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G_α Encoding Gene Family of the Malaria Vector Mosquito *Anopheles gambiae*: Expression Analysis and Immunolocalization of AG_{αq} and AG_{αo} in Female Antennae

Michael Rützler, Tan Lu, and Laurence J. Zwiebel*

Department of Biological Sciences, Program in Developmental Biology, Centers for Molecular Neuroscience and Chemical Biology, Institute for Global Health, Vanderbilt University, Nashville, Tennessee 37235

Abstract

To initiate a comprehensive investigation of chemosensory signal transduction downstream of odorant receptors, we identified and characterized the complete set of genes that encode G-protein α subunits in the genome of the malaria vector mosquito *An. gambiae*. Data are provided on the tissue-specific expression patterns of 10 corresponding *aga*-transcripts in adult mosquitoes and pre-imago developmental stages. Specific immunoreactivity in chemosensory hairs of female antennae provides evidence in support of the participation of a subset of AG_{αq} isoforms in olfactory signal transduction in this mosquito. In contrast, AG_{αo} is localized along the flagellar axon bundle but is absent from chemosensory sensilla, which suggests that this G-protein α subunit does not participate in olfactory signal transduction.

Indexing terms

RT-PCR; olfaction; insect; G-protein

Olfaction plays a critical role in modulating a wide range of behaviors across all insect taxa (for review, see Dahanukar et al., 2005; Rützler and Zwiebel, 2005). Indeed, chemosensory-driven behaviors have profound effects on the capabilities of many insects to transmit a range of human and animal pathogens (reviewed by (Zwiebel and Takken, 2004). Following the identification of genes encoding candidate insect chemoreceptors in the *Drosophila melanogaster* genome (Clyne et al., 1999; Gao and Chess, 1999; Vosshall et al., 1999), several homologs have been identified in a number of nonmodel insects, including the malaria vector mosquito *Anopheles gambiae* (Fox et al., 2001; Hill et al., 2002). More recently, studies in *D. melanogaster* and other systems have facilitated functional characterization of members of this gene family (Chyb et al., 2003; Dobritsa et al., 2003; Hallem et al., 2004a,b; Sakurai et al., 2004; Goldman et al., 2005; Kreher et al., 2005; Nakagawa et al., 2005; Neuhaus et al., 2005). In a similar fashion to odorant receptors (ORs) in vertebrates and nematodes, insect ORs are thought to function by affecting the guanosine nucleotide exchange rate of heterotrimeric guanosine 5'-triphosphate (GTP) binding (G) proteins (Jones and Reed, 1989; Zwaal et al., 1997), although the precise mechanisms of

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*Correspondence to: L.J. Zwiebel, Department of Biological Sciences, Program in Developmental Biology, Centers for Molecular Neuroscience and Chemical Biology, Institute for Global Health, VU Station B, Box 35–1634, Vanderbilt University, Nashville, TN 37235. l.zwiebel@vanderbilt.edu.

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peripheral olfactory signal transduction remain unclear (for review, see Krieger and Breer, 1999; Rützler and Zwiebel, 2005). In one study using the cockroach, exposure of antennal preparations to pheromone resulted in a rapid increase in inositol-1,4,5-trisphosphate (IP₃) levels (Breer et al., 1990). This effect can be inhibited by pertussis toxin, indicating that it is likely to be dependent on either a G_{ai} or G_{ao} heterotrimeric G-protein subunit (Boekhoff et al., 1990). Moreover, the localization of G_{aq}-proteins to the dendritic membrane of *Bombyx mori* olfactory receptor neurons further implicates these G-proteins in the generation of second-messenger signals in response to OR-odorant binding (Laue et al., 1997). Both reports suggest that at least some aspects of insect olfactory signal transduction involve phospholipase C (PLC); indeed, several mutant alleles of *norpA*, encoding a PLC enzyme, display reduced electrophysiological responses to odorant stimuli in *D. melanogaster* maxillary palpi (Riesgo-Escovar et al., 1995).

