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Identification of a *Drosophila* G protein α subunit ($dG_q\alpha$-3) expressed in chemosensory cells and central neurons

(olfaction/GTP-binding protein/signal transduction/RNA)

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ABSTRACT **We have identified another *Drosophila* GTP-binding protein (G protein) α subunit, $dG_q\alpha$-3. Transcripts encoding $dG_q\alpha$-3 are derived from alternative splicing of the dGqα locus previously shown to encode two visual-system-specific transcripts [Lee, Y.-J., Dobbs, M.B., Verardi, M.L. & Hyde, D.R. (1990) *Neuron* 5, 889–898]. Immunolocalization studies using $dG_q\alpha$-3 isoform-specific antibodies and LacZ fusion genes show that $dG_q\alpha$-3 is expressed in chemosensory cells of the olfactory and taste structures, including a subset of olfactory and gustatory neurons, and in cells of the central nervous system, including neurons in the lamina ganglionaris. These data are consistent with a variety of roles for $dG_q\alpha$-3, including mediating a subset of olfactory and gustatory responses in *Drosophila*, and supports the idea that some chemosensory responses use G protein-coupled receptors and the second messenger inositol 1,4,5-trisphosphate.**

Heterotrimeric G proteins play a central role in a wide variety of signal-transduction pathways in organisms ranging from yeast to human (for review, see refs. 1–7). G proteins have been implicated in signal-transduction events underlying olfaction and vision (for review: see ref. 8). Vertebrate and invertebrate animals use heterotrimeric G proteins to mediate light responses (for review, see refs. 8 and 9). In *Drosophila*, light-activated rhodopsin molecules activate G α subunit proteins encoded by a $dG_q\alpha$ gene (10–12). This gene produces two photoreceptor-cell-specific transcripts encoding two putative α subunits, $dG_q\alpha$-1 and $dG_q\alpha$-2. $dG_q\alpha$-2 is identical to $dG_q\alpha$-1, except that it lacks a single exon near the C terminus. Recent evidence indicates that the $dG_q\alpha$-2 protein is not produced (12). $dG_q\alpha$-1 is 353 amino acids in length and encodes the functional G α subunit required for vision (11, 12). Loss-of-function mutations in the visual-specific $dG_q\alpha$ gene products abolish the light response, demonstrating the absolute requirement for $dG_q\alpha$ gene products in visual transduction (12).

$dG_q\alpha$-1 is thought to activate a inositolphospholipid phospholipase C encoded by the *norpA* gene (11, 13). norpA cleaves the membrane lipid phosphatidylinositol bisphosphate into inositol 1,4,5-trisphosphate ($InsP_3$) and diacylglycerol. Increases in intracellular $InsP_3$ concentration trigger downstream events, ultimately leading to the opening of cation channels in the photoreceptor cell membrane and the generation of a receptor potential (for review: see refs. 9 and 14).

Vertebrate odorant receptors are thought to activate heterotrimeric G proteins of the stimulatory G protein class (G_{olf}; ref. 15), that in turn activate adenylyl cyclase (16), producing cAMP. The rise in cAMP triggers the opening of cyclic nucleotide-gated cation channels (17, 18), resulting in cell depolarization. In addition to cAMP, some odorant responses may be mediated through $InsP_3$, nitric oxide (NO), or carbon monoxide (CO) second messengers (for review: see ref. 19). The signal-transduction components and second messengers mediating olfaction in invertebrates like *Drosophila* are not well-characterized (for review: see ref. 20). Members of the putative odorant receptor family have not been identified in invertebrates. G protein α-subunit expression in the olfactory system has not been explored in detail. However, work from several laboratories has implicated $InsP_3$ signaling mechanisms mediating olfactory responses in invertebrates including *Drosophila* (21–26). This evidence, in turn, suggests that members of the G_q α-subunit class may transduce some odorant responses in invertebrates. We report here the identification of another isoform of $dG_q\alpha$; this isoform is expressed in the chemosensory cells of the olfactory and gustatory structures, as well as in the gut, thoracic ganglion, and some central nervous system‡ neurons.

