Neuronal expression of a newly identified *Drosophila melanogaster* G protein α_0 subunit

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Guanine nucleotide-binding proteins (G proteins) mediate signals between activated cell-surface receptors and cellular effectors. A bovine G-protein α -subunit cDNA has been used to isolate similar sequences from *Drosophila* genomic and cDNA libraries. One class, which we call DG α_0 , hybridized to position 47A on the second chromosome of *Drosophila*. The nucleotide sequence of the protein coding region of one cDNA has been determined, revealing an α subunit that is 81% identical with rat α_0 . The cDNA hybridizes strongly to a 3.8 kb mRNA and weakly with a 5.3 kb message. Antibodies raised against a *trp-E*-DG α_0 fusion protein recognized a 39 000 Da protein in *Drosophila* extracts. In situ hybridization to adult *Drosophila* sections combined with immunohistochemical studies revealed expression throughout the optic lobes and central brain and in the thoracic and abdominal ganglia. DG α_0 message and protein were also detected in the antennae, oocytes, and ovarian nurse cells. The neuronal expression of this gene is similar to mammalian α_0 , which is most abundantly expressed in the brain.

Introduction

Guanine nucleotide-binding proteins (G proteins) play a central role in coupling hormone, neurotransmitter, and growth-factor receptors to different cellular effectors, including adenylate cyclase, phospholipases, phosphodiesterases, and ion channels (Stryer and Bourne, 1986; Gilman, 1987; Neer and Clapham, 1988). All known G proteins consist of three subunits, α , β , and γ . Ligand binding to a cell-surface receptor causes the α subunit to exchange GDP for GTP. This nucleotide exchange leads to dissociation of the heterotrimer to α and $\beta\gamma$. Both α and $\beta\gamma$ have

been demonstrated to modulate effector activities. Hydrolysis of GTP to GDP at the active site of the α subunit leads to reassociation of α and $\beta\gamma$ into the inactive heterotrimer.

Mammals express a bewildering variety of G proteins, each distinguished by their α subunit. One class of nonretinal G protein, G_i/G_o, is defined by its ability to act as a substrate for ADP ribosylation by *Bordetella pertussis* toxin. This class contains at least four different α subtypes, α_{i1} , α_{i2} , α_{i3} , and α_0 , which are products of different genes. G_i/G_o α subunits have been implicated in modulating several different cellular effectors, including adenylate cyclase, phospholipases, and ion channels. The three α_i subtypes share ~85% sequence identity, while α_0 is more divergent, sharing ~70–75% identity with the three α_i subunits. In addition to this sequence similarity, all G_i/G_o α subunits contain a cysteine residue four amino acids from the carboxyl terminus, which is a site for ADP ribosylation by pertussis toxin. This toxin has been useful for assigning some functions to G_i/G_o because its activity uncouples G proteins from receptors, thus preventing GTP/GDP exchange. While pertussis toxin has permitted assignment of some functions to the G_i/G_o family, it has been difficult to ascribe activities to individual α subunits because many cells express multiple members of this class (Kim *et al.*, 1988). The observation that α_0 is 5- to 10-fold more abundant in bovine brain cortex than α_i (Huff *et al.*, 1985) raises the possibility that α_0 may be important to neuronal function. Recent evidence has implicated G_o in regulation of voltage-sensitive calcium channels in neurons (Hescheler *et al.*, 1987; Harris-Warrick *et al.*, 1988). Ultrastructural studies (Gabrion *et al.*, 1989) have localized α_0 to the cell membrane, especially in regions of cell-cell contact. It was also found associated with the cell matrix but was absent from synaptic regions. These observations have led Gabrion *et al.* (1989) to speculate that α_0 modulates neurotransmitter release rather than playing a direct role in synaptic transmission.