It is now well accepted that individual G-protein-coupled receptors (GPCRs), in a given agonist or antagonist bound state, may display specific efficacies for the activation of individual signal transduction pathways depending on their cellular environment (for review, see Kenakin, 2003). It follows that the nature and stoichiometry of signal transduction components (e.g., G-proteins) present in a cell will directly influence the observations made whenever receptors are functionally characterized. This concept may be especially important for the study of ORs in heterologous expression systems because they often elicit significant responses to a broad range of chemical stimuli. Heterologous expression inevitably inserts ORs into a nonnative signal transduction environment. A recent study overcame this obstacle by coexpressing components of the native signal transduction machinery, particularly G_{ao}, along with vertebrate ORs (Shirokova et al., 2004). In this case, OR function in HeLa cells was similar to that previously observed in olfactory epithelium. In order to develop a reliable heterologous expression system for the functional characterization of *An. gambiae* odorant receptors (AgORs), it is therefore necessary to gain a better understanding of the signal transduction molecules that are present in *An. gambiae* chemosensory organs and, more specifically, in olfactory receptor neurons (ORNs).

We identified the complete set of G_α-genes in the *An. gambiae* genome and characterized the expression of their transcripts in several adult tissues as well as during preadult stages. In addition, the cellular localizations of AG_{ao}, AG_{aq2}, and AG_{aq3} were determined within the brain and the olfactory apparatus in female adults.

MATERIALS AND METHODS

Bioinformatics

Drosophila G_α-proteins (GenBank identifiers 121029: G_{as}, 120973 and 24652446: G_{ao}, 120999: *concertina*, 17137790: G_{ai}, 585177: G_{af}, 417034 and 944924: G_{aq}) were used in BLAST (Altschul et al., 1990) searches against the *An. gambiae* genome and annotated proteome (<http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/agambiae.html>) to identify potential *An. gambiae* G_α (AG_α)-proteins and corresponding *aga*- transcripts. Potential coding regions were refined with respect to homology and consensus exon-intron splice sites as well as expressed sequence tag (EST) information that has been annotated at The Institute for Genomic Research (TIGR). The following sequences were determined in the course of this study and submitted to GenBank: AY724801 (*agoa*), AY724802 (*agon*), AY724803 (*agq1*), AY724804 (*agq2*), AY724805 (*agq3*), AY724806 (*agq4*), AY724807 (*agq* 381 basepair (bp) fragment), AY724808 (*agq* 618bp fragment), DQ182013 (*agc*), DQ182014 (*agm*), DQ182015 (*agq2* full-length open reading frame (ORF)), DQ182016 (*agi* full-length ORF), and DQ182017 (*ags* full-length ORF). These sequences established alternative splicing in the case of *ago* and *agq* transcripts. Suggestions for updating all of the G_α sequences, based on our refinements, were communicated to the GenBank RefSeq Database.

Bootstrap analysis was carried out in ClustalX v1.62b (Swofford, 1998) with a gap open penalty of 50 for the initial sequence alignment. A neighbor-joining tree was calculated in 1,000 bootstrap trials, in which gaps in the sequence alignment were disregarded. The tree was plotted utilizing NJ-plot software (M. Gouy, Univ. Lyon, France) whereby the *Saccharomyces cerevisiae* G α -protein of the pheromone response pathway Gpa1p was introduced as an out-group.

Reverse transcribed-polymerase chain reaction

Cold-anesthetized mosquitoes (*An. gambiae* s.s., G3 strain) were dissected over dry ice and the following amounts of tissues were collected to generate cDNA: antennae, maxillary palpi, and proboscides from 50 mosquitoes; five heads minus appendages; two bodies including wings but without heads and legs; and 60 legs. In addition, the following amounts of tissues for expression analysis in various developmental stages were collected from 1) four sexually mature females prior to and 20 hours after a blood meal: heads including appendages, thoraces including wings and legs, and abdomens; 2) 60–80 embryos collected from 24–48 hours after a blood meal; 3) 10–15 early larvae (1 week old); 4) 8–10 late larvae (2 weeks old); and 5) five to six pupae.