MATERIALS AND METHODS

PCR and DNA Sequence Analysis. cDNA was prepared from mRNA isolated from appendages of Oregon-R flies. Degenerate primers, oMP19 and oMP20, specific to G protein α-subunit genes, were originally described in ref. 27. Amplification was done according to Strathman *et al.* (27). PCR products were subcloned into Bluescript (Stratagene) and sequenced using the dideoxynucleotide chain-termination procedure of Sanger *et al.* (28) with Sequenase kits (United States Biochemical). cDNA and genomic clones were subjected to *Exo* III nested deletions (Promega) and sequenced as above.

$dG_q\alpha$-3 Antisera. Antiserum specific to $dG_q\alpha$-3 was raised in New Zealand White rabbits using the peptide MFVDLNPDSEKIIY conjugated to tuberculin PPD (Statens Seruminstitut, Copenhagen). Immune serum was affinity-purified using Affi-Gel columns (Bio-Rad). Immunofluorescence localization was done as described in Smith *et al.* (29) and analyzed by using a Bio-Rad MRC 1000 confocal microscope. Rabbit anti-β-galactosidase antiserum was purchased from Cappel. Mouse anti-ELAV was the gift of Helmut Kramer (University of Texas Southwestern Medical Center).

LacZ Fusion Constructs. Approximately 8 kb of genomic sequence upstream of the $dG_q\alpha$ coding region, including the initiator methionine and nucleotides encoding the first 10 amino acids of $dG_q\alpha$, were fused in-frame to a LacZ gene containing a simian virus 40 large T antigen nuclear localization signal (29, 30). Transgenic flies were generated by *P*-element-mediated germ-line transformation as described by Karess and Rubin (31). LacZ expression was examined in frozen-tissue sections of transgeneic flies as described in ref. 29.

RESULTS AND DISCUSSION

Identification and Isolation of the $G_q\alpha$ Molecule. Using PCR with degenerate oligonucleotides corresponding to con-

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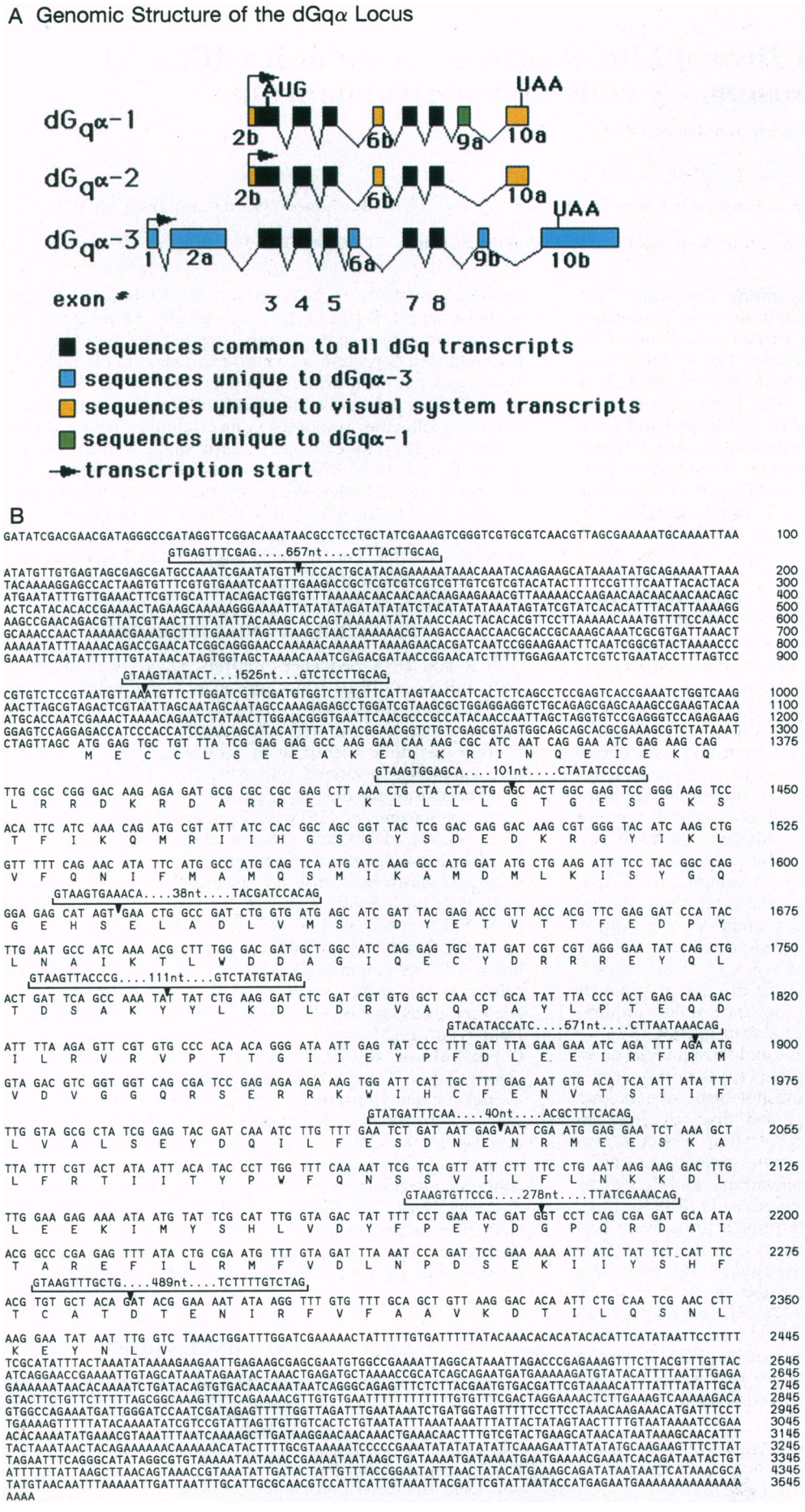
Abbreviation: $InsP_3$, inositol 1,4,5-trisphosphate.

