Chem. Senses 35: 183–193, 2010
doi:10.1093/chemse/bjp095
Advance Access publication January 4, 2010

Expression Analysis of the 3 G-Protein Subunits, Gα, Gβ, and Gγ, in the Olfactory Receptor Organs of Adult *Drosophila melanogaster*

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Accepted November 30, 2009

Abstract

In many species, olfactory transduction is triggered by odorant molecules that interact with olfactory receptors coupled to heterotrimeric G-proteins. The role of G-protein–linked transduction in the olfaction of *Drosophila* is currently under study. Here, we supply a thorough description of the expression in the olfactory receptor organs (antennae and maxillary palps) of all known *Drosophila melanogaster* genes that encode for G-proteins. Using RT-polymerase chain reaction, we analyzed 6 *Gα* (G_s, G_i, G_q, G_o, G_f, and *concertina*), 3 *Gβ* ($G_{\beta 5}$, $G_{\beta 13F}$, and $G_{\beta 76C}$), and 2 *Gγ* genes ($G_{\gamma 1}$ and $G_{\gamma 30A}$). We found that all Gα protein-encoding genes showed expression in both olfactory organs, but G_f mRNA was not detected in palps. Moreover, all the *Gβ* and *Gγ* genes are expressed in antennae and palps, except for $G_{\beta 76C}$. To gain insight into the hypothesis of different G-protein subunits mediating differential signaling in olfactory receptor neurons (ORNs), we performed immunohistochemical studies to observe the expression of several Gα and Gβ proteins. We found that Gs, Gi, Gq, and $G_{\beta 13F}$ subunits displayed generalized expression in the antennal tissue, including ORNs support cells and glial cells. Finally, complete coexpression was found between Gi and Gq, which are mediators of the cyclic adenosine monophosphate and IP3 transduction cascades, respectively.

Key words: *Drosophila*, G-protein, olfaction, olfactory reception, signal transduction

Introduction

Heterotrimeric G proteins are composed of 3 subunits, α, β, and γ, and mediate many cellular signaling processes. When activated, the Gα subunit (which binds guanosine triphosphate) and the Gβγ heterodimer act on their effectors. Once the stimulus ends, the Gα subunit returns to the basal state in which it is bound to guanosine diphosphate (GDP), and an inactive heterotrimer Gαβγ is again formed (Gilman 1984).

In many species of vertebrates as well as invertebrates, olfactory reception is mediated by odorant receptors that belong to the G-protein–coupled receptor (GPCR) family (Mombaerts 1999). In mammals, olfactory stimulation leads to an increase of cyclic adenosine monophosphate (cAMP) due to G_{olf} activation and subsequent cell depolarization caused by the opening of cyclic nucleotide-gated channels (Ronnett and Moon 2002). However, olfactory transduction in insect olfactory receptor neurons (ORNs) remains unclear. G-protein inhibition in insect antennal homogenates impairs odor-evoked increases in IP_3, which is a presumptive olfactory second messenger (Boekhoff et al. 1990), and treatment of moth ORNs with the G-protein activator NaF leads to pheromone-evoked responses (Laue et al. 1997).

Gene expression studies signal the presence of several G-protein subunits in the olfactory receptor organs. In lobsters, ORNs express 2 classes of Gα proteins, G_q and G_s (McClintock et al. 1997; Xu et al. 1997). The expression of different classes of Gα proteins has also been studied in the olfactory organs of silk moths and *Anopheles* (Miura et al. 2005; Rützler et al. 2006). In *Drosophila*, some Gα proteins have been found in the antennae, such as G_q (Talluri et al. 1995; Kalidas and Smith 2002), which triggers the IP3 cascade, and Go (Schmidt et al. 1989), which has effectors that are not well determined.

From a functional viewpoint, recent electrophysiological studies in heterologous systems expressing *Drosophila* odorant receptor molecules showed contradictory results regarding the role of G-protein–linked transduction in olfactory reception. In some reports, olfactory receptors were proposed to be ligand-gated ion channels independent of

G-proteins (Sato et al. 2008; Smart et al. 2008), whereas in others, the Gα subunit was shown to become activated in response to odorants (Wicher et al. 2008). Moreover, behavioral and electrophysiological studies in *Drosophila Gq* mutants (Kalidas and Smith 2002; Kain et al. 2008) showed altered responses to odors, which have been interpreted as a sign of G-protein involvement in olfactory reception.

In the *Drosophila* genome, 6 genes have been described that encode for Gα proteins: G_s, G_i, G_q, G_o, G_f, and *concertina* (*cta*). Each gene activates different transduction pathways depending on its particular effectors. The associated pathways for G_s, G_i, and G_q are well known and produce increases in cAMP (G_s), decreases in cAMP (G_i), and the release of IP_3 and diacylglycerol (G_q). The signal transduction pathways downstream of G_o, G_f, and *cta* are unknown. In addition, some of the G-protein genes undergo alternative splicing and produce different transcripts. Sometimes these encode different proteins, but in most cases, these transcripts differ in untranslated regions and produce the same protein but in different expression patterns (Talluri et al. 1995; Wolfgang et al. 2001; Ray et al. 2007). In the case of the G_q and G_o genes, some of the transcripts do produce different amino acid sequences, and their expression is analyzed in this report.

The other 2 components of the G protein heterotrimer are the Gβ and Gγ subunits. Three genes in the *Drosophila* genome encode the β subunits, $G_{\beta 5}$, $G_{\beta 13F}$, $G_{\beta 76C}$ (Yarfitz et al. 1988; Dolph et al. 1994), and 2 genes encode the γ subunits, $G_{\gamma 1}$ and $G_{\gamma 30A}$ (Ray and Ganguly 1992; Schulz et al. 1999). Each gene produces a single protein variant. Gβγ-regulated effectors include K+ and Ca2+ channels, adenylyl cyclase, phospholipase C-β, phosphoinositide 3-kinases, and some protein kinases. They stabilize the binding of GDP to Gα to suppress spurious activation, and provide membrane contact points for GPCR kinases and also help to anchor the Gα subunits to the plasma membrane, which promotes interaction of Gα with receptors (Gilman 1987; Clapham and Neer 1997).

Expression studies usually offer a basic source of information about the possible biological function of gene products and have been extensively applied to G-proteins in *Drosophila melanogaster*. In some cases, restricted expression in specific tissues of genes encoding for different subunits has been described. Thus, the Gq-1 isoform (Lee et al. 1990) and the Gβ76C subunit (Yarfitz et al. 1991) showed specific expression in photoreceptor cells, pointing to their role in phototransduction. On the other hand, Gγ30A also constitutes a visual subunit, although it is expressed not only in photoreceptor cells but also in the brain (Schulz et al. 1999).