The material was broken with a disposable pestle in buffer RLT (RNeasy, Qiagen, Chatsworth, CA) and RNA was isolated following the RNeasy procedure according to the manufacturer's guidelines. One-fifth of each RNA sample was used for first-strand synthesis with Superscript II reverse transcriptase (Invitrogen, La Jolla, CA) and, subsequently, as a template in each set of polymerase chain reactions (PCRs) with the following primers, which were tested in all their gene-specific combinations (target in parentheses): AgGAsfw 5'-ACGGCGTGCTCTAGTTA-TAATAT-3' and AgGAsrev 5'-GTCGCAGATGCATCCG-CT-3' (*ags*-gene); GmF2 5'-GGTCGAAGCTGGTGGACAT-3' and AgGq/srev 5'-CTTGGAAGGTTTTTCGTCA-3' (*agm*-gene); AgconF3 5'-TGAGTTTACGATAAGGATACAGAACA-3' and AgcocR2 5'-CGAACGTGGTATTGTTTACG-3' (*agc*-gene); AgGA01altNfwd 5'-TCGCCCGGTGCAAGCA-3' (*ago*-gene exon 1N) and AgGA01rev 5'-ATACTCGTTGCTGCGGCA-3' (*ago*-gene exon D) and AgGA01anotfwd 5'-CCGCTAGGA-GCCGCCT-3' (*ago*-gene exon 1A, Fig. 2A); AgG05fwd 5'-CACTCTCGGGATACGATCT-3' and AgG05rev 5'-ATT-CTTGATGATCACATCCGA-3' (*agi*-gene); AgAqex4altfwd 5'-ATTGATCTGGATAGCATCAT-3' (*agq*-gene exon D*) and AgAqex4altrev 5'-CTCCTTTAGGTTGGACTGAAG-T-3' (*agq*-gene exon H*) and AgAqex8altfwd 5'-TCCCTT-CGATCTGGAGGAA-3' (*agq*-gene exon D) and AgAqex8altrev 5'-TTCTTTCAGAGCAGTTTGCATA-3' (*agq*-gene exon H, Fig. 2B). 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. To control for cDNA quality, the ribosomal protein S7 (*rpS7*) (Salazar et al., 1993) encoding transcript was amplified utilizing the primers Rps7.5' 5'-AGAACCAGCAGACCACCATC-3' and Rps7.3' 5'-GTTCTCTGGGAATTCGAACG-3'. All reverse-transcribed (RT)-PCR results are shown for 34 PCR cycles.

Real-time quantitative RT-PCR

Antennae from 51, 42, and 47 female mosquitoes were collected at zeitgeber 0.5, 7, and 12, respectively. The mosquito colony was maintained at a regular 12:12-hour light:dark cycle at 27°C and 70% relative humidity. The mosquito-rearing protocol was approved by the institutional Animal Care and Use Committee. RNA was prepared as described above. For cDNA synthesis, one-third of the RNA was utilized as above and diluted in buffer EB (Qiagen) to a final volume of 70 μ L prior to real-time PCR, 3 μ L of which was used as template in a 10- μ L PCR reaction with QuantiTect SYBR Green PCR Kit (Qiagen) and 3

pmol of each primer. Each experimental value was determined in duplicate and compared with a standard dilution of linearized target DNA. Furthermore, a no-reverse-transcriptase sample was included in every experiment and additional experimental samples were included occasionally that were spiked with known amounts of target DNA to control for the presence of PCR inhibitors. PCR reactions were carried out in a Roche (Indianapolis, IN) LightCycler, and products were analyzed by means of agarose gel electrophoresis. At least one band for each primer pair was cloned and sequenced to confirm primer specificity. Utilizing Primer3 software (Rozen and Skaletsky, 2000), primers for real-time PCR were designed to span presumptive or previously determined exon–exon boundaries to avoid amplification of genomic DNA. Primers for real-time PCR were as follows: Gq4.1/5 5'-GA-GGAAATTCGGTTTAGGATGG-3' and Gq7.1/8.1 5'-CG-TATATTTTCGGTGTCTGTCTG-3' (*agq2*-transcript) Gq4.2/5 5'-ATCTGGATAGCATCATCTTTAGGA-3' and Gq7.1/8.1 (*agq3*-transcript) GmF2 and AgGq/srev (*agm*-transcript) AgconcF3 and AgconcR2 (*agc*-transcript) AGsF2 5'-GTA-TTTGGAATAATCGATGGTTACGG-3' and AGsR2 5'-ACT-CGCTGTCGATATACGCAA-3' (*ags*-transcript) AgG05fwd and AgG05rev (*agi*-transcript) Go706F 5'-CACGA-AGATGAAACCACGAA-3' and Go892R 5'-CGTACTC-CTGTCCACCCGTAT-3' (*ago*-transcripts) Rps7F3 5'-TTCAACAACAAGAAGGCGATCAT-3' and Rps7R3 5'-CGCAAAGTGTCAACTTTGTGTTC-3' (*rpS7*-transcript).