‡The sequence reported in this paper has been deposited in the GenBank data base (accession no. U31092).

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          M  E  C  C  L  S  E  E  A  K  E  Q  K  R  I  N  Q  E  I  E  K  Q
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ACA TTC ATC AAA CAG ATG CGT ATT ATC CAC GGC AGC GGT TAC TCG GAC GAG GAC AAG CGT GGG TAC ATC AAG CTG    1525
 T  F  I  K  Q  M  R  I  I  H  G  S  G  Y  S  D  E  D  K  R  G  Y  I  K  L

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 V  F  Q  N  I  F  M  A  M  Q  S  M  I  K  A  M  D  M  L  K  I  S  Y  G  Q
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AAAA
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FIG. 1. (*A*) Transcripts encoded by the dG$_q\alpha$ locus. dG$_q\alpha$-1 and dG$_q\alpha$-2 are photoreceptor-specific and are identical except for the lack of exon 9 in dG$_q\alpha$-2. dG$_q\alpha$-3 uses specific forms of exons 1, 2, 6, 9, and 10. Splicing differences in exons 6, 9, and 10 result in clustered amino acid substitutions in dG$_q\alpha$-3 protein. (*B*) cDNA sequence and translation of dG$_q\alpha$-3. The sequence of dG$_q\alpha$-3 with conceptual translation of the encoded protein is shown. Location, size, and sequence of splice junctions appear above the sequence. The only significant differences noted between sequences reported here and those of Lee *et al.* are the presence of a thymine in exon 9B not reported in ref. 10 and an 18-base perfect repeat in exon 8 reported in ref. 10 not observed in either dG$_q\alpha$-3 cDNAs or genomic clones spanning this region.

served regions of the G proteins α-subunit family and reverse-transcribed mRNA isolated from *Drosophila* appendages, we identified transcripts encoding G protein α subunits homologous to vertebrate $G_q\alpha$. Subcloned $G_q\alpha$ PCR fragments were used as probes to screen an appendage cDNA library for corresponding cDNAs. We isolated cDNAs ranging in size from 2.5 to 3.5 kb. All cDNAs were 2.0 kb reported for the visual-system-specific $dG_q\alpha$ transcripts as determined by Northern blots (10), suggesting the presence of a specific $G_q\alpha$ gene in *Drosophila* or a previously unsuspected splicing variant of the $dG_q\alpha$ locus.

We sequenced the longest cDNA obtained from the appendage library, $dG_q\alpha$-3. $dG_q\alpha$-3 is 3549 nt in length and contains a single long open reading frame preceded by a consensus translation initiation ATG (32). $dG_q\alpha$-3 has long stretches of nucleotide-sequence identity with the visual-system-specific $dG_q\alpha$ transcripts but also has regions of divergence (Fig. 1). The predicted protein encoded by $dG_q\alpha$-3 is 353 amino acids in length and is 89% identical to the visual-system-specific $dG_q\alpha$-1, differing only in two domains, resulting from the use of alternatively spliced exons (Fig. 1*A*). Therefore, the $dG_q\alpha$ locus encodes at least three different transcripts, which encode distinct putative protein products (see below).

To characterize the gene structure of $dG_q\alpha$-3 and to look for additional potential coding exons, we sequenced 8.2 kb of genomic DNA encoding the $dG_q\alpha$-3 transcript. The complete intron–exon structure of $dG_q\alpha$-3 is shown in Fig. 1. $dG_q\alpha$-3 has 10 exons separated by nine introns, ranging in length from 62 to 1549 nt (Fig. 1*B*). Scanning the genomic sequence for homology to G protein α subunits, we found no evidence for additional alternate exons encoding $dG_q\alpha$ domains.

$dG_q\alpha$-3 transcription initiation is >3 kb upstream from the visual system transcription initiation (Fig. 1*A*) and is regulated by a different promotor (ref. 11, see below). $dG_q\alpha$-3 uses polyadenylylation signals ≈1 kb downstream from the visual-specific polyadenylylation signal (10). We propose the exon numbering system used in Fig. 1*A*, which accounts for all $dG_q\alpha$ transcripts.

$dG_q\alpha$-3 Protein Sequence. $dG_q\alpha$-3 is 89% identical to $dG_q\alpha$-1, differing only in two domains. Alternative forms of exon six encode 11 amino acid substitutions between $dG_q\alpha$-1 and $dG_q\alpha$-3 over this 39-amino acid region. Five differences result in charge substitutions (Fig. 2). Exon six encodes a portion of the protein that contacts the guanine nucleotide (34, 35). This may have important effects on the kinetics of activation or deactivation of $dG_q\alpha$-3 compared with $dG_q\alpha$-1. The second domain that differs between $dG_q\alpha$-3 and $dG_q\alpha$-1 is at the C terminus. This region is important for receptor interactions (34, 35). Therefore, $dG_q\alpha$-3 may be activated by nonrhodopsin G protein-coupled receptors. Interestingly, the C-terminal region of $dG_q\alpha$-3 is more similar to the mammalian G_q and G_q11 (33) than it is to $dG_q\alpha$-1 (94% identity with mammalian G_q and 54% identical to $dG_q\alpha$-1; Fig. 2). This result suggests that $dG_q\alpha$-3 was the prototypic *Drosophila* G_q gene product and that the visual-system-specific molecules have subsequently evolved from it.

Mammalian G protein α subunits, including G_q, have two potential initiator methionines, one corresponding to the *Drosophila* protein start site and one six residues further upstream (Fig. 2). Whether the upstream site is actually used in mammals is the subject of controversy. However, *Drosophila* lack this upstream methionine, suggesting that the putative long form is not biologically relevant in mammals or that this variation evolved after the diversification of insects and mammals.

$dG_q\alpha$ Expression Is Not Limited to the Visual System. To define the number and identity of the cells expressing $dG_q\alpha$-3, we generated transgenic animals expressing β-galactosidase under control of the $dG_q\alpha$ promoter. The β-galactosidase gene we used encoded a simian virus 40 nuclear localization signal to facilitate the identification of $dG_q\alpha$-positive cells. Frozen tissue sections were prepared from transgenic flies and stained for β-galactosidase activity. We identified expression in the retina, the antenna, the maxillary palps (accessory olfactory organs), the lamina, the proboscis, epithelial cells of the gut, some thoracic neurons, and scattered cells throughout the central nervous system (Fig. 3A–D). $dG_q\alpha$-1 isoform-specific antibodies only detect protein in the retina (12), indicating other sites of β-galactosidase expression reflect $dG_q\alpha$-3. This complex pattern suggests that $dG_q\alpha$-3 mediates a variety of signal-transduction processes in *Drosophila*, potentially including olfaction, gustation, and visual processing.

We used a synthetic peptide to generate antiserum specific to the $dG_q\alpha$-3 isoform (see underlined sequence in Fig. 2) and used it to immunolocalize $dG_q\alpha$-3 expression in frozen head-tissue sections. We detected $dG_q\alpha$-3 protein in the third antennal segment, maxillary palps, the tip of the proboscis and in the brain, especially prominant in the lamina ganglionaris (Fig. 3*B*). Some of the immunoreactive cells in the antenna are olfactory neurons, as dendrites can be seen entering some

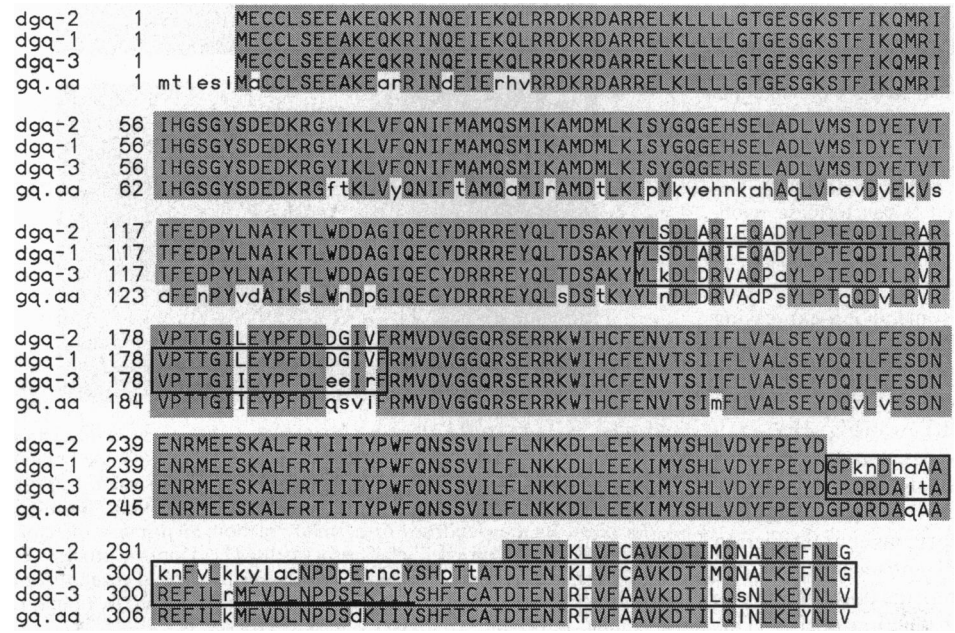

FIG. 2. Amino acid alignment of the three proteins encoded by $dG_q\alpha$ (dgq) and mouse G_q (gq) (33). The sequence used to generate synthetic peptide antisera specific to $dG_q\alpha$-3 is underlined. Boxed regions are encoded by alternate $dG_q\alpha$ exons. $dG_q\alpha$-3 is more similar to the mammalian protein at the C terminus than it is to $dG_q\alpha$-1. Shaded regions indicate an identical residue in the majority of the sequences. Lowercase letters indicate an amino acid not found in the other sequences.

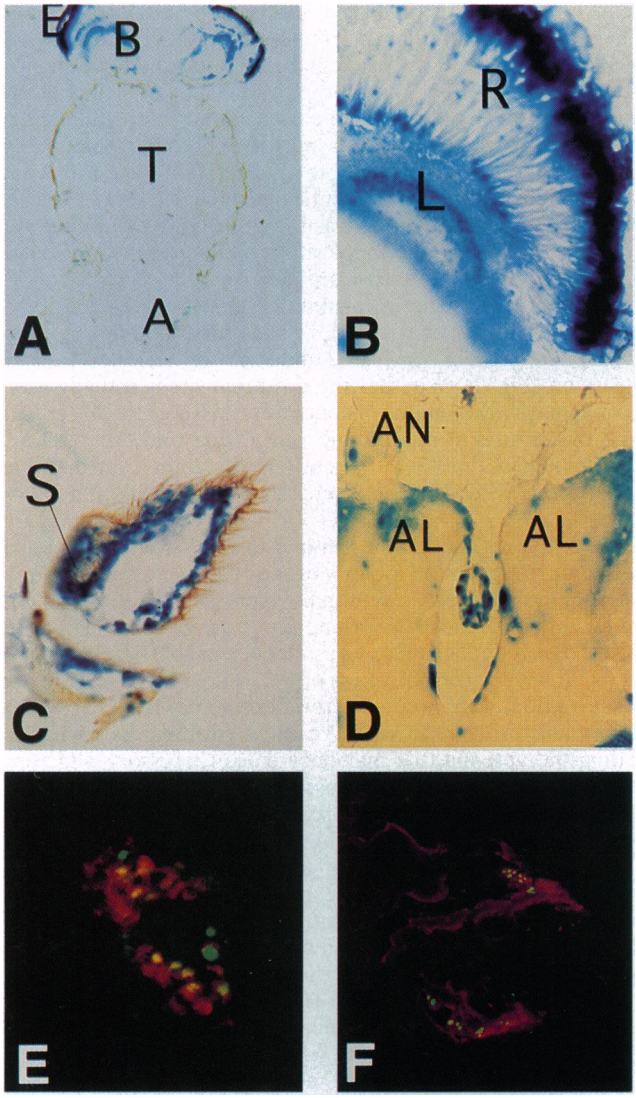

FIG. 3. Expression of nuclear-localized β-galactosidase in transgenic flies expressing LacZ under control of the $dG_q\alpha$ promoter. (*A*) Frozen section through an adult fly. Expression is detected in the compound eye (E) and the brain (B). T, thorax; A, abdomen. (*B*) Frozen-tissue section through transgenic-fly head. E, compound eye; L, lamina ganglionaris. (*C*) Frozen section through the third antennal segment. Accessory cells as well as neurons express $dG_q\alpha$-3. S, sacculus. (*D*) In contrast to muscarinic receptors (36), few cells of the antennal lobes express $dG_q\alpha$-3. AN, antennal nerve; AL, antennal lobes. (*E*) Confocal immunofluorescent image of third antennal segment from dG_q-LacZ transgenic fly treated with anti-ELAV (green) and anti-β-galactosidase (red). ELAV is specifically expressed in chemosensory neurons in the antenna and proboscis (37). Colocalization of ELAV and nuclear localized β-galactosidase is observed in some, but not all, ELAV-positive cells. (*F*) β-Galactosidase colocalizes with a subset of ELAV-positive cells in the proboscis.

sensillae (Fig. 3*A*), and the nuclei of some $dG_q\alpha$-expressing cells are positive for the olfactory neuron nuclear marker ELAV (Fig. 3*E*). However, other antennal cells that express $dG_q\alpha$-3 appear to be nonneuronal accessory cells (Fig. 3*E*). Some gustatory neurons also express $dG_q\alpha$-3 (Fig. 3*F*).

$dG_q\alpha$-3 and Chemoreception. $dG_q\alpha$-3 is expressed in many cells in the antenna, including a subset of olfactory neurons (Figs. 3*A*, 4 *C* and *E*). The presence of $dG_q\alpha$-3 in a subset of these neurons indicates it does not perform a general function common to all olfactory neurons. Indeed, antiserum specific to the $dG_q\alpha$-3 isoform localizes it in the dendritic portion of those neurons (Fig. 3*A*) where olfactory transduction occurs. This makes $dG_q\alpha$-3 a candidate for mediating a subset of olfactory responses in *Drosophila* and supports a model whereby different primary olfactory neurons express different signaling molecules to transduce different olfactory responses. Alternatively, $dG_q\alpha$-3 may transduce local paracrine signals between nonneuronal support cells or between the neuron and the support cells.

$dG_q\alpha$ is also expressed at the tip of the proboscis, where taste transduction occurs (for review, see ref. 38). We observed a subset of chemosensitive neurons expressing $dG_q\alpha$ (Fig. 4*F*). This result reflects expression of $dG_q\alpha$-3, as the isoform-specific antibody detects protein in this area (data not shown).

The expression pattern of $dG_q\alpha$-3 is reminiscent of the vertebrate olfactory G protein, G_{olf}, which is enriched in the cilia of the olfactory neurons (15), and is also expressed in other parts of the central nervous system not associated with chemosensation (39).

$dG_q\alpha$-3 and Visual Processing. $dG_q\alpha$-3 is expressed in cells of the central nervous system and is strongly expressed in the lamina ganglionaris (Fig. 3*B*). This region of the brain is the synaptic target of six of the eight photoreceptor cells from each ommatidium or unit eye. These R1–6 photoreceptor cells are of a single class, express the opsin Rh1 (40), and project directly to the lamina where they synapse on interneurons (for review, see ref. 9). $dG_q\alpha$-3 may have a role in synaptic transmission or information processing of visual information from the R1–R6 photoreceptor cells. Histamine is the neurotransmitter released by the R1–R6 photoreceptor axon terminals at the lamina (41). Histamine signaling is mediated by G protein-coupled receptors, and histamine type 1 receptors activate phospholipase C in vertebrates (for review, see ref. 42). However, in blowflies histamine released by the R1–R6 photoreceptor neurons activates laminar interneurons via histamine-gated Cl^- channels (43). Therefore, $dG_q\alpha$-3 may be

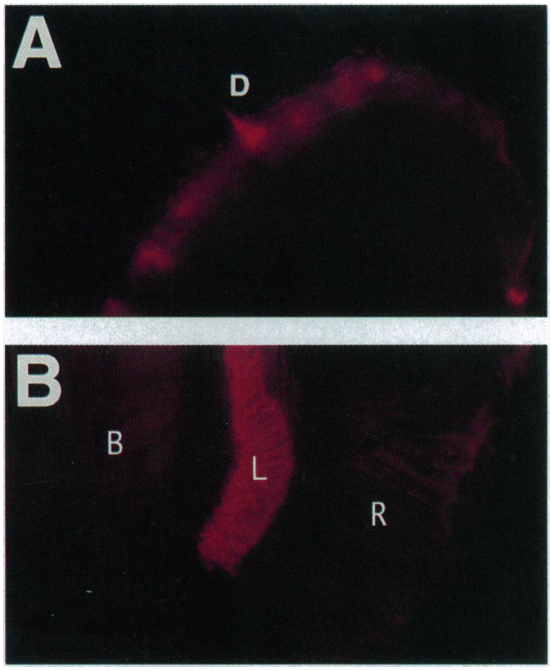

FIG. 4. Immunolocalization of $dG_q\alpha$-3. (*A*) Frozen tissue section through a third antennal segment incubated with anti-$dG_q\alpha$-3 peptide antibody and Texas Red-conjugated goat anti-rabbit serum. Approximately 30 olfactory neurons are present in this section. $dG_q\alpha$-3 is localized in the dendrites of a small fraction of primary olfactory neurons and is expressed in accessory cells. The dendritic process (D) of one neuron can be seen entering the sensillum. (*B*) Frozen section through the head, demonstrating $dG_q\alpha$-3 in the lamina. R, retina; L, lamina; B, brain.

part of a feedback mechanism involved in the visual processing of R1–R6 visual input.

$dG_q\alpha$-3 Is Not Highly Expressed in Antennal Lobes. Muscarinic acetylcholine receptors are G protein-coupled receptors in animals, including *Drosophila* (44, 45). The *Drosophila* receptors stimulate production of $InsP_3$ when expressed and activated in Y1 adrenocarcinoma cells (44). Muscarinic receptors are highly expressed in the glomeruli of the antennal lobes (36). We examined the LacZ staining pattern in the antennal lobes of transgenic flies expressing β-galactosidase under control of the $dG_q\alpha$ promoter. $dG_q\alpha$-3 expression does not parallel that of the muscarinic recepter in the glomeruli of the antennal lobes (Fig. 4*D*), indicating that these receptors do not use $dG_q\alpha$-3 for signaling. Therefore, either undiscovered *Drosophila* G_q genes are expressed in the antennal lobes or these receptors function through other second messengers.

Genes Encoding Signal-Transduction Molecules Expressed in Olfactory and Photoreceptor Neurons Are Shared. $dG_q\alpha$ encodes signaling molecules that appear to mediate both chemosensory and visual transduction in *Drosophila*. Interestingly, other genes encoding signaling molecules may be used in both olfactory and visual transduction. Mutants in the phospholipase C gene *norpA* are devoid of light-evoked receptor potentials in photoreceptors (46, 47) and have specific olfactory defects (48). Mutations in the *rdgB* gene result in light-dependent photoreceptor degeneration (46) and also appear to disrupt the normal response kinetics to specific odorants (20, 26). rdgB has homology to inositolphospholipid transfer proteins, suggesting a role in phosphatidylinositol bisphosphate metabolism (49). Because single gene mutations result in both olfactory and visual transduction defects, the olfactory and visual transduction pathways must use an overlapping set of genes encoding molecules important for signaling (48). The visual defects are more striking compared with the olfactory defects, suggesting that visual and olfactory transduction mechanisms only partially overlap. This hypothesis is consistent with the idea that vision utilizes a single transduction cascade, whereas olfaction may utilize several. $dG_q\alpha$-3 expression in some primary olfactory neurons suggests that a subset of odorant responses are mediated by this protein and that other mechanisms must be present in the remaining olfactory neurons.

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