ gene was detected in genomic Southern blots of *D. melanogaster* Canton-S DNA cut with either *Eco*RI, *Bam*HI, or *Hin*dIII (Fig. 2). The *Eco*RI band of 2.6 kb is identical in size to the insert in Tβ1J. None of the three restriction enzymes used cuts within the region covered by the probe. The same bands were detected at both high and low stringency with the *Drosophila* genomic insert probe and at low stringency with the bovine cDNA probe. The nucleotide identity between the coding region of Tβ1J and the bovine transducin β cDNA (7) is 70.3%. This compares to 79.3% identity between human $Gβ_1$ (11) and $Gβ_2$ (10). Since all the detected restriction fragments correspond to the *Drosophila* genomic clone, it appears that the *Drosophila* genome contains no other sequence as closely related to the Gβ gene as mammalian $Gβ_1$ is related to $Gβ_2$.

DNA Sequence. The complete DNA sequence of the *Drosophila* Gβ genomic clone Tβ1J and portions of the three corresponding cDNA clones is shown in Fig. 3. An open reading frame of 1023 bases follows a *Drosophila* consensus translational start site (35) 907 bases from the 5′ end of the genomic clone. The proposed initiator methionine codon is preceded by stop codons in each cDNA reading frame. Conceptual translation of the open reading frame predicts a protein of 340 amino acids with a high degree of identity to mammalian Gβ proteins. On the basis of restriction mapping

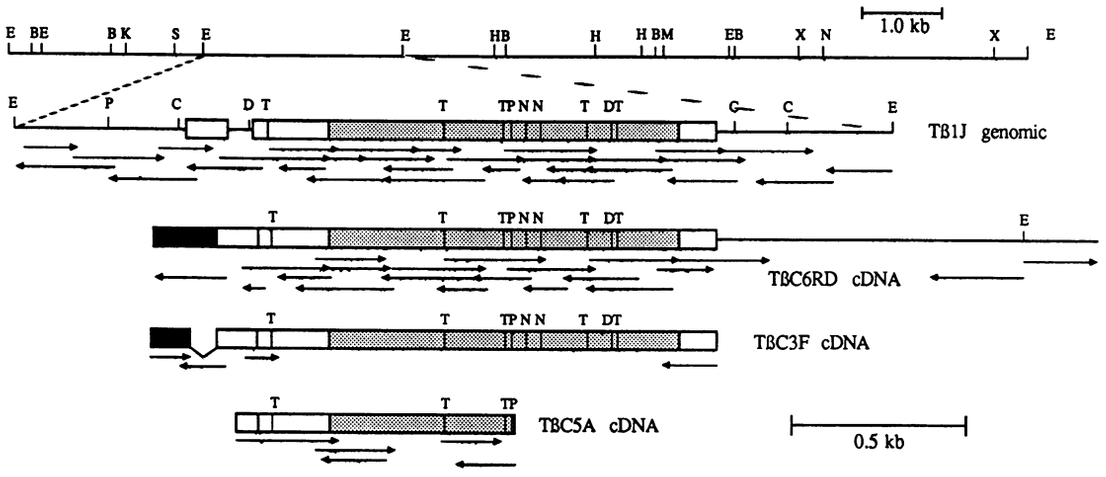

FIG. 1. Restriction map of the *Drosophila* Gβ gene. The top line is a map of the *Drosophila* insert of the genomic clone λCH42A3; the second line is an expansion of the 2.6-kb *Eco*RI insert in Tβ1J that contains the coding region; the lower three maps are of cDNA clones. TβC6RD is a composite of two cDNA subclones, TβC6R and TβC6D. Boxed areas correspond to exons; the coding region is shaded gray, the white boxes represent noncoding exons contained on Tβ1J, and the black boxes represent additional exons not found on the 2.6-kb genomic *Eco*RI fragment. Small arrows depict separate DNA sequence determinations. Restriction sites: E, *Eco*RI; B, *Bgl* II; K, *Kpn* I; S, *Sst* I; H, *Hin*dIII; M, *Bam*HI; X, *Xho* I; N, *Nar* I; P, *Pst* I; C, *Hin*cII; T, *Taq* I; D, *Nde* I. The *Taq* I map of the 3′ end of TβC6RD is incomplete.