Immunoblots, immunolabeling, and confocal microscopy

For immunoblots, mosquito heads of mixed-sex cold-anesthetized animals were dissected and transferred into 300 μ L buffer (125 mM Tris pH 6.8, 2 mM ethylenediaminetetraacetic acid (EDTA), 0.1% sodium dodecyl sulfate (SDS), 0.5% 2-mercaptoethanol). Following homogenization with a disposable pestle, 40 μ L 1M dithiothreitol (DTT) and 100 μ L loading buffer (250 mM Tris pH 6.8, 4 mM EDTA, 0.2% SDS, 50% glycerol, 0.1% bromophenol blue) were added, prior to incubation at 55°C for 15 minutes and electrophoresis using 10% polyacrylamide gels under denaturing conditions with SDS (Laemmli, 1970). Protein was transferred to polyvinylidene fluoride membrane prior to immunolabeling with the following dilutions of antisera (Table 1): rabbit anti-Gq/11 (1:20000), rabbit anti-DGq1 (1:2000), and guinea pig anti-Go (1:20000). Secondary antibodies and dilutions utilized were: antirabbit horseradish peroxidase (HRP) conjugate (1:20000; Pierce Biotechnology, Rockford, IL) and antiguinea pig HRP conjugate (1:100000; Sigma, St. Louis, MO), respectively. For preadsorption experiments, primary antibodies were diluted in 5% nonfat dry milk / phosphate-buffered saline (PBS) / 0.05% Tween20 along with a ~100-fold molar excess of the Gq/11 cognate peptide compared to anti-Gq/11 serum or anti-DGq1 serum and 1 μ g of recombinant rat $G_{\alpha o}$ (Calbiochem, La Jolla, CA) per ~10 ng of anti-Go serum, respectively, and incubated for 30–60 minutes on ice, prior to application to immunoblot membranes. All cognate and presumptive heterologous antigens are listed in Table 1. Immunolabeling was visualized with SuperSignal West Femto Maximum Sensitivity Substrate (Pierce Biotechnology) and subsequent exposure to X-ray film.

Heads from female *An. gambiae* were fixed in 4% paraformaldehyde (PFA) / PBS / 0.01% Triton X-100 (T) for 30 minutes at 4°C, followed by two 5-minute washes in PBS and overnight incubation in 25% sucrose/PBS/0.01%T. Cryo-sections of 15 μ m were obtained after embedding the heads in Tissue-Tek O.C.T (Sakura, Torrance, CA) on a Leica CM1900 Cryostat. Sections were then dried for 1 hour prior to fixation for 30 minutes in 4% PFA/ PBS and two subsequent washes in PBS for 5 minutes each. Slides were dried again; areas containing sections were encircled with a PAP PEN hydrophobic marker (Research Products International, Mt. Prospect, IL). Then slides were incubated in blocking solution (5% normal

goat serum/PBS/ 0.3%T) for 30 minutes. Primary and secondary antibodies were each applied overnight at 4°C, followed by three 5-minute washes in PBS 0.1%T. For preadsorption controls, anti-Gq/11 serum was diluted in blocking solution and incubated on ice for 30–60 minutes along with a ~ 100-fold molar excess of its cognate peptide and anti-Go serum was similarly preadsorbed in the presence of 1 µg rat G_{αo} per 40 ng of antiserum. Anti-horseradish peroxidase serum Fluorescein (1:100) or Cyanine 3 (1:800) conjugates (Jackson ImmunoResearch, West Grove, PA) were applied along with each secondary antibody at the indicated dilutions. Toto-3 nuclear stain (Molecular Probes Eugene, OR) was applied at a 1:2000 dilution in PBS for 30 minutes, followed by a short wash in PBS, immediately prior to mounting of slides in Vectashield (Vector Laboratories, Burlingame, CA) and fluorescence microscopy.