Some reports have previously studied the expression of the G-protein subtypes G_q and G_o in the olfactory receptor organs, but a complete analysis has not been performed. In this report, we systematically examine the expression pattern of all described G-protein subunits and their variants in the third antennal segments and maxillary palps of adult *Drosophila* using RT-polymerase chain reaction (PCR), as a first step in unraveling their role in olfactory reception "in vivo." To examine more closely the expression patterns of G-proteins in antennal tissue, immunohistochemical studies of different Gα and Gβ proteins have been performed.

Materials and methods

Sequence analysis

Protein sequences of *Drosophila* and human G-proteins were obtained from Flybase and National Center for Biotechnology Information databases (Gs: CG2835; Gi: CG10060; Gq: CG17759; Go: CG2204; Gf: CG12232; cta: CG17678; Gβ5: CG10763; Gβ13F: CG10545; Gβ76C: CG8770; Gγ1: CG8261; Gγ30A: CG3694/CG18511; GNAS: NM_000516; GNAL: NM_182978; GNAI1: NM_002069; GNAI2: NM_002070; GNAI3: NM_006496; GNAT1: NM_000172; GNAT2: NM_005272; GNAT3: XM_294370; GNAQ: NM_002072; GNA11: NM_002067; GNAO: NM_020988; G12: NM_007353; G13: NM_006572; GNB1: NM_002074; GNB2: NM_005273; GNB3: NM_002075; GNB4: NM_021629; GNB5: AF017656; GNG5p2: NG_002699; GNG2: AK001024; GNG3: NM_012202; GNG4: NM_004485; GNG5: NM_005274; GNG7: NM_052847; GNG8: AF493875; GNG12: AF119663; GNG13: NM_016541; AC091046.2: AC091046) and aligned with Bioedit (Hall 1999). We selected penalty values for gap opening (10) and gap extension (0,1). A neighbor joining tree was calculated, and 100 bootstrap trials were performed to upgrade reliability. The evolutionary distances were computed using the Jones, Taylor, and Thorton matrix-based method (Jones et al. 1992). All positions containing gaps and missing data were eliminated from the data set. Phylogenetic analyses were conducted in MEGA4 (Tamura et al. 2007).

Fly stocks

Wild-type Canton-S flies from the Bloomington Stock Center were used for gene expression studies by RT-PCR.

Inmunohistochemical experiments were performed in Canton-S flies as well as in *Elav-Gal4/UAS-GFP* flies (P{GawB}elav[C155], P{UAS-mCD8::GFP.L}LL4, P{hsFLP}1, w[*], stock 5146 of the Bloomington Stock Center, donated by L. Luo, Stanford University, CA) and *OR83b-Gal4/UAS-GFP* heterozygotes to identify antennal neurons or olfactory neurons by the presence of green fluorescent protein (GFP). The *OR83b-Gal4/UAS-GFP* flies were generated by crossing the *OR83b-Gal4*; *TM2/TM6B* stock (donated by L. Vosshall, Rockefeller University, NY) and P{UAS-mCD8::GFP.L}LL5 (stock 5137 of the Bloomington Stock Center, donated by L. Luo, Stanford University, CA). GFP expression in these hybrids was used as a marker for ORNs that contained the broadly expressed Or83B receptor.

Reverse transcription-PCR

A reverse transcription (RT) experiment was performed to test for the presence of native G-protein mRNA in head, third antennal segment, and maxillary palp fractions of Canton-S individuals. About 50 heads deprived of olfactory organs (antennae and maxillary palps), 300 third antennal segments and 50 maxillary palps were collected after sieving complete flies that were frozen in liquid nitrogen. Total RNA was isolated with Nucleospin RNA II (Macherey-Nage) according to manufacturer's instructions, followed by an additional acid phenol/chloroform extraction step and RNA precipitation. First strand cDNA was synthesized from the whole amount of the isolated RNA using the SuperScript first-strand synthesis system for reverse transcriptase-PCR (Invitrogen) with random primers.

All primer sets were selected to span intron regions or presumptive exon–exon boundaries in order to discriminate between amplification of genomic DNA and cDNA targets. A pair of primers was designed for each G-protein gene. For *Gq* and *Go*, specific primers were also designed against 2 different parts of the gene, each common to a specific group of transcripts that encode the same protein isoform. Each group of transcripts was named according to one of the primer pairs. Thus, the *Gq-RA*, *Gq-RD*, *Go-RA*, and *Go-RB* groups were studied.

The following primers were designed after being tested in all their gene-specific combinations (target in parentheses): Gsfwd 5′-CATGTCGACGGATTTTCTGA-3′ and Gsrev 5′-TGAAGAACGCCCTTGTCTTT-3′ (G_S-gene); Gifwd 5′-AGATGGGTTGTGCCGTGAG-3′ and Girev 5′-GCGACGATACTCCTCGCATT-3′ (G_i-gene); Gqfwd 5′-AGGACAAGCGTGGGTACATC-3′ and Gqrev 5′-AAAGCGTTTTGATGGCATTC-3′ (*Gq*-gene); Gq-RAfwd 5′-CCCTTGGTTTCAAAATTCGT-3′ and Gq-RArev 5′-AACTCTCGGGCCGTTATTG-3′ (Gq-RA group of transcripts); Gq-RDfwd 5′-TCGAATGGAGGAATCTAAAGC-3′ and Gq-RDrev 5′-AAATGCGAATAGCATTGACG-3′ (Gq-RD group of transcripts); Gofwd 5′-CTGGCATCGTTGAGGTACAC-3′ and Gorev 5′-TGTCCGTGAACCATTTGTTG-3′ (*Go*-gene); Go-RAfwd 5′-AGGAGTCGCCTCATCGAGCGC-3′ and Go-RArev 5′-TGTTTAAAGTCCTCCGCAGTG-3′ (Go-RA group of transcripts); Go-RBfwd 5′-GCCATCCAGCGATCCAAA-3′ and Go-RBrev 5′-CTGTAGACAACCGGTCGATATT-3′ (Go-RB group of transcripts); Gffwd 5′-GAGGGACACCACCGTTAAGA-3′ and Gfrev 5′-TATAGTCCGCCGATCTTTCG-3′ (*Gf*-gene); ctafwd 5′-TTTGCTTGCTTTCGATGTTG-3′ and ctarev 5′-CCATGAATGATTCGCATTTG-3′ (*cta*-gene); Gβ13Ffwd 5′-AATAAACGCCGTATGCAAGC-3′ and Gβ13Frev 5′-GCGTAGATTTTCGCCAAATG-3′ (*Gβ13F*-gene); Gβ76Cfwd 5′-TATCGCTGGCTTATCGCTTT-3′ and Gβ76Crev 5′-GGTTCATACTGGGCGATTTG-3′ (*Gβ76C*-gene); Gγ1fwd 5′-GCCCGTAGAGTTCGAGATTT-3′ and Gγ1rev 5′-GCAGGGATGATGACATTACG-3′ (*Gγ1*-gene); Gγ30Afwd 5′-GATCGGGACGCATTAAAGAA-3′ and Gγ30Arev 5′-ATAACGCATTTGCCCTTTTC-3′ (*Gγ30A*-gene).