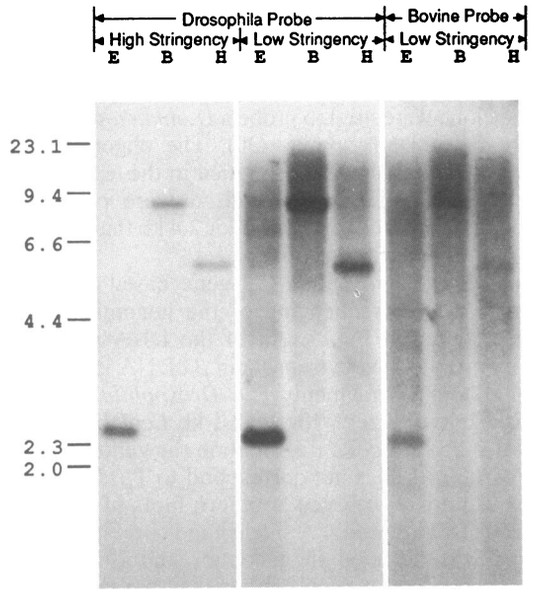

FIG. 2. Southern blot analysis of *Drosophila* genomic DNA. Canton-S DNA (15 μg) was digested with a 6.7-fold excess of *Eco*RI (E), *Bam*HI (B), or *Hin*dIII (H), and ≈5 μg was loaded per lane. The DNA was fractionated by electrophoresis in a 0.9% agarose gel in 0.04 M Tris acetate buffer (pH 8.2) and transferred to a Nytran filter (Schleicher & Schuell). The *Drosophila* probe was a gel-purified 1.15-kb *Pst* I doublet that contains all but 0.3 kb of 5′ sequence from the insert in Tβ1J. The bovine probe was a gel-purified 1.35-kb *Eco*RI fragment from pTβ112-5. The probes were labeled with ^{32}P by nick-translation; hybridization and washing were at 56°C. High stringency: hybridization, 5× Denhardt's solution/6× SSC/1% $NaDodSO_4$/50% formamide containing 100 μg of salmon sperm DNA per ml; washes, 6× SSC/0.5% $NaDodSO_4$ (20 min), 0.5× SSC/0.5% $NaDodSO_4$ (20 min), and 0.1× SSC/0.5% $NaDodSO_4$ (20 min). Low stringency: hybridization, 5× Denhardt's solution/6× SSC/1% $NaDodSO_4$ containing 100 μg of salmon sperm DNA per ml; wash, 6× SSC/0.5% $NaDodSO_4$ (60 min). Markers at left (kb) show positions of *Hin*dIII-digested phage λ DNA.

and DNA sequencing of the genomic and cDNA clones, there are no introns in what appears to represent the coding region of the *Drosophila* gene, although there are at least two introns in the 5′ noncoding region. The 5′ ends of TβC6R and TβC3F are identical for 112 bases, but the first exon of TβC6R is 73 bases longer at its 3′ end. This difference is the result of alternative splicing (data not shown). The DNA sequence of the cDNA clone TβC6R is colinear and identical to the genomic sequence within the 1023-base open reading frame with the exception of a silent polymorphism at position 628 (cytosine instead of thymine). There is an additional polymorphism in the 5′ noncoding region at position −113 (thymine instead of guanine). None of the cDNA clones represents a full-length message; TβC5A terminates within the coding region, and TβC3F terminates in an adenine-rich stretch in the 3′ noncoding region at position 1131. The 3′ end of the 2.6-kb cDNA is apparently a cloning artifact; its sequence diverges from Tβ1J in the same adenine-rich stretch where TβC3F terminates and it contains poly(dG·dC) at its 3′ end.