Sources and antigens of primary antisera are listed in Table 1. The following dilutions of each serum were utilized: rabbit anti-Gq/11 (1:300), guinea pig anti-Go (1:200), rabbit anti-DGq1 (1:100). Secondary antibodies utilized were goat antirabbit Cyanine 3 conjugate (1:800) and donkey antiguinea pig Cyanine 2 conjugate (1:200) (Jackson ImmunoResearch). Confocal images were captured with a Zeiss Axioplan fluorescent microscope using the LSM 510 META system. Confocal stacks were projected along the y-axis utilizing Zeiss LSM Image examiner software. For presentation, images were arranged in Adobe Photoshop (San Jose, CA), without alteration of color intensity or contrast.

In situ hybridizations

Probes for in situ hybridization were amplified from *An. gambiae* head cDNA samples utilizing the following PCR primers: AgGA01anotfwd/Go892R (*ago*; described above) and TANS6-F 5'-CGATGATACGAGCGATGGAT-3' and TANS6-R 5'-TATCCTTTACGGCGCAGAAC-3' (*agq1*). PCR products were ligated to pCRII-Topo (Invitrogen) and clones for both insert orientations were selected. Digoxigenin-labeled RNA probes were generated for sense and antisense orientations utilizing SP6 (*agq1*) and T7 (*ago*) RNA polymerase, respectively. A detailed description of the tissue embedding, RNA hybridization, and visualization protocol are provided elsewhere (Kwon et al., submitted). Briefly, Digoxigenin RNA probes were hybridized to 8 µm paraffin sections at 65°C (*agq1*) and 60°C (*ago*) for 16 hours. Colorimetric detection of probes was achieved utilizing Fast Red substrate (Roche). All images were captured and processed using confocal microscopy as described above.

RESULTS

Identification of G_α-encoding sequences in the *An. gambiae* genome

In order to identify *An. gambiae* sequences that potentially encode α-subunits of heterotrimeric G-proteins, we used *D. melanogaster* G_α-protein sequences as initial queries in BLAST-searches (Altschul et al., 1990) to screen 1) the whole *An. gambiae* genome and 2) open reading frames that have been annotated in the course of the *An. gambiae* genome project. This approach revealed six candidate G_α-encoding genes. Two of these, which potentially encode G_{αo}- and G_{αq}-protein homologs, encompass significant stretches of similar sequences. Since many G_α-proteins share a specific primary structure (Conklin and Bourne, 1993), it is possible that these stretches of sequence similarity could encode alternatively spliced exons. To test this hypothesis, we designed oligonucleotide primers for PCR-based expression analyses of potential G_α-encoding transcripts that facilitate discrimination of alternatively spliced transcripts in the amplified region. In addition, we determined the putative coding regions for AG_{αq2}, AG_{αs}, and AG_{αi}, which have been submitted to GenBank along with partial sequences obtained from the expression analysis (see Materials and Methods). Phylogenetic grouping revealed that each *D. melanogaster* G_α-

protein has at least one clearly defined homolog in *An. gambiae* (Fig. 1). It is noteworthy that the *Drosophila concertina* protein (Parks and Wieschaus, 1991) has a 70–80 amino acid (aa) elongated amino terminus when compared with that of the presumptive mosquito protein AG_{ac} (whose amino terminus is supported by analysis of EST clones BM586532 and BM609491) and its closest mammalian homologs G_{α12/13} (Strathmann and Simon, 1991).

Tissue-specific expression of *Anopheles* G_α-transcripts

In order to define G_α-proteins that might be involved in *An. gambiae* olfactory signal transduction, we investigated expression of the presumptive G_α-genes in adult tissues with established olfactory function (antennae, combined maxillary palpi and proboscides) (for review, see Clements, 1999) as well as in tissues with potential cryptic olfactory sensing (legs) (Fox et al., 2001) and in those (heads and carcasses) with no evidence of any role in olfaction (Fig. 3A). Furthermore, we also examined the pre-imago expression patterns of *ago* transcripts in developmental stages (Fig. 3C) to investigate the significance of transcripts that were only moderately expressed in adult tissues. The results of these studies are summarized in Table 2.