The $G_{\beta 5}$ gene lacks introns, and therefore, genomic DNA due to sample contamination could not be distinguished from tissue cDNA by size. For this gene, the primers previously described (Ishimoto et al. 2005, Supplementary Material) were used (Gβ5fwd 5′-CATCTCAGAGCAACCATGTC-3′ and Gβ5rev 5′-CGTGAGGATTTGCTGATCGG-3′), and RT-PCR was performed using aliquots of the same sample with and without RT to evaluate the contribution of both genomic DNA and cDNA and to deduce the actual expression of the gene.

In all cases, PCR was carried out in a final volume of 20 μL in the presence of 1 μL of head cDNA, 1 μL of antenna cDNA, 3 μL of palp cDNA, or 1 μL of genomic DNA (used as control) and the Taq polymerase (Promega). Samples were subjected to 35 cycles of PCR. Each cycle included 30-s denaturation at 95 °C, 30-s annealing at a temperature dependent on the gene, and 1-min elongation at 72 °C. After amplification, 10 μL aliquots of each sample were analyzed by 2% agarose gel electrophoresis.

Immunohistochemistry

Drosophila heads including antennae were frozen and cut into 10-μm sections. Antennal sections were fixed and stained with primary and secondary antibodies as previously described (Larsson et al. 2004).

The following primary antibodies were used: rabbit anti-Gs/olf at 1:50 and rabbit anti-Gq/11 at 1:75 (Santa Cruz Biotechnology Inc.). Although these antibodies were generated to recognize mammal epitopes, they have been previously used in *Drosophila* (anti-Gs/olf in Ueno et al. 2006; anti-Gq/11 in Ratnaparkhi et al. 2002). We also performed western blot experiments for *Gs* and *Gq* to test if the appropriated proteins were recognized in *Drosophila* (see the Supplementary Material, Figure S1).

The other primary antibodies used in this report were generated against *Drosophila* epitopes: mouse anti-Gi at 1:250, rabbit anti-Gi at 1:100, and rabbit anti-Gβ13F at 1:250 (kindly provided by F. Yu from the Temasek Life Sciences Laboratory; J. Knöblich from the IMBA, Vienna and F. Matsuzaki from the Center for Developmental Biology RIKEN, respectively); mouse anti-Repo at 1:1000 (developed by Corey Goodman and obtained from the Developmental Studies Hybridoma Bank, established under the auspices of the National Institute of Child Health and Human Development and maintained by the University of Iowa, Department of Biological Sciences).

For secondary antibodies, goat anti-rabbit and goat anti-mouse Cyanine 3 (Jackson Immunoresearch Laboratories) and goat anti-rabbit Alexa 488 conjugates (Molecular Probes Inc., Invitrogen) were used.

Sections were analyzed by Leica confocal microscopy TCS-SP2-AOBS (Leica Microsystems). Images were processed with the Leica Confocal Software Lite.

Results

Comparative structure of G-proteins

We performed a comparison between the amino acid sequences of all the *Drosophila* G-protein subunits and their variants. These data were related to corresponding human G-protein orthologs, illustrating the relative evolutionary conservation of different subunits in the same species as compared with other strongly separated species.

The 3 main branches corresponding to each type of G-protein subunit, Gα, Gβ, and Gγ, are clearly separated in the phylogenic tree shown in Figure 1. This result was expected given that the subunits differ in size and composition. In *D. melanogaster*, Gα variants range from 350 to 450 amino acids, Gβ contains approximately 300 amino acids, and Gγ contains approximately 70 amino acids. Each subfamily of G-proteins has one representative gene in *Drosophila*; however, there are larger number of genes in mammals, with more members in each subfamily. The human genome contains more than 15 genes encoding for the Gα subunits, 5 encoding for the Gβ subunits, and 10 encoding for the Gγ subunits (Berg et al. 2002). As a rule, different variants of the same subunit within the same species displayed a lower identity level than the same variant among species.

The Gα group in *Drosophila* has the highest number of variants and each protein has up to 80% identity with their human counterparts, whereas they are only approximately 40–50% identical within the group (Parks and Wieschaus 1991). The Gf protein has no equivalent in humans, and