Homology to Mammalian Gβ Gene Products. The degree of similarity of the deduced amino acid sequence of *Drosophila* Gβ to the mammalian homologs is striking. In Fig. 4, the sequence encoded by the *Drosophila* Gβ gene is compared to the published amino acid sequences of bovine transducin β_1 (7) and human HL-60 β_2 (10). The *Drosophila* protein is 84% identical to β_1 and 82% identical to β_2; 79% of the amino acids are identical in all three proteins. The *Drosophila* region is unique at 15% of the amino acid positions. Most of the amino acid differences are scattered, but as in the comparison of β_1 and β_2 (10), there are clusters of nonconservative substitutions between residues 25 and 38 and between residues 175 and 199. Overall, the amino-terminal 40 amino acids are the most divergent. In this region 40% of the *Drosophila* residues are unique and only 53% are identical in all three proteins. The repetitive segmental structure described for β_1 (7) and β_2 (10) is maintained, with few nonconservative substitutions in the residues comprising the repeat motif. The three proteins are identical in 87% of the amino acids identified as elements of the repeat pattern in β_1 (7).

Developmental Regulation of Expression. At least six different-sized transcripts were detected on RNA gel blots by using *Drosophila* Gβ hybridization probes. Fig. 5 shows a blot of total RNA extracted from flies of different developmental stages and probed with 2.3 kb of the 2.6-kb *Drosophila* Gβ genomic clone Tβ1J. A control probe, pGRP49, which detects ribosomal protein 49 message (28), was used to monitor the integrity of the RNA. Transcripts of 5.2, 4.2, 3.3, 3.0, and 1.9 kb hybridized with the β-subunit probe and are expressed in all developmental stages tested from mid-embryo through adult. The highest level of expression is in late embryo and pupae. In the early embryo the larger transcripts are much reduced in abundance, while there are additional transcripts at 3.1, 2.5, and 2.0 kb. The 1.9- and 2.0-kb messages are the predominant transcripts in the early embryo; these are expressed at low levels in the other developmental stages. Expression is low in the larvae (predominantly third-instar) and barely detectable in adult bodies. The probe used in this blot was double-stranded and extended beyond the coding region. Nevertheless, we believe the RNAs detected represent authentic Gβ messages, since identical adult patterns were obtained with an antisense RNA probe complementary to the 5′ end of the coding region (Fig. 3, bases 19–545) on blots of poly(A)$^+$ RNA (data not shown).

Chromosomal Location. The *Drosophila* Gβ gene was mapped to 13F on the X chromosome by *in situ* hybridization to polytene larval salivary chromosomes (Fig. 6).

DISCUSSION

The identification and cloning of a *Drosophila* Gβ gene provide further evidence that vertebrates and invertebrates use similar signal-transduction machinery. There have been several reports consistent with a role for G proteins in insects and other invertebrates. Light activates a GTPase activity in *Musca* eye membranes (18), and GTP analogs induce membrane depolarization in *Musca* photoreceptors (17). Light-dependent GTP binding and increased inositolphospholipid turnover are exhibited by fly (*Musca* and *Drosophila*) eye membranes (36). Pertussis toxin modifies specific substrates with properties similar to G-protein α subunits in octopus (21), and light stimulates inositolphospholipid turnover in octopus (37), *Limulus* (20), and squid (38). Both cholera and pertussis toxin substrates are found in *Drosophila* heads, and pertussis toxin substrates are present in *Manduca* eyes, brain, and antennae (19). Two groups have reported the cloning of a yeast G-protein α-subunit gene: Nakafuku *et al.* (39) isolated a G_i α-subunit clone from *Saccharomyces cerevisiae* by cross-hybridization with a mammalian probe, and Dietzel and Kurjan (40) identified and cloned the same gene by genetic means. In addition, our laboratory has also recently cloned a *Drosophila* G-protein α-subunit gene (41).

The high degree of amino acid sequence conservation over the long evolutionary distance between insects and mammals suggests that the *Drosophila* gene product and mammalian Gβ proteins may have similar functions. It has been argued that this type of conservation can be expected for proteins that interact with several other macromolecules (42). Gβ interacts with an α subunit and a γ subunit, and it promotes the interaction between α subunits and receptors (5). The

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GGTAGAGTGC AAATGAAAAG TTACAGATTG GTGGCTTCGT TAACAAAATT TTCAGTTATT TGTGTAGTTG AAGACGGGAA TTTGTTTTGC GTATAAAAAG
TTAACACTGC GTGTAAGTAA CGTATTGTCG TCATCAACAA ACTGTCCAGT GAATGAAAAT TGAAAGAAAA ATTACTGATC TATGA

-906     GAATTC GAAATCCAGC ACATAAAAAG CATAAATCAC ATCAAAAAAT TCGCATTTTC TCTGGTCCTT AGCACCAGAT CGCTTTTTAT GCATCCAATT
-810  CGGTTTCCTC TTTTTTTTTA GTATACCATC CCATCCGCGA GATAACATTC TGTATTAAAC AAAACGCCCA ACATCCTAAT TACAAATCAG ACGGGACGGA
-710  AAATCGAAAA AACTATAGGA ACCTCTCTCT CCCCCCCCAC TAACGTAGTT TATGTGTATT TAACAAACGA AGAACTTTCT GCAGAGGTCG AAATACACCA
-610  ATCGCACATA CCAAACAAAA AGTAATAAAA ACGAAATAAT TGAAAAACCA AATAGTTAAA TTGGCAGAGC AAACACAGGA CGACGACTAT GAACTAAAGT
-510  CAGAAATTAA TGGACATCGT ACCGAGAATA TAATCCGTGA ATAGTACTAT ACGATAAAAC AGAAAAAGAA AAAAAGTCAA CTAAAAAGCA AAAAACCAAC
-410  TCTTGCAGAA CACAGTTCAT GATTTATCCT ATCCATACTA GAAGTGCACA AAATAAACGC CGTATGCAAG CGTGATCATC CAAGGCTCAT TATCCGACTA
-310  AGCTACTGGC CAGCTTAAAA AAAGTAAGCG TAAGTACACC CACAATTGAC CATATAACTA GGTTAATGAA CGCCTCCATT TACTACTTCC CCATATGTAT
-210  AGCACCATCT AACAAGAGCG AATCGAATCG AATCGACTTG GACGACCGAC CGACCGACCG CACAAAGAAG GATCTATCTG GAAAGCCGGC TAGCCGATTC
-110  CCGCATCCGA TTCCGAATAC GAATACGTAT CCGTATCCGA ATCCGAATTT GAAACCGAAT CCTCAACCAA ATCATCCCAT AAGGACGCCG CTGTCTTCAA
 -10  CGATCACAAG

   1  ATG AAT GAA CTA GAC AGT CTC AGG CAG GAA GCC GAG TCC CTA AAG AAC GCC ATT CGG GAT GCC CGG AAG GCG GCC TGC GAC
      Met Asn Glu Leu Asp Ser Leu Arg Gln Glu Ala Glu Ser Leu Lys Asn Ala Ile Arg Asp Ala Arg Lys Ala Ala Cys Asp
  82  ACA TCA CTG TTG CAA GCG GCC ACC TCG CTG GAA CCC ATC GGC CGC ATA CAG ATG CGC ACC CGT CGT ACA TTA CGC GGC CAT
      Thr Ser Leu Leu Gln Ala Ala Thr Ser Leu Glu Pro Ile Gly Arg Ile Gln Met Arg Thr Arg Arg Thr Leu Arg Gly His
 163  TTG GCG AAA ATC TAC GCC ATG CAT TGG GGC AAC GAT TCA AGG AAT CTC GTA TCA GCC TCA CAG GAC GGC AAA CTG ATC GTT
      Leu Ala Lys Ile Tyr Ala Met His Trp Gly Asn Asp Ser Arg Asn Leu Val Ser Ala Ser Gln Asp Gly Lys Leu Ile Val
 244  TGG GAC TCG CAT ACC ACG AAC AAA GTC CAT GCC ATT CCA CTG CGA TCC TCG TGG GTG ATG ACC TGT GCG TAC GCC CCA TCC
      Trp Asp Ser His Thr Thr Asn Lys Val His Ala Ile Pro Leu Arg Ser Ser Trp Val Met Thr Cys Ala Tyr Ala Pro Ser
 325  GGT AGC TAT GTG GCC TGC GGT GGC CTC GAC AAC ATG TGT TCA ATT TAC AAC CTA AAG ACG CGC GAG GGC AAC GTC CGG GTG
      Gly Ser Tyr Val Ala Cys Gly Gly Leu Asp Asn Met Cys Ser Ile Tyr Asn Leu Lys Thr Arg Glu Gly Asn Val Arg Val
 406  TCC CGT GAG CTG CCC GGC CAT GGT GGC TAT CTA TCG TGC TGC CGC TTC CTG GAC GAC AAT CAG ATC GTG ACC AGC TCC GGT
      Ser Arg Glu Leu Pro Gly His Gly Gly Tyr Leu Ser Cys Cys Arg Phe Leu Asp Asp Asn Gln Ile Val Thr Ser Ser Gly
 487  GAT ATG TCG TGC GGA TTG TGG GAT ATC GAG ACG GGA CTG CAG GTA ACC TCG TTT TTG GGC CAC ACC GGC GAT GTG ATG GCC
      Asp Met Ser Cys Gly Leu Trp Asp Ile Glu Thr Gly Leu Gln Val Thr Ser Phe Leu Gly His Thr Gly Asp Val Met Ala
 568  CTC TCA CTG GCG CCC CAA TGC AAA ACG TTC GTA TCC GGC GCC TGC GAT GCG TCC GCC AAG CTA TGG GAC ATC CGG GAG GGT
      Leu Ser Leu Ala Pro Gln Cys Lys Thr Phe Val Ser Gly Ala Cys Asp Ala Ser Ala Lys Leu Trp Asp Ile Arg Glu Gly
 649  GTC TGT AAA CAA ACC TTC CCC GGC CAC GAA TCC GAT ATC AAT GCG GTC ACA TTT TTC CCG AAT GGT CAG GCA TTC GCC ACC
      Val Cys Lys Gln Thr Phe Pro Gly His Glu Ser Asp Ile Asn Ala Val Thr Phe Phe Pro Asn Gly Gln Ala Phe Ala Thr
 730  GGT TCG GAC GAC GCA ACC TGT CGA TTG TTC GAT ATC CGT GCC GAT CAG GAG TTG GCC ATG TAT TCG CAC GAC AAC ATC ATA
      Gly Ser Asp Asp Ala Thr Cys Arg Leu Phe Asp Ile Arg Ala Asp Gln Glu Leu Ala Met Tyr Ser His Asp Asn Ile Ile
 811  TGC GGC ATC ACA TCG GTG GCA TTC TCG AAG AGC GGA CGT CTG TTA TTA GCG GGC TAC GAT GAT TTC AAC TGC AAT GTA TGG
      Cys Gly Ile Thr Ser Val Ala Phe Ser Lys Ser Gly Arg Leu Leu Leu Ala Gly Tyr Asp Asp Phe Asn Cys Asn Val Trp
 892  GAC ACG ATG AAG GCA GAA CGG TCT GGC ATA CTC GCT GGC CAC GAC AAC CGT GTA TCC TGT TTG GGT GTC ACC GAG AAC GGC
      Asp Thr Met Lys Ala Glu Arg Ser Gly Ile Leu Ala Gly His Asp Asn Arg Val Ser Cys Leu Gly Val Thr Glu Asn Gly
 973  ATG GCG GTG GCA ACA GGA TCG TGG GAC TCC TTC TTG CGT GTA TGG AAC TAA
      Met Ala Val Ala Thr Gly Ser Trp Asp Ser Phe Leu Arg Val Trp Asn

1024  AAATAAAACA GAAACAGAAA CAGCAGCATA AGCAGAAACA GAAACAAAAC AAAACAAAAA ACAAGAGAAA ACCAAACAAA CAAAGAACAA CAAAAAAAAG
1124  AGGAAAAAGA AAAAGAACTT GAAAATGCAT TAAAGGGTGG GGTGGGGTGT CAGGTCAACT GTTGCGTCAG TCTGCGGATA GTGTTTCACC CATCATCTAC
1224  ATTGTCATCT AATGTATATG ATTCGGTGTT ACCGTCTCTG TGTGAAATAT TAAATGAGTT GCCGGATCGT TTTGAATATG TGGGCGTCAC GTAGTTGACT
1324  GAACGTAGAC CACAACAACA AAGAACTTTA TATACAACAT TATTTTATAT TGCTATATAC TTATTAACTA AAAATTATAC AAAAAAAACA AAATAAAACC
1424  AAAAAAAAAA AACAACAAAA ACCGAAAAAA TCGCAGCAAC ATACAAATAG ACATACACAC GCATACAAGA ATATTATTAG TGTAAATGGA AAATTGCAGA
1524  AAAGAAAAAA TGAAAAACCA AGAACAACCG ATCAAGAAGA AACAAAACGA TAAAGAAATG ATGGAGTAAC GATGGGCCCG CTGATAGTCA GAAGAGTAAG
1624  AGTCAGTTGT TTAGTCAGAA TTC
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FIG. 3. DNA sequence of the *Drosophila* Gβ gene. The numbered sequence is that of the genomic clone Tβ1J, which is 2552 bases long. Numbering begins at the proposed translational start site. The unnumbered sequence is that of the 5′ ends of cDNA clones TβC6R and TβC3F. Noncoding exons are underlined, and the exon sequence unique to TβC6R is doubly underlined. Potential polyadenylylation signal sequences are marked with stars. The oligonucleotides used to probe the cDNA library were CTCGTATCAGCCTCACAG (positions 208–225, sense) and CTGTGAGGCTGATACGAG (positions 292–309, antisense).

sequence identity between *Drosophila* Gβ and mammalian β_1 (84%) and β_2 (82%) is remarkably high. The identity between the two mammalian proteins is 92% (10); among mammals β_1 and β_2 proteins are conserved across species, and it has been proposed that they have distinct but related functions (10). The two mammalian forms of Gβ may have resulted from a gene duplication that occurred after the divergence of insects and vertebrates. An estimation of evolutionary distances between the genes by analysis of third-base position differences (43) supports this conclusion. The average number of base substitutions per third-base site in all codons (mean ± SD) are 0.72 ± 0.08 between human β_1 and β_2, 1.28 ± 0.20 between *Drosophila* Gβ and human β_1, and 0.95 ± 0.1 between *Drosophila* Gβ and human β_2.

There is a repetitive segmental structure to the amino acid sequence of mammalian β_1 and β_2 (7, 10). The conservation of this motif suggests that it is functionally significant. A similar but unrelated internal repeat pattern has been reported for members of the lipocortin family (42, 44, 45). Such a structure suggests multiple binding sites for multiple copies of the same ligand or of closely related ligands. In the case of calpactin I heavy chain, it has been argued that each of the repeat units corresponds to a potential Ca^{2+} binding site (42). No such ligand for Gβ has been identified, but the stoichiometry of the βγ complex has not been precisely measured and it is possible that multiple γ subunits bind to each β.

The results reported here are consistent with the idea that there is only a single *Drosophila* Gβ gene, in contrast to at

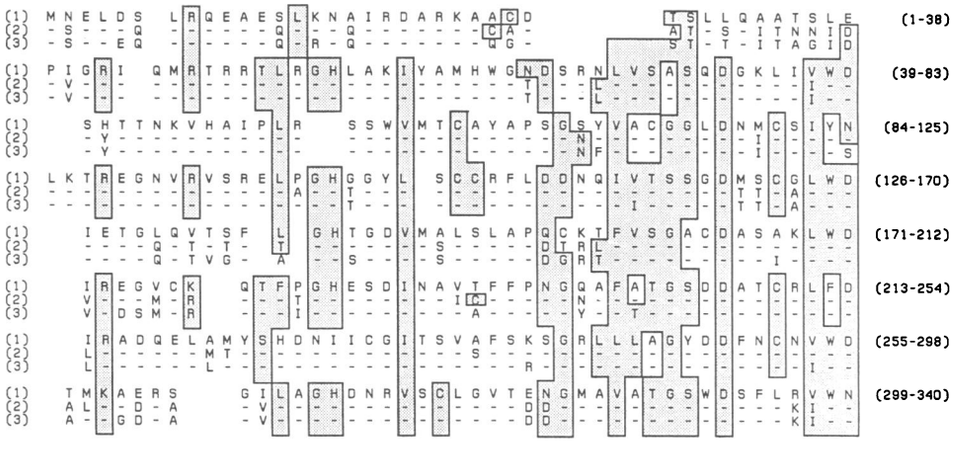

FIG. 4. Amino acid sequence comparison of *Drosophila* and mammalian Gβ proteins. (1) *Drosophila* Gβ; (2), bovine transducin β; (3), human HL-60 $G\beta_2$. Sequences are given in standard one-letter code and are aligned according to the repetitive segmental pattern (7), and the amino acids that comprise the repeats are shaded. Identical amino acids are indicated by dashes.

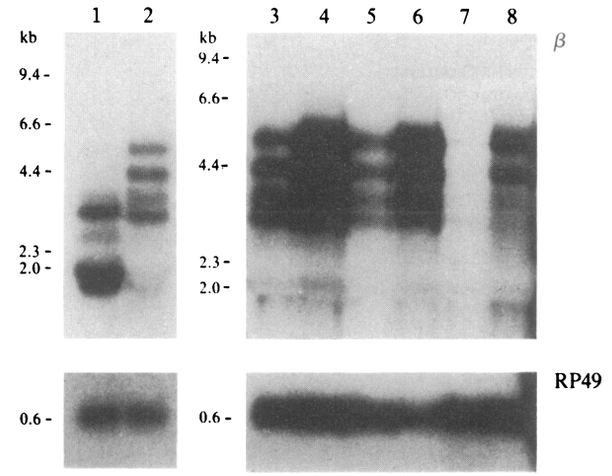

FIG. 5. Expression of *Drosophila* Gβ RNA at different developmental stages. RNA gel blots were hybridized with Tβ1J (*Upper*) or with pGRP49 (*Lower*) as a control for degradation. The Tβ1J probe was a gel-purified 1.15-kb *Pst* I doublet that contains all but 0.3 kb of the *Drosophila* insert. Total RNA (15 μg per lane) was fractionated in 1% agarose/2.2 M formaldehyde gels in 0.04 M Mops buffer (pH 7.0) and transferred to Nytran filters (Schleicher & Schuell) in 20× SSC. The filters were hybridized with nick-translated probes in 5× Denhardt's solution/6× SSC/30% deionized formamide/1% Na-DodSO$_4$ containing 100 μg of salmon sperm DNA per ml at 63°C and washed twice in 2× SSC at 20°C and once in 0.5× SSC/0.5% NaDodSO$_4$ at 63°C. Lanes: 1, embryos at 0–3 hr; 2 and 3, embryos at 3–12 hr; 4, embryos at 12–24 hr; 5, larvae; 6, pupae; 7, adult bodies; 8, adult heads.

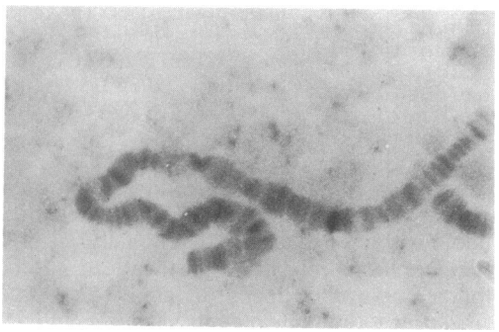

FIG. 6. Localization of the *Drosophila* Gβ gene by *in situ* hybridization of polytene chromosomes with biotinylated pTβC5A.

least two closely related Gβ genes in mammals (6, 10). A single gene without introns in the coding region codes for multiple RNA transcripts. The boundaries of the different transcriptional units have not been determined, but there is evidence for differential mRNA processing in the 5′ noncoding region. We have defined the coding region on the basis of homology to mammalian proteins and the structure of three cDNA clones. It is also possible, but not likely, that additional or alternative coding exons exist in *Drosophila*.

The Gβ clones can now be used for *Drosophila* transformation experiments. On the basis of DNA blotting (41), toxin labeling (19), and guanosine 5′-[γ-thio]triphosphate binding (36), there appear to be multiple G-protein α-subunit genes in *Drosophila*. If a single β subunit interacts with different α subunits in diverse signal-transduction pathways, mutations in the *Drosophila* Gβ gene are likely to have pleiotropic effects.

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