Transcripts derived from each of the identified G_α-genes, including the multiple transcripts derived from the genes encoding G_{αo} and G_{αq}, were identified and characterized. Of these, *ago* was transcribed into two (*agon* and *agoa*) and *agq* into at least four (*agq1-4*) mRNAs (schematized in Fig. 2A,B). This is similar to data from *D. melanogaster*, where the apparent *ago* homolog *G-oo47A* encodes two distinct G_{αo}-proteins (de Sousa et al., 1989; Yoon et al., 1989). In *An. gambiae*, the 5' coding exon of the *ago* gene is situated ~23 kb upstream of the next coding exon, thus creating a genomic locus spanning more than 56 kb (Fig. 2A). In order to carry out our expression analyses, oligonucleotide primers were designed to distinguish between the two expected *ago* transcripts (Fig. 2A). In these studies, both *ago* transcripts were barely detectable in adults (Fig. 3A), yet were consistently amplified from larvae and pupae (Fig. 3C). DNA sequence analyses of the RT-PCR products confirmed the expression of two alternatively spliced transcripts. In addition, both *ago* transcripts contained a sequence stretch that was not sequenced as part of the *An. gambiae* genome project (Holt et al., 2002). When the novel sequence of the RT-PCR fragments was combined with the hypothetical cDNA sequence deduced from the mosquito genome, the corresponding protein sequences for AG_{αoA} and AG_{αoN} shared high sequence identity (>94%) with the two identified *D. melanogaster* G_{αo}-homologs (Fig. 1) (de Sousa et al., 1989; Yoon et al., 1989).

At least four transcripts originating from the *agq* locus were identified (*agq1-4*), equaling the number of *dgq* transcripts derived from *Gα49B* described in *D. melanogaster* (Lee et al., 1990; Talluri et al., 1995; Ratnaparkhi et al., 2002). Of these, *agq1* expression was limited to adult heads (Fig. 3A), larvae, and pupae (Fig. 3C). Its intron/exon configuration (Fig. 2B) closely resembles that of the *Drosophila dgq1* transcript, which encodes a G_α-protein that is functional in phototransduction (Lee et al., 1994; Scott et al., 1995). A composite of EST clones consistent with this *agq*-isoform has been annotated at TIGR (TC40117). A second transcript, *agq2*, was expressed in all adult tissues that were assayed (Fig. 3A), as well as throughout preadult development (Fig. 3C). Its exon configuration and expression pattern resembled the *Drosophila dgq3* transcript (Ratnaparkhi et al., 2002). Two additional transcripts, *agq3* and *agq4* (Fig. 3A), have no corresponding isoforms in *D. melanogaster*, yet both yielded conceptual proteins that include all elements of a composite α-chain (Conklin and Bourne, 1993). In adults the *agq3* transcript fragment was amplified predominantly from bodies, but was also (see below and Fig. 3A) detected inconsistently and very weakly from olfactory tissues (Fig. 3A). *agq4* was only appreciable at low levels in heads and bodies (Fig. 3A). Interestingly, both transcripts showed robust preimago expression (Fig. 3C), suggesting an important role for *agq3* and *agq4* during those stages.

Furthermore, a moderate increase in PCR products derived from adult female heads and abdomens was consistently observed for several of the studied genes after a blood meal. This effect was most prominent for *agq3* and *agq4* (Fig. 3C) in female abdomens and may indicate maternal contribution of these transcripts to embryogenesis or a function in oogenesis. Two additional bands of unexpected size were amplified while characterizing *agq4* (Fig. 3A,C, asterisks). DNA sequence analysis revealed that the smaller product of 381 bp lacked a G or G* exon, while the larger product of 618 bp contained both the D and D* exon. A similar transcript that also lacks a G-type exon was identified in *D. melanogaster* and termed *dgq2* (Lee et al., 1990). No protein corresponding to *dgq2* was detected in wildtype flies (Scott et al., 1995). Furthermore, ectopic expression of *dgq2* in photoreceptor cells resulted in detectable protein formation, but did not affect phototransduction (Scott et al., 1995). The D and D* exons in the 618-bp *agq* product have the potential to encode two sequential homologous peptides of 42 amino acids at the carboxyl terminal end of insert region 1 of the composite α -chain (Conklin and Bourne, 1993). This region contains a part of the catalytic pocket of the GTPase-activating domain that is highly conserved in all known G_{α} -proteins (Markby et al., 1993). It therefore seems likely that the 618-bp fragment represents a premature transcript.