To further define the function of these α subunits, it would be helpful to identify α genes and cDNAs in an organism that is amenable to ge-

DROSOPHILA α_0

ACTTCGCAGATCCACCAACTGCTCTGGAGACTGCCTATCTGCTCCTTGACACTAGCTATTTTCGTTGTTTCAGCGATTGCATTTCGAGTGGTAAGACCACTT
 AAAGCCGAAACTGGACAGCTGCTGGAACCTGCATTTCCCAAGGAGGGCGGAACCAACAGTCTCCGCCAGATCCGGAAGAAACAAAAGCCGAAACCGAA
 AAGCCCCGTGTAAATCCGAATCCGAATCCAAATCAGTATCCAAACCAACCAATGGGCTGCACCACATCCGCCGAAGAACGCCGCCATCCAGCGAT
 MetGlyCysThrThrSerAlaGluGluArgAlaAlaIleGlnArgS
 CCAAACAGATCGAGAAGAATCTAAAGGAGGATGGAATCCAGGCGGCCAAGGACATCAAGCTCTGCTGCTGGGTGCCGGTGAGTCGGCAAGAGCACAAAT
 erLysGlnIleGluLysAsnLeuLysGluAspGlyIleGlnAlaAlaLysAspIleLysLeuLeuLeuLeuGlyAlaGlyGluSerGlyLysSerThrIl
 AGTCAAACAGATGAAATCATTACGAGAGCGGCTTCACTGCGGAGGACTTTAAACAATATCGACCGGTTGTCTACAGCAACACAATACAATCATTAGTT
 eValLysGlnMetLysIleIleHisGluSerGlyPheThrAlaGluAspPheLysGlnTyrArgProValValTyrSerAsnThrIleGlnSerLeuVal
 GCAATATTGCGCGCATGCCAACCTTAAGTATTAGTACAGCAATAACGAGCGGAGAGCGATGCCAAGATGGTGTTCGACGTATGCCAACGCATGCACG
 AlaIleLeuArgAlaMetProThrLeuSerIleGlnTyrSerAsnAsnGluArgGluSerAlaLysMetValPheAspValCysGlnArgMetHisA
 ACACCGAGCCCTTCTCGGAGGAGCTGCTGGCCGCCATGAAACGCCTCTGGCAGGACGCCGGTGTCCAGGAGTGCTTCTCGCGCAGCAACGAATACCAACT
 spThrGluProPheSerGluGluLeuLeuAlaAlaMetLysArgLeuTrpGlnAspAlaGlyValGlnGluCysPheSerArgSerAsnGluTyrGlnLe
 AAACGATTCCGCAAAATATTTCTGGACGATTTGGATCGGTTAGGCGCCAAGGATTACCAGCCAACCTGAACAAGATATCTTGGCGACTCGCGTCAAGACC
 uAsnAspSerAlaLysTyrPheLeuAspAspLeuAspArgLeuGlyAlaLysAspTyrGlnProThrGluGlnAspIleLeuArgThrArgValLysThr
 ACTGGCATCGTTGAGGTACACTTCTCCTTCAAAAACCTCAACTTTAAATTGTTTACGTGGGCGGTGACGCGCTCGGAACGTAAGAAATGGATACACTGCT
 ThrGlyIleValGluValHisPheSerPheLysAsnLeuAsnPheLysLeuPheAspValGlyGlyGlnArgSerGluArgLysLysTrpIleHisCysP
 TCGAAGATGTCACGGCGATCATTTTCTGCGTGGCCATGTCCGAGTACGATCAAGTCTTGCATGAGGATGAAACCACGAACCGCATGCAAGAGTCGCTGAA
 heGluAspValThrAlaIleIlePheCysValAlaMetSerGluTyrAspGlnValLeuHisGluAspGluThrThrAsnArgMetGlnGluSerLeuLy
 ACTGTTTGACTCGATCTGTAACAACAAATGGTTACGGACACCTCGATTATTCTATTCTGAACAAGAAGGATTGTTTCGAGGAGAAGATTCCGAAGAGT
 sLeuPheAspSerIleCysAsnAsnLysTrpPheThrAspThrSerIleIleLeuPheLeuAsnLysLysAspLeuPheGluGluLysIleArgLysSer
 CCCCTGACGATTGCTTCCCGAATACACAGGTGGACAGGAGTACGGCGAGGCGGCTGCTTACATTACGGCTCAATTTGAAGCGAAAAACAATCAACCT
 ProLeuThrIleCysPheProGluTyrThrGlyGlyGlnGluTyrGlyGluAlaAlaAlaTyrIleGlnAlaGlnPheGluAlaLysAsnLysSerThrS
 CAAAAGAAATCTACTGCCACATGACGTGTGCCACAGATACCAATAACATTAGTTTGTATTTCGATGCTGTACCGATGTCATCATAGCAACAACCTGCG
 erLysGluIleTyrCysHisMetThrCysAlaThrAspThrAsnAsnIleGlnPheValPheAspAlaValThrAspValIleIleAlaAsnAsnLeuAr
 CGGCTGTGGACTGTACTAAGATGGATTCCAGGCCGGATCC
 gGlyCysGlyLeuTyrEnd