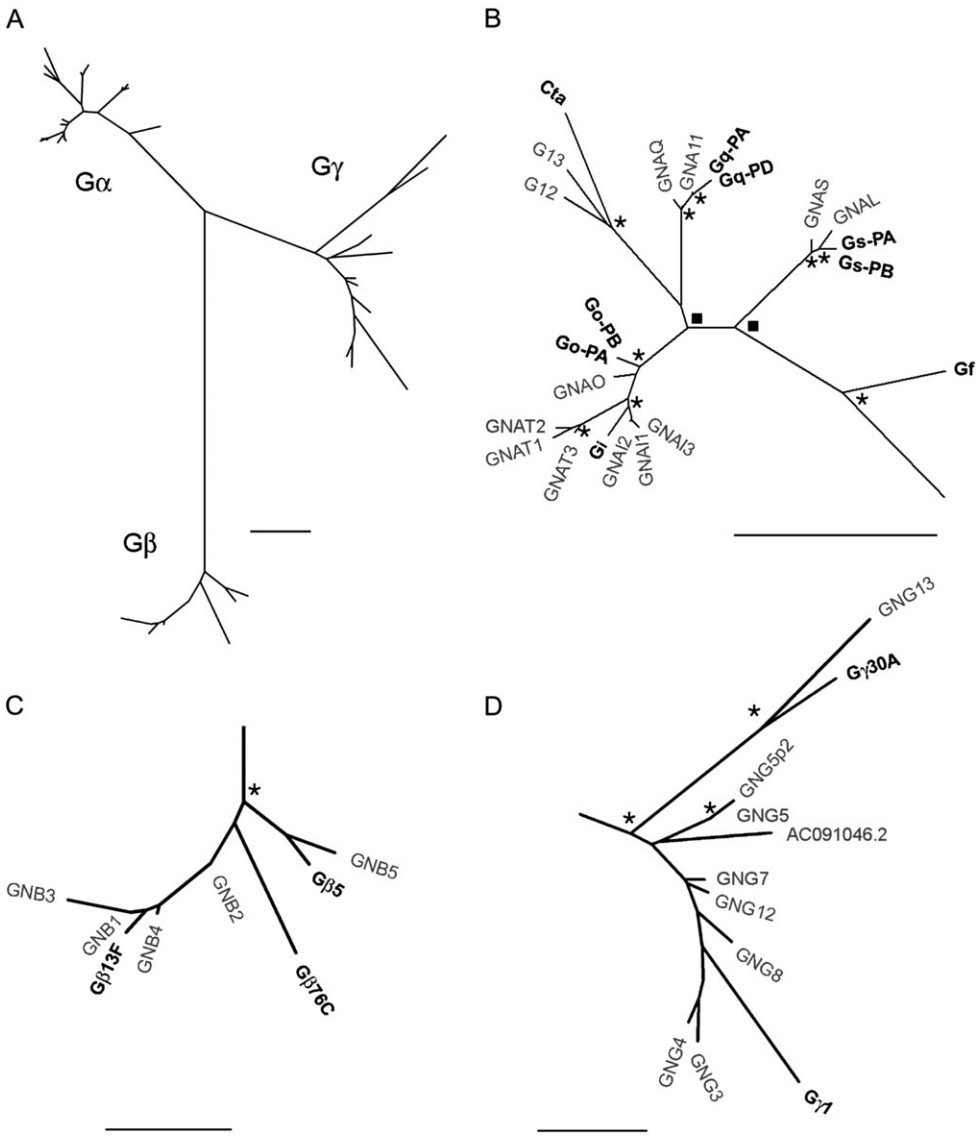

Figure 1 Neighbor joining tree of *Drosophila melanogaster* G-proteins compared with their human orthologs. Branches with bootstrap values between 70% and 100% are marked with an asterisk and can be considered to be significant. Values between 60% and 70% are marked with a square and can be considered somewhat significant. The tree is drawn to scale, with branch lengths in units of the number of amino acid substitutions per site.

although it may define an additional family, appears closely related to the Gs branch. Similarly, human transducins (GNAT1, GNAT2, and GNAT3) have no homologue in *Drosophila*, but, because they belong to the Go/i subfamily, they show a high degree of similarity to Go/i proteins in the fly.

Within the β group, Gβ13F has a higher number of related sequences, whereas Gβ76C, although similar to human proteins, is divergent.

There is a remarkable increase in the number of γ subunit family members in humans compared with *Drosophila*, in which Gγ1 appears to have many related sequences, whereas Gγ30A has just one clear homologue in humans, GNG13. Like for Gα, identity among *Drosophila* Gγ proteins (25%) is lower than the identity between correspondent orthologs in *Drosophila* and humans (31–41%).

The results confirm that the G-protein family is well conserved through evolution, as the fly proteins stay strongly linked to the corresponding sequence or sequences in humans in each of the 3 main branches. These data suggest the existence of a strong selective pressure to maintain the physiologic functions of G-proteins.

G-protein gene expression in olfactory receptor organs

A complete analysis was performed using RT-PCR to determine expression in the adult olfactory receptor organs (third antennal segment and maxillary palps) of all known *D. melanogaster* genes that encode for G-protein subunits and transcripts that translate to different protein variants. This information is intended to define those G-proteins that might be involved in olfactory reception in *Drosophila*. To assess tissue specificity, gene expression was studied in heads (deprived of antennae and palps) as well as in the primary (antennae) and secondary (maxillary palps) olfactory receptor organs. Given that G-proteins are especially abundant in nervous tissue, we used the heads as a positive expression control.

Results for the 6 genes that encode Gα subunits, *Gs*, *Gi*, *Gq*, *Go*, *Gf*, and *cta*, are shown in Figure 2A. Primers against regions common to all the different transcripts of each gene were selected. All the *Gα*-encoding genes were expressed in antennae and maxillary palps, except for *Gf*, whose expression was restricted to the antennae. Expression of *Gf* in adults was previously shown to be barely above the detection threshold (Quan et al. 1993), although we did find it in the head. Regarding *Gi* and *cta*, genomic DNA does not appear in the figure because the selected primers would amplify big fragments of 5262 and 8375 bp, respectively. This explanation also applies to other figures where no band is seen in the genomic DNA line.

According to Flybase, *Gi* and *Gf* are each transcribed into a single mRNA and encode a single protein variant, whereas *cta* is transcribed into 2 different mRNAs but also encodes a single protein. *Gs*, on the other hand, encodes 2 different

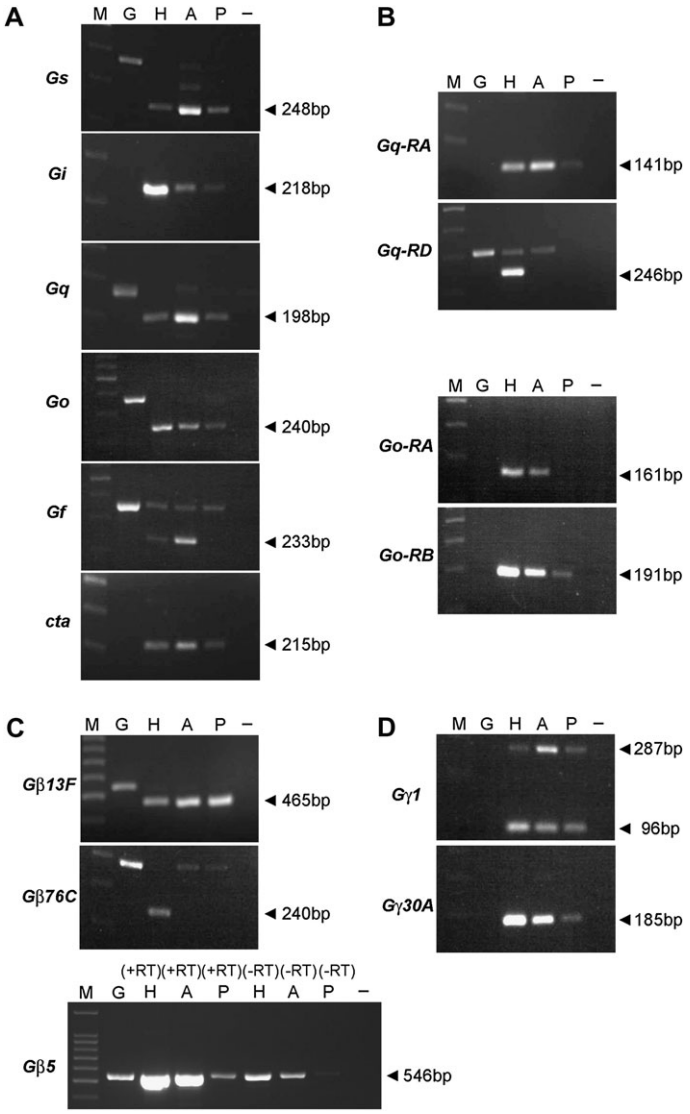

Figure 2 Expression analysis of G-protein transcripts in adult *Drosophila* olfactory tissues using RT-PCR. Target genes are denoted on the left margin and cDNA amplification size on the right margin, expressed in base pairs. Original RNA was extracted from the tissues identified at the top of each figure as follows: M: 100 bp ladder; G: genomic DNA; H: head used as control; A: third antennal segment; P: maxillary palp; –: negative control. **(A)** Gα gene expression. **(B)** Expression of Gα transcripts, including transcripts corresponding to different protein variants encoded by Gq and Go. Each label represents a group of transcripts that encode the same protein. **(C)** Expression of Gβ subunits. For *Gβ5*, (+RT) indicates amplification results from reactions with reverse transcriptase, whereas (−RT) indicates results from reactions carried out without reverse transcriptase. This comparison was used to identify genomic DNA contamination in the original sample. **(D)** Expression results for the Gγ subunit.

amino acid sequences due to alternative splicing involving a nonconsensus splice site (Quan and Forte 1990). Considering that these proteins only differ by 4 amino acids, we could not design appropriate primers to distinguish between them. Thus, only the expression pattern that includes both of the Gs variants could be analyzed in this study. *Gq* encodes 2

protein variants from 8 different mRNAs. Each group of transcripts was named according to the protein variant it is translated into (*Gq-RA* and *Gq-RD*, corresponding to Gq3 and Gq1 in previous reports; Talluri et al. 1995). *Go* also encodes 2 different amino acid sequences translated from 9 different transcripts. We named these 2 groups of transcripts *Go-RA* and *Go-RB*, respectively.

Specific expression of the different groups of transcripts is presented in Figure 2B. The protein variant encoded by the *Gq-RA* transcripts is expressed in the head as well as in the 2 olfactory receptor organs, whereas the variant encoded by the *Gq-RD* transcripts is not expressed in the olfactory receptor organs, in agreement with previous reports (Talluri et al. 1995). Differences in expression between transcripts encoding for protein variants of the *Go* gene were also found. The *Go-RA* transcripts are expressed only in the head and antennae but not in the maxillary palps, whereas the *Go-RB* transcripts are present in both olfactory receptor organs.

In Figure 2C, results for the 3 genes that encode Gβ subunits, *Gβ5*, *Gβ13F*, and *Gβ76C*, are displayed. The *Gβ5* gene is expressed in both olfactory receptor organs, as evidenced by RT-PCR amplification levels in the bands with RT clearly exceeding the corresponding ones without RT (see Materials and methods). *Gβ13F* is expressed in antennal as well as palp tissues. However, the *Gβ76C* gene is not present in olfactory receptor organs but only in head extracts.

Figure 2D shows the expression of the 2 *Gγ* genes, *Gγ1* and *Gγ30A*. Similar to *Gβ*, each gene encodes a single protein. However, because the coding region of the *Gγ1* gene lacks introns, we designed specific primers flanking the nontranslated region and the coding region, separated by an intron, in order to distinguish tissue cDNA from genomic DNA. This explains why there are 2 bands present in the head and olfactory receptor organ samples; these bands correspond to the *Gγ1-RB* transcript (287 bp) and the *Gγ1-RA*, *Gγ1-RC*, *Gγ1-RD,* and *Gγ1-RE* transcripts (96 bp), differing as a consequence of the untranslated fragment. The *Gγ30A* gene is also expressed in both olfactory receptor organs.

Table 1 summarizes the expression data for all the genes and transcript groups. Most of the subunits appear in olfactory organ extracts, except for the *Gq-RD* transcript and the *Gβ76C* gene, which have been shown to specifically mediate phototransduction processes (Dolph et al. 1994; Hardie et al. 2002). The *Gf* and *Go-RA* transcripts are expressed in the third antennal segments but not in maxillary palps.

Immunohistochemistry

Differential expression of some G-protein genes and transcripts suggests that specific G-protein subunits may play a role in ORN signaling. To characterize the expression patterns of these subunits at the cellular level, we carried out immunohistochemical studies of the Gα and Gβ proteins that show expression in antennae (Gs, Gq, Gi, and Gβ13F) by RT-PCR.

Figure 3 shows the expression of 3 Gα proteins, Gs, Gq, and Gi, in the antennae of *Elav-Gal4/UAS-GFP* and

Table 1 RT-PCR expression results of G-protein transcripts in adult *Drosophila* olfactory receptor organs

Gene (synonym)	Accession number	Map	Transcript groups	Head	Antenna	Maxillary palp
Gs	CG2835	60A12-13	*Gs-RA, Gs-RB, Gs-RC*	+	+	+
Gi	CG10060	65D5	*Gi-RA*	+	+	+
Gq	CG17759	49B8-9	***Gq-RA***, *Gq-RB*, *Gq-RC*, *Gq-RE*, *Gq-RG*, *Gq-RH*	+	+	+
			Gq-RD, *Gq-RF*	+	–	–
Go (bkh, drogpad)	CG2204	47A7-9	***Go-RA***, *Go-RC*, *Go-RI*	+	+	–
			Go-RB, *Go-RD*, *Go-RE*, *Go-RF*, *Go-RG*, *Go-RH*	+	+	+
Gf	CG12232	73B5	*Gf-RA*	+	+	–
cta	CG17678	40A-40B	*cta-RA*	+	+	+
	CG40010					
Gβ5	CG10763	7D16	*Gβ5-RA*	+	+	+
Gβ13F	CG10545	13F16	*Gβ13F-RA*, *Gβ13F-RB*, *Gβ13F-RC*, *Gβ13F-RD*, *Gβ13F-RE*, *Gβ13F-RF*	+	+	+
Gβ76C (Gβe)	CG8770	76C1	*Gβ76C-RA*	+	–	–
Gγ1 (bro4)	CG8261	44F3-5	*Gγ1-RA*, *Gγ1-RB*, *Gγ1-RC*, *Gγ1-RD*, *Gγ1-RE*	+	+	+
Gγ30A (Gγe)	CG3694	30A7	*Gγ30A-RA*, *Gγ30A-RB*, *Gγ30A-RC*	+	+	+
	CG18511					

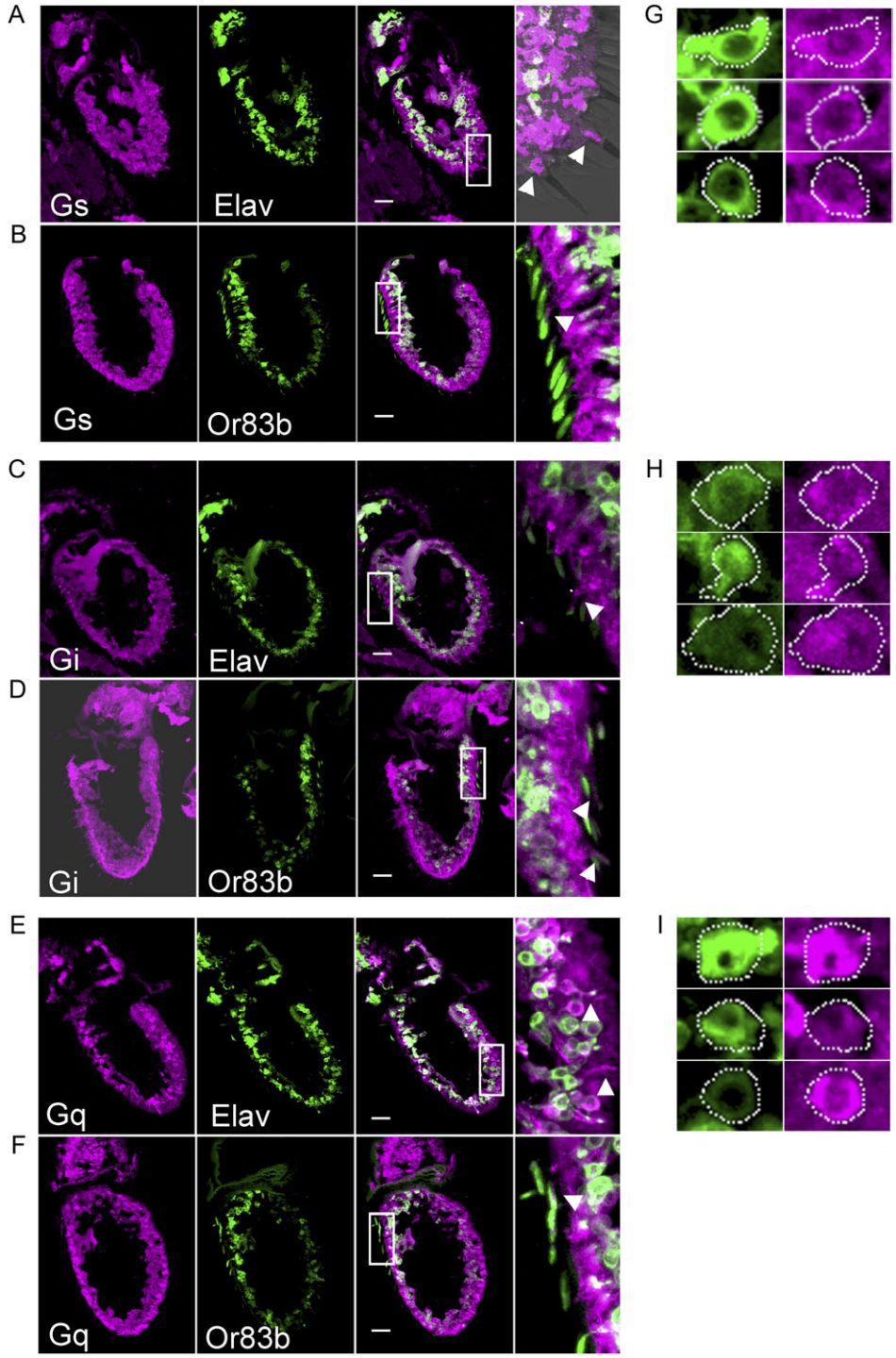

Figure 3 Immunolocalization of Gα subunits in adult antennae. Magenta staining corresponds to the Gα subunit, and green staining represent GFP signal in Elav-GFP and OR83b-Elav hybrids. White rectangles in merged images are enlarged to the right of each panel. Scale bars = 20 μm. **(A, B)** Expression of Gs in Elav- and OR83b-expressing cells, respectively. Arrowheads indicate the expression of this protein at the base of olfactory sensilla. **(C, D)** Expression of Gi in Elav- and OR83b-expressing cells. Arrowheads indicate the expression of Gi along the sensilla. **(E, F)** Expression of Gq in Elav- and Or83b-expressing cells. Arrows in E indicate the expression in dendrites of the cells. In **F**, arrows point to the expression in OR83b-expressing sensilla. (**G–I)** Detail where the outline of several cells is drawn in order to identify them, showing that they are expressing both Elav and the corresponding G-protein (G: Gs; H: Gi, and I: Gq).

OR83b-Gal4/UAS-GFP heterozygotes. In all cases, the G-protein is labeled in magenta, whereas the green staining corresponds to GFP expressed either in all neurons (as Elav is a pan-neuronal marker) or in the great majority of ORNs, where OR83b is expressed (Larsson et al. 2004). For each anti G-protein antibody, negative control slides were

prepared where no primary antibody was used. Data are shown for anti Gβ13F (Figure 4). In the other cases, the level of staining is even less marked (data not shown).

Gα-protein expression is very generalized among sections. Gs is expressed in most, if not all, neurons in the third antennal segment, as well as in the nerve bundle. Compared with the bright-field image, Gs also seems to appear in the basal segments of the sensilla. Although staining at this region may relate to transduction processes in the ORNs, at this level of resolution we cannot exclude the possibility that it could represent protein expression in support cells that also localize in this region of the sensilla (Shanbhag et al. 2000).

A very similar pattern was observed when staining was carried out with Gi antiserum, showing generalized expression in most of the cells and in the nerve bundle, with higher expression at the base of the sensilla and prolonging the staining inside the hair.

The staining of adult antennal sections with anti-Gq/11 antibody showed that the Gq protein is expressed in most cells, including neurons, neuronal processes, and nonneuronal tissue. These results are consistent with previous studies that observed Gq in ORNs (Kain et al. 2008).

We also analyzed the expression of the Gβ13F subunit (Figure 4) in the third antennal segment. It is less broadly expressed compared with the Gα subunits studied, although it was located in all Elav-expressing cells. Thus, it may be less abundant in support cells surrounding the neurons. Gβ13F is also expressed in all Or83b-positive cells and, as previously suggested for Gq, shows a high level of expression in olfactory sensilla where olfactory transduction should take place.

We observed anti-Gs immunoreactivity at the base of the olfactory sensilla and Gi, Gq, and Gβ13F expression along these sensilla, thereby suggesting that these proteins may play a role in olfactory transduction in adult *Drosophila*. Thus, previous reports on olfactory transduction proteins in *Anopheles* and *Drosophila* antenna localize arrestins at the base of the sensilla (Merril et al. 2002).

In order to identify other cell types in the third antennal segment, double-labeling experiments with anti-Repo (which marks glial nuclei) and anti-G-protein antibodies were performed. Figure 5 shows expression of Gs in glial cells. Note

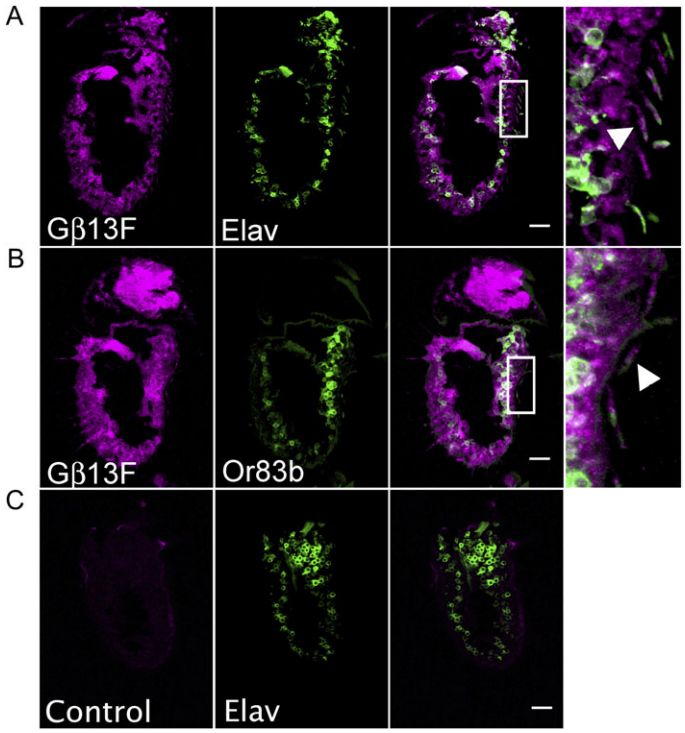

Figure 4 Immunolocalization of Gβ13F subunit in **(A)** Elav-expressing cells and **(B)** in Or83b-positive neurons in antennal sections. Magenta staining corresponds to Gβ13F expression, and green staining corresponds to the GFP signal in Elav-GFP and OR83b-Elav hybrids. White rectangles in merged images are enlarged to the right of each panel. Arrowheads indicate the presence of Gβ13F in olfactory sensilla. **(C)** Example of a negative control where no primary antibody was used. Scale bars = 20 μm.

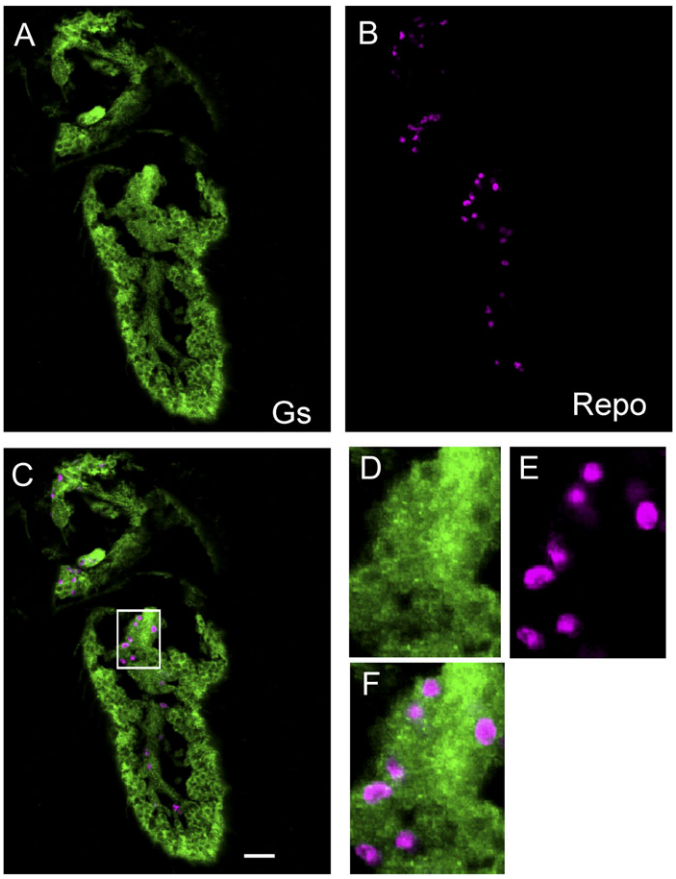

Figure 5 Localization of G-proteins in antennal glial cells. **(A)** Anti-Gs labeling is marked in green. **(B)** Glial nuclei marked with anti-Repo are shown in magenta. **(C)** Merged image where all the Repo-expressing nuclei correspond to Gs-expressing cells. White rectangle is enlarged in **D** (Gs), **E** (Repo), and **F** (merge), showing that labeled nuclei correspond to Gs-positive citoplasms. The same results were observed for Gi, Gq, and Gβ13F (data not shown). Scale bar = 20 μm.

that, in this case, no signal overlap but perfect color exclusion is expected as a sign for coincident expression in the same cell because Repo is a nuclear protein, whereas trimeric G protein subunits are expressed in cytoplasm and membrane-related regions. Here, the magenta (Repo) labeling corresponds to the nuclei of Gs-expressing cells. Similar results were found for Gq, Gi, and Gβ13F (data not shown).

The broad expression of all the G-proteins tested, which appeared in potentially all Elav and OR83b-positive neurons, suggests that several subunits are expressed in the same cell. To test this hypothesis, we carried out double-staining assays (Figure 6) and found complete coexpression of Gi with Gs and Gi with Gq. Most cells also express both Gi and Gβ13F subunits, although Gi is more intensely labeled in the periphery of the tissue, reinforcing the idea of less generalized expression of Gβ13F in the antennae of *Drosophila*.

Discussion

Different studies have previously suggested that olfactory reception, like other sensory modalities including vision and taste, is mediated by G-protein–linked signal transduction in invertebrates and vertebrates (Boekhoff et al. 1990; Ronnett and Moon 2002). However, this issue remains controversial in *Drosophila* (Kain et al. 2008; Sato et al. 2008; Smart et al. 2008; Wicher et al. 2008). Previous reports have shown specificity of expression of certain G-protein subunit variants in the visual system. The Gq-1 isoform (Lee et al. 1990) and the Gβ76C subunit (Yarfitz et al. 1991) showed specific expression in photoreceptor cells, pointing to their role in phototransduction. By systematically studying the expression pattern of all known variants of each G-protein subunit, we examined whether specific G-proteins may play specific roles in olfaction.

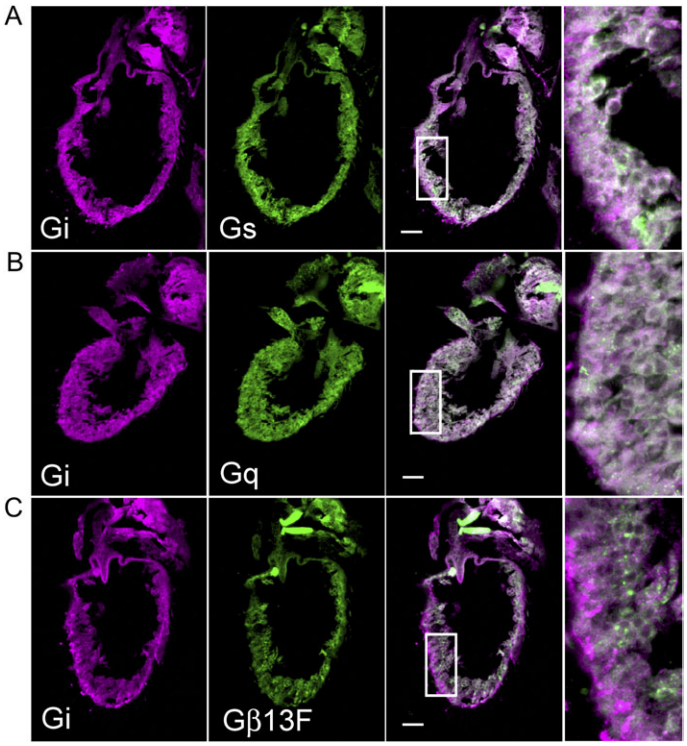

Figure 6 Double immunostaining of different G-protein subunits in third antennal segment. In all cases, Gi is shown in magenta, whereas **A**: Gs, **B**: Gq, and **C**: Gβ13F labeling appear in green. White color in merged images indicates complete colocalization, and sections in white rectangles are enlarged on the right. Scale bar = 20 μm.

Our results do not support the hypothesis of specific expression in ORNs because we found a generalized pattern for almost all the Gα, Gβ, and Gγ genes. Only some of the variants described as photoreceptor cell-specific were absent in both olfactory receptor organs. A Gα subunit, the *Gq-RD* variant, and a *Gβ* gene encoding the Gβ76C protein were not present in olfactory tissue; however, the *Gγ30C* gene that mediates phototransduction (Schulz et al. 1999) was present in olfactory receptor organs.

Although specific gene expression in certain cellular types could be indicative of their role in these cells, the converse cannot be directly concluded. It has been reported that many mammalian signaling molecules are restricted in their placement within the plasma membrane to "raft domains" that include GPCRs, G proteins and effectors, including ion channels and adenylate cyclases (Cooper 2005). Cyclic nucleotide signaling is dependent on compartmentalization (Baillie 2009), observed locations of cAMP and cyclic guanosine monophosphate are discrete, suggesting the existence of microdomains of action. If cAMP increases were not compartmentalized and its concentration increased in a uniform manner throughout the cell, all pools of cAMP effectors would be activated, leading to perturbations of normal cell function.

If the compartmentalization of transduction pathway components plays a role in olfactory signaling in *Drosophila*, we would expect to find G protein subunits localized in olfactory neuron dendrites rather than in cellular locations involved in other processes. This matches our results, as we are able to localize Gi, Gs, Gq, and Gβ13F in these regions.

Some expression differences in antennae and maxillary palps were observed, with the maxillary palps showing a more restricted pattern of expression. These differences could be due to the larger size of the antennae, so that greater amounts of rare mRNA could be more easily extracted, but also due to the more complex composition of the antennae, which displays a larger repertoire of cell types. For example, the antennae of *Drosophila* harbor 3 types of sensilla, basiconic, trichoidea, and coeloconic, compared with the maxillary palps, which only contain basiconic sensilla. At the cellular level, the antennae also contain more ORN types (more than 40 in antennae compared with around 10 in maxillary palps, Laissue and Vosshall 2008). Differential expression between antennae and palps were observed for 2 Gα-encoding genes, the *Go-RA* variant and *Gf*. In fact, *Gf* was previously believed to have a developmental role with almost no expression in the adult stage (Quan

et al. 1993). However, our data did not agree with such an observation, as we found gene expression in the head and antennae.

We also analyzed G-protein expression specificity in the ORNs of the antennae using immunohistochemistry against several Gα and Gβ subunits. Our results showed a lack of expression specificity in the olfactory receptor organs, compared with other sensory organs, at the cellular level. Thus, although Gs, Gi, Gq, and Gβ13F proteins are expressed in ORNs containing the Or83b receptor and other antennal neurons, they also appeared in glial cells and other tissues. Gs, Gi, Gq were also likely expressed in the support cells of the sensilla.

The generalized expression patterns of G-protein subunits in the antennae, expressed in both olfactory and nonolfactory cells, probably reflect the central role of G-protein–mediated transduction in many cellular processes (Svoboda et al. 2004). This idea is also consistent with their high level of conservation in evolution, from *Drosophila* to humans, which was portrayed in the phylogenetic tree in Figure 1.

Coexpression studies for several G-protein variants in the antennae revealed that the same cells express the Gs, Gi, Gq, and Gβ13F proteins. Coexpression of different G-protein subunits in ORNs has previously been described in invertebrates. Gq and Gs have been found in the trichoid sensilla of *Bombyx mori* (Miura et al. 2005), and both Gα subunits are expressed in all ORNs in the lobster (McClintock et al. 1997; Xu et al. 1997). However, as indicated previously, similar cellular location does not necessarily mean that the proteins are involved in the same cellular function. Thus, the subcellular localization may provide important information. In our study, the location of some variants (such as Gi, Gq, and Gβ13F) in the base and within the sensillum could indicate a role in olfactory transduction because these regions correspond to the location of the ORN dendrites.

Our results also showed that different G protein subunits like Gs and Gi, which interact differently with transduction cascades, colocalize at the base of the sensilla. Because Gs and Gi trigger the cAMP transduction cascade, increasing or decreasing cAMP, respectively, our results are compatible with differing responses within the same ORN. In fact, it has been previously reported that a single ORN may display an excitatory or inhibitory response depending on the odorant (de Bruyne et al. 2001). In addition, coexpression of Gs and Gq, mediators of the cAMP and IP3 pathways, respectively, is compatible with a previous observation in rats that both transduction cascades function in the same ORNs, with possible cross talk between them (Vogl et al. 2000).

However, based upon our data, we cannot exclude the possibility that G-proteins are not directly involved in olfactory reception. Only functional studies could provide the final evidence for an active role of G-proteins in olfaction. Previous reports have suggested a role for cAMP (Martin et al. 2001; Gomez-Diaz et al. 2004) and IP3 (Gomez-Diaz et al. 2006) signaling in olfaction using functional studies in mutants of other transduction-related genes. However, completing the functional studies in vivo in G-protein mutants, a process that has been already initiated with *Gq* (Kalidas and Smith 2002; Kain et al. 2008), will be crucial to establishing the actual role of each G-protein subunit and variant in olfactory function.

Supplementary material

Supplementary material can be found at http://www.chemse.oxfordjournals.org/

Funding

This work was supported by the Spanish Ministry of Education and Science [Biologia Fundamental BFU2005-04525 and BFU2008-1256]. T.B. is a Formacion de Profesorado Universitario fellow of the Spanish Ministry of Education and Science and C.G-D. is a predoctoral fellow of the Plan de Ciencia, Tecnologia e Innovacion Program of the Principado de Asturias.

Acknowledgements

We thank Y. Borrel and F. Gomez-Romano for technical help in the construction of the phylogenetic tree. J. Knoblich, F. Yu, and F. Matsuzaki kindly provided the anti-Gi and anti-Gβ13F antibodies.

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