The genomic organization of $G_{\alpha i}$ (*agi*) and $G_{\alpha s}$ (*ags*) homologs suggests that these genes encode single proteins. This conclusion was supported by sequence analysis of multiple cDNA clones that encode identical putative full-length proteins. Both *agi* and *ags* were expressed in all investigated tissues (Fig. 3A,C). In *Drosophila*, two transcripts are derived from the $G_{\alpha s}$ -gene *G-sa60A*, $G_{\alpha s}S$ and $G_{\alpha s}L$; they arise from alternative splicing involving a nonconsensus splice site and differ by only 4 aa (Quan and Forte, 1990). PCR primers were designed to assess whether a similar alternative splicing event occurs in the *ags* gene product. Sequence analysis of a total of 11 independent RT-PCR products representing all investigated tissues did not indicate multiple *ags*-transcripts.

Similarly, *agm* and *agc*, homologs of the *D. melanogaster* *dgf* or *Ga73B* (Quan et al., 1993) and *cta* (Parks and Wieschaus, 1991) genes, respectively, likely encode single transcripts. The overall sequence identity (50.5%) between the conceptual $AG_{\alpha m}$ -protein and the $DG_{\alpha f}$ -protein is remarkably low for two G_{α} -proteins of presumably the same subtype (Fig. 1). In *Drosophila*, *dgf* expression is predominantly observed in embryonic, larval, and early pupal stages and in adults is confined to ovarian nurse cells and developing oocytes (Quan et al., 1993). Our RT-PCR-based analysis suggests a similar expression pattern for the *agm* gene, which was barely detectable in mixed-sex adult bodies (Fig. 3A). In further analyses of preimago stages, which included female adult tissues to study possible expression of maternal effect genes, we found *agm* expression in adult abdomens as well as in embryos, larvae, and pupae (Fig. 3C). In *Drosophila*, *cta* is a maternal effect gene that is ubiquitously present in the precellularized embryo (Parks and Wieschaus, 1991). Expression of *agc* in *An. gambiae* was readily detectable in all investigated adult tissues (Fig. 3A) and throughout preimago stages (Fig. 3C).

Quantification of *aga* transcripts in female antennae

To gain additional information about expression levels of individual *aga* transcripts in an olfactory organ, the relative levels of *agq2*, *agq3*, *ags*, *agi*, *ago*, and *agc* in female antennae were quantified by means of real-time RT-PCR. Female *An. gambiae* antennae were exclusively used in this study because of the importance of the female mosquito as a disease vector. Male mosquitoes do not take blood meals that are required for completion of the female-specific gonadotropic cycle and, accordingly, do not transmit the pathogens responsible for malaria and other diseases (Zwibel and Takken, 2004). Furthermore, we did not quantify *agq1*, *agq4*, and *agm* levels, which have been shown by conventional RT-PCR to be absent in female antennae. *agq3* was occasionally detected as a very faint conventional

PCR product and therefore was included in quantitative analyses. This analysis also included *ago* because the primers for the conventional RT-PCR analysis were designed to distinguish between two alternatively spliced 5'-exons (1N and 1A, respectively; Fig. 2A). Since 3' ends of cDNAs are generated more efficiently, it is reasonable to expect that the amount of *ago* might not be comparable to those of other *agα* transcripts whose primers were restricted to the 3' end of the conceptual ORFs. The primers for real-time RT-PCR analysis were therefore designed to amplify regions close to the 3' end of the *ago* ORF and do not distinguish between the two transcripts. Comparison of individual collections did not indicate any potential variation of *agα*-transcripts over