Figure 1. DNA sequence and translation of DG α_0 coding region.

netic manipulation. To this end, we have studied a member of the G/G α α gene family in *Drosophila*. For the study of *Drosophila* G protein homologues to be an informative model for their mammalian counterparts, they would have to share significant structural similarity. Furthermore, the *Drosophila* and mammalian homologues should be expressed in similar cell types. Here we describe a *Drosophila* α subunit with remarkable similarity to mammalian α_0 , which, like α_0 , is predominantly expressed in the nervous system.

Results and Discussion

A *Drosophila* genomic library was screened (Maniatis *et al.*, 1978) with a DNA fragment isolated from a bovine α_{11} cDNA (Michel *et al.*, 1986). Two distinct clones, denoted DG α_1 and DG α_0 , were isolated. In situ hybridization to *Drosophila*

polytene chromosomes revealed that DG α_1 hybridized to 65C on the third chromosome, while DG α_0 hybridized to 47A on the second chromosome (data not shown). Since DG α_1 has been well characterized (Provost *et al.*, 1988) we have focused our attention on DG α_0 . A head-specific cDNA library was screened using an 8.5 kb *EcoRI* fragment from the genomic clone. The largest cDNA isolated was 2.4 kb, and the DNA sequence of the protein coding region of this clone was determined (Figure 1). The largest open reading frame revealed the complete coding region for a G-protein α subunit. Several peptide regions common to G proteins, labeled A, C, E, and G in Figure 2, can be identified in DG α_0 . X-ray crystallographic studies of the ribosomal elongation factor EF-Tu and the *ras* oncogene product (Jurnak, 1985; la Cour *et al.*, 1985; De Vos *et al.*, 1988) have revealed that these regions form the guanine nucleotide-binding site. A, C,

COMPARISON OF G PROTEIN α SUBUNITS

[illegible]

Figure 2. Alignment of the amino-acid sequence of DG α_0 with rat α_0 and α_{11} (Jones and Reed, 1987), *Drosophila* DG α_1 (Provost *et al.*, 1988) and human α_2 (Fong *et al.*, 1988). Dots (·) indicate identity with the DG α_0 amino acid. # indicates gaps inserted to maximize similarity. Regions A, C, E, and G have been implicated in binding guanine nucleotides. Table indicates the percent of amino acids identical with DG α_0 .

and E contact the sugar or phosphate residues, while G appears to confer specificity for binding guanine nucleotides. Also, as with other members of the G/G₀ gene family, DG α_0 contains a cysteine residue four amino acids from the carboxyl terminus, which could serve as a site for pertussis-toxin modification. Hopkins *et al.* (1988) have shown that a 39 000 Da protein extracted from *Drosophila* head was labeled by pertussis toxin. It is conceivable that this pertussis-toxin substrate was encoded by DG α_0 . The product of the DG α_1 gene does not contain a cysteine residue at this position and is presumably not modified by pertussis toxin (Provost *et al.*, 1988).

Comparison of the predicted amino-acid sequence of α subunits reveals that DG α_0 shows greatest similarity with mammalian α_0 (Figure 2). 81% of the amino acids are conserved between rat α_0 and this *Drosophila* α subunit. If conservative substitutions are permitted, this similarity increases to 90%. In contrast, DG α_0 is only 64% identical to DG α_1 . DG α_1 shows greatest similarity to a mammalian α_1 isoform; it is 78% identical to both human and rat α_{11} . The observation that the two *Drosophila* genes show greater similarity to

mammalian α subunits than to each other implies that the two forms arose before the divergence of arthropods and chordates. If DG α_0 and DG α_1 diverged after this separation, then the *Drosophila* proteins would show greater similarity to each other than to their mammalian counterparts. In addition to these α subunits, *Drosophila* also express a β subunit that is 84% identical to mammalian β (Yarfitz *et al.*, 1988). Furthermore, genes encoding α -, β -, and γ -like proteins have recently been identified in yeast, where they are involved in modulating the mating pheromone response pathway (Dietzel and Kurjan, 1987; Nakafuka *et al.*, 1987; Whiteway *et al.*, 1989). These observations suggest that G proteins may have arisen in unicellular organisms before the evolution of multicellular organisms.

The sizes of mRNA transcribed from DG α_0 were determined by hybridization of the 2.4 kb cDNA to poly A⁺ RNA isolated from adult *Drosophila* (Figure 3). One band of 3.8 kb hybridized strongly, and a second of 5.3 kb reacted weakly. The larger transcript is probably not from the DG α_1 gene, which expresses a 2.3 kb mRNA in adults (Provost *et al.*, 1988). The 5.3 kb message may be a precursor RNA, an alternative splicing product of the

Figure 3. Blot hybridization analysis of $DG\alpha_0$ in adult *Drosophila*. Poly A⁺ RNA was prepared as described in Maniatis *et al.* (1982). Complete $DG\alpha_0$ cDNA was labeled with ^{32}P dCTP by the hexamer labeling method (Feinberg and Vogelstein, 1983). RNA sizes are given in kilobases.

$DG\alpha_0$ gene, or the product of yet another α -subunit gene. The size of these mRNAs indicates that the 2.4 kb cDNA was only a partial copy of a $DG\alpha_0$ transcript. This cDNA did not contain a poly A tail, so presumably cDNA synthesis was primed within the body of the message.

In situ hybridization to adult Drosophila

To determine the tissue distribution of $DG\alpha_0$, a ^{35}S -labeled *Bam*HI/*Cla*I fragment from the 3' non-coding region of the cDNA was hybridized to frozen tissue sections of adult *Drosophila*. As a control, a ^{35}S -labeled *Pvu*II fragment from Bluescript (Stratagene) was used for nonspecific background. Nonspecific hybridization was only noted in the lumens of the crop and intestinal tract (not shown). The $DG\alpha_0$ probe specifically hybridized throughout the cellular layers of the nervous system (Figure 4). In the head, hybridization was found in the region of nerve-cell bodies throughout the optic lobes, including the lamina, medulla,

lobula, and lobula plate. In addition, two regions of the optic-lobe neuropil, one in the lamina and the second lying between the medulla and lobula/lobula plate, also showed weak hybridization. This could represent message in either nerve fibers or glial cells. Little or no signal was detected over the photoreceptor of the eye. $DG\alpha_0$ RNA was also detected throughout the cellular regions of the central brain, but no hybridization was observed in regions of central-brain neuropil. In addition, weak hybridization was also noted in the antennae. In the thoracic and abdominal ganglia, signal was detected throughout those regions that contain neural cell bodies but was absent from the central neuropil. These observations suggest that many or perhaps all of the nerve cells contain $DG\alpha_0$ message, although in situ hybridization experiments cannot tell which cells actually contain $DG\alpha_0$ proteins. Finally, in the abdomen, reproducible signal was only seen in ovarian nurse cells and oocytes (data not shown). The presence of message in oocytes may indicate that $DG\alpha_0$ is a maternal message. If so, then this protein may play a role in modulating signals during development.

Antibodies to trp-E- $DG\alpha_0$ fusion protein

Antisera isolated from two rabbits (R21 and R22) injected with a *trp-E-DGα₀* fusion protein were tested on Western blots with unfractionated extracts from *Drosophila* along with membrane and cytosol fractions (Figure 5). The $DG\alpha_0$ cDNA predicts a protein of 40 500 Da, and the antibodies raised against the fusion protein recognize a protein with an apparent molecular weight of 39 000 Da, which is not recognized by preimmune serum. The molecular weight of $DG\alpha_0$ was confirmed by in vitro translation. When RNA is transcribed from the $DG\alpha_0$ cDNA by the use of the T7 promoter of the Bluescript plasmid (Stratagene) and then translated in a rabbit reticulocyte lysate, the in vitro synthesized protein comigrates with the 39 000 Da protein detected in Western blots of head extracts (not shown). This protein was absent from reticulocyte lysates to which antisense message was added. We conclude that the apparent molecular weight of $DG\alpha_0$ on SDS-PAGE is ~39 000.

An additional protein of 45 000 Da was recognized by R21 antiserum (lanes 4–6), but not by R22 antiserum (lanes 10–12). The 45 000 Da protein was not detected by R21 antiserum in Western blots of head extracts (not shown). There was no difference in the pattern of immunohistochemical staining in the head and thorax with

Figure 4. Hybridization of $DG\alpha_0$ to sections of adult *Drosophila*. Anterior is up. A ^{35}S -labeled DNA fragment from the 3' noncoding region of the $DG\alpha_0$ cDNA was hybridized to 7- μm horizontal sections of adult *Drosophila*. (A) Phase contrast and (B) dark field of *Drosophila* head exhibits silver grains in the cellular layers of the midbrain (mb) and in the antenna (a). In the optic lobes, probe hybridized to the cellular layers of the lamina (l), medulla (m), lobula (lo), and lobula plate (lp). Hybridization was not detected in the neuropil of the midbrain and was generally absent from the neuropil of the optic and antennal lobes, with the following exceptions: faint but reproducible signal was observed in the lamina and between the lobula/lobula plate and medulla. This may represent signal in either neural fibers or glial cells. Signal was absent from retina (r). (C) Phase contrast and (D) dark field reveal hybridization in the cellular regions of the thoracic ganglion, with no hybridization detected in the neuropil. prn, prothoracic neuromere; msn, mesothoracic neuromere; mtn, metathoracic neuromere; abd, abdominal ganglion. Only the most ventral cellular layer of the abdominal ganglion is visible in this section. Bars are 50 μm .

Figure 5. Western-blot analysis of $DG\alpha_0$ protein detected by rabbit 21 (R21) and rabbit 22 (R22) antisera. Whole *Drosophila* were homogenized in 50 mM Tris-HCl pH 7.6, 3 mM benzimidazole. 50 μ g of total lysate protein were loaded onto lanes 1, 4, 7, and 10; 50 μ g of membrane protein were loaded onto lanes 2, 5, 8, and 11; 50 μ g of supernatant protein were loaded onto lanes 3, 6, 9, and 12. Samples were then fractionated on an 11% NaDodSO₄ polyacrylamide gel, transferred to nitrocellulose, and probed with either R21 preimmune (lanes 1–3), R22 preimmune (lanes 7–9), or anti-*trpE*- $DG\alpha_0$ immune serum (R21, lanes 4–6; R22, lanes 10–12) followed by ¹²⁵I-labeled goat anti-rabbit immunoglobulin. The molecular weight of the 39 000 Da (39 kDa) protein was established by the use of purified bovine α_0 as a marker (not shown). Lanes 13 and 14 were loaded with 0.05 μ g of purified bovine brain G_0 and analyzed as above. Lane 13 was probed with R21 antiserum, and lane 14 was probed with antiserum to bovine α_0 , which we have described previously (Huff *et al.*, 1985).

the two antisera tested. Therefore we do not believe that the 45 kDa protein contributes to the observed immunohistochemical patterns. Figure 5 (lanes 13–14) also shows that the antibody to $DG\alpha_0$ (R21) recognizes bovine α_0 much less well than antibody to bovine α_0 (Huff *et al.*, 1985). The difference in immunoreactivity is ~ 10 -fold, as determined by densitometry of the radioautograph. Furthermore, the anti- $DG\alpha_0$ antibody (R21) recognizes the *Drosophila* α_0 more efficiently than the anti-bovine α_0 antibody. When Western blots of *Drosophila* membranes were analyzed in parallel with R21 antiserum and anti-bovine α_0 serum, the band at 39 000 Da was 10-fold darker with the R21 antiserum than the bovine- α_0 antiserum (not shown). Taken together, these experiments show that anti- $DG\alpha_0$ antiserum can discriminate between bovine α_0 and *Drosophila* α_0 , even though these two proteins are 81% identical. It is impossible, at this stage, to rule out cross-reactivity with as-yet-unidentified *Drosophila* G-protein α subunits.

Immunohistochemical staining of adult *Drosophila*

To localize the $DG\alpha_0$ protein, adult flies were sectioned and then stained with the anti-*trpE*- $DG\alpha_0$ antibody. As with the in situ hybridization experiments, most immune staining was observed in the nervous system (Figure 6). In contrast to the hybridization experiments, however, the highest concentration of immunoreactivity was observed in the neuropil, and little, if any, staining was seen in neuronal cell bodies. Staining was observed throughout the neuropil of the optic lobes, including the lamina, medulla, lobula, and lobula plate, but was absent from the photoreceptor cells, except possibly for their projections to the lamina and medulla. The neuropil of the central brain and of the antennal and protocerebral lobes were labeled as intensely as the optic lobes. Two regions, the anterior portions of the antennal and protocerebral lobes, stand out as more intensely labeled than the remainder of the neuropil. Immunoreactivity was also observed in the antenna

Figure 6. Immunohistochemistry of R21 polyclonal antibody directed against *trp-E-DG α_0* fusion protein on frozen sections of adult flies. (A1) Brightfield and (A2) immunofluorescent staining show a portion of a section through the head of an adult *Drosophila*. The second antennal segment (a) contains a few labeled filaments and vessicles. The neuropil of the antennal (al) and protocerebral (pc) lobes are stained throughout, but more heavily so in the anterior portions of each lobe. A small portion of the central-brain neuropil is seen at the bottom of A2 and is uniformly labeled. (B) Immunofluorescence staining. The third antennal segment is the most heavily stained antennal region, displaying a filamentous network of labeled structures. Arrow points to autofluorescing cuticle. (C1) Brightfield and (C2) immunofluorescent staining show a section through the thoracic and abdominal ganglia. Neuropil in the thoracic and abdominal ganglia is uniformly stained. prn, prothoracic neuromere; msn, mesothoracic neuromere; mtn, metathoracic neuromere; abd, abdominal ganglion. * in C1 indicates neural cell bodies of the ganglion that are not stained in C2. Arrows in C2 point to autofluorescing structures. (D) Immunofluorescent staining. In a head section containing the retina (r) and optic lobe, staining appears only in the neuropil of the lamina (l), the medulla (m), the lobula (lo), and the lobula plate (lp). Arrow points to autofluorescing cuticle. For location of neural cell bodies, please refer to Figure 4A. (E1) Brightfield and (E2) immunofluorescent staining. A portion of an adult head stained with R21 preimmune serum reveals no specific staining. The only fluorescence seen in E2 is the autofluorescence of the cuticle and lenses. Rabbit 22 antisera gave results identical to these (not shown). Bars are 25 μ m, and anterior is up.

and in the neuronal tract running from the antennae to the antennal lobe (tract staining not shown). In the thorax, staining was seen throughout the neuropil of the thoracic and abdominal ganglia; in the female abdomen, the oocytes and ovarian nurse cells were stained (not shown). Neither preimmune serum nor antibodies raised against a *trp-E-rat α_{1-1}* fusion protein gave signal in any of the regions described above. The difference in the localization of DG α_0 mRNA and protein in the nervous system is consistent with translation of the message in the neuronal cell body followed by transportation of DG α_0 protein into the neural

cell fibers. In rat brain, α_0 protein was also found in the neuropil (Worley *et al.*, 1986; Asano *et al.*, 1988), while the α_0 mRNA was predominantly in the cellular layers (Largent *et al.*, 1988).

Our observations that *Drosophila* contains a G-protein α subunit that is 81% identical to mammalian α_0 and that, like mammalian α_0 , this protein is predominantly located in the neuropil of the central nervous system (CNS) suggest that the protein may have a similar function in *Drosophila* and mammals. The fact that α_0 is abundant in the CNS in both fruit flies and mammals suggests that it is particularly adapted to a specifically neu-

ronal function. In many cases, the CNS expresses neuronal forms of receptor and effectors. Therefore it is possible that a particular form of α subunit has evolved to couple these membrane proteins and that the whole neuronal transmembrane-signaling system has been conserved over many millions of years of evolution. One would expect that the interacting domains of different proteins may be severely constrained in the amino-acid substitutions that can be tolerated (Saris *et al.*, 1986; Yarfitz *et al.*, 1988). Given the extensive conservation of α_0 , one prediction would be that some of the receptors and effectors that α_0 modulates would also be conserved between *Drosophila* and mammals.

The gene, cDNA, and antibody described here will provide important tools for determining the function of DG α_0 . Knowledge of the chromosomal position of this gene combined with the localization of mRNA and protein reported here should provide useful guidelines for identifying DG α_0 mutations. For example, the observation that DG α_0 mRNA is present in the oocyte suggests that this gene product may be essential to early development. If this is true, then identifying embryonic lethals that map to the 47A region might yield mutations in the DG α_0 gene. Regardless of the screen used, the mutant phenotype should provide important clues regarding the function of DG α_0 . Furthermore, given the extensive conservation of α_0 , these mutants may help predict the function of α_0 in mammals.

Methods

Molecular cloning

A *Sma*I-*Xba*I DNA fragment that spans the carboxyl terminal coding region of bovine α_{L-1} (Michel *et al.*, 1986) was used to screen a *Drosophila melanogaster* genomic DNA library (Maniatis *et al.*, 1978) with the use of standard procedures (Maniatis *et al.*, 1982). Hybridization to plaque lifts were carried out at 50°C in 6× SSC (1× SSC is 0.15 M NaCl/0.015 M Na Citrate), 5× Denhart solution (1× Denhart is 0.02% Ficoll/0.02% polyvinylpyrrolidone/0.02% BSA), and 0.5% NaDodSO₄. Filters were then washed three times at 50°C in 1× SSC/0.2% NaDodSO₄. A *Drosophila melanogaster* head-specific cDNA library (Paul Salvaterra, City of Hope, Duarte, CA) was screened with the use of an 8.5 kb *Eco*RI fragment isolated from one class of *Drosophila* genomic clone. Hybridization to the cDNA library was conducted in 6× SSC/5× Denhart solution/0.5% NaDodSO₄ at 65°C, and filters were washed in 0.2× SSC/0.2% NaDodSO₄ at 60°C.

DNA sequencing

Overlapping deletions in both directions were generated with the use of the Exo/Mung Bean deletion kit (Stratagene) and transferred to m13mp9, and sequence was determined by the dideoxy chain termination method (Sanger *et al.*, 1977).

RNA blot analysis

Total RNA and poly A⁺ RNA were isolated by standard techniques (Maniatis *et al.*, 1982). RNA was fractionated in a 1.2% agarose gel containing 2.2 M formaldehyde and 0.04 M MOPS buffer and then transferred to a nitrocellulose filter. DNA was labeled by the random priming method (Feinberg and Vogelstein, 1983) and hybridized to the filter in 6× SSC/50% formamide/5× Denhart solution/0.2% NaDodSO₄ at 42°C. The filter was then washed in 0.2× SSC/0.2% NaDodSO₄ at 60°C.

In situ hybridizations

Adult flies (*yw*) were sectioned at 7 μ m on a Reichert-Jung cryotome and collected on TESPA (3-aminopropyltriethoxysilane, Pierce Chemicals)-coated, formaldehyde-activated slides (Berger, 1986). The slides were air dried for 1 h, dehydrated in an ethanol series, and stored at -80°C. For hybridization, the sections were thawed and immediately fixed in 4% paraformaldehyde (freshly prepared) in PBS (containing in mM 137 NaCl, 2.7 KCl, 4.3 Na₂HPO₄, and 1.4 KH₂PO₄; pH 7.3) for 10 min at room temperature. Slides were presoaked in 4× SSC, 0.02% Ficoll, 0.02% polyvinylpyrrolidone, and 0.2% SDS for 4 h at 37°C. Each slide was then hybridized with 5 × 10⁶ cpm of ³⁵S-labeled probe for 18 h. Slides were then washed four times in 1× SSC (0.15 M NaCl, 15 mM sodium citrate; pH 7) at 37°C for 30 min, dried in a graded series of ethanol, and then mounted in Permount.

DG α_0 fusion protein production and purification

To produce DG α_0 in *E. coli*, we made use of the pATH-10 vector, which directs production of a *trp*-E-DG α_0 fusion protein under the control of the *trp*-E promoter (Dieckmann and Tzagoloff 1985). A 1.2 kb *Xho* II fragment from the DG α_0 cDNA containing the complete coding region was subcloned into the *Bam*HI site of pATH-10 to produce a *trp*-E-DG α_0 fusion protein. For purification of the fusion protein, bacteria were initially grown in M9 (Maniatis *et al.*, 1982) plus ampicillin (25 μ g/ml) and tryptophan (0.2 mg/ml) medium at 37°C until the cells reached an OD₆₅₀ of 1.2. Cells were then starved for tryptophan by diluting into M9 + ampicillin, and *trp*-E expression was induced by addition of indoleacrylic acid (0.01 mg/ml). Cells were incubated for 5–7 h at 30°C and then harvested by centrifugation at 10 000 × g for 10 min. The cell pellet was washed in 10 mM Tris, pH 7.4; pelleted and resuspended in 50 mM Tris, pH 7.4, 5 mM NaEDTA, and 8 mg/ml lysozyme; and incubated on ice for 2 h. The suspension was then adjusted to 0.5 M NaCl, 1% Nonidet-P40 (Sigma), and the cells were then disrupted with a Brinkman Polytron at setting 6 for 30 s. The insoluble fraction was pelleted by centrifugation at 10 000 × g and then resuspended in 10 mM Tris pH 7.5. For rabbit injections, the suspension was adjusted to 4 M Urea and injected with Freund's adjuvant (1:1).

Western blots

Adult *Drosophila* (*yw*) were homogenized in 50 mM Tris/HCl pH 7.4, 3 mM benzamidine (TB buffer) in a 1.5-ml microfuge tube and then spun at 3000 rpm for 2 min to remove particulate matter. The supernatant was then spun at 14 000 rpm for 10 min in an Eppendorf microfuge. The membrane pellet was resuspended in TB. Membrane proteins were size fractionated on an 11% polyacrylamide/NaDodSO₄ gel (Laemmli, 1970) and electroblotted onto a nitrocellulose membrane (Towbin *et al.*, 1981). The membrane was blocked in 10 mM Tris, pH 7.4, 150 mM NaCl, and 3% BSA (TNB) for 1 h and then incubated with a 1:1000 dilution of the anti-DG α_0 serum in TNB overnight. The filter was then washed twice for 10 min in 10

mM Tris, pH 7.4, 150 mM NaCl (TN) and once in TN + 0.5% Nonidet P40 for 10 min, followed by two more washes in TN. The filter was then incubated for 1 h with 100 000 cpm/ml of ^{125}I goat anti-rabbit IgG, and then the wash procedure was repeated. The blot was then autoradiographed at -70°C overnight with Kodak AR film.

Immunohistochemistry

Young adult *Drosophila* (*yw*) were anesthetized on ice and sectioned at $7\ \mu\text{m}$ on a Reichert-Jung cryotome, and the sections were collected on TESPA-coated slides (Berger, 1986). The slides were air dried for 1 h and stored at -80°C until use. For the immunohistochemical assay, the slides were removed from -80°C to a humidified chamber at ambient temperature and immediately overlaid with 4% paraformaldehyde freshly prepared in 150 mM sodium phosphate buffer, pH 7.0, 0.075 M lysine, and 0.01 M sodium periodate (McLean and Nakane, 1974) for 20 min. After fixation, the slides were rinsed for 5 min with 0.1 M Tris, pH 7.4, and then for 5 min in 20 mM sodium phosphate, pH 7.0, 150 mM NaCl, 0.3% Triton X-100 (PBST). The slides were incubated for 30 min with either immune serum diluted 1:300 in PBST containing 10% normal goat serum and 3% bovine serum albumin (SDB) or with preimmune serum diluted 1:300 in the same buffer. The slides were then rinsed for 5 min in PBST and incubated for 20 min with rhodamine-conjugated goat anti-rabbit antibody (Boehringer Mannheim) diluted 1:100 in SDB. After this incubation, the slides were rinsed for 5 min in PBST and then for 5 min in PBS without Triton X-100. Slides were then mounted with 85% glycerol, 10 mM Tris, pH 7.4, and 5% n-propyl gallate (Giloh and Sedat, 1982) and viewed by epifluorescence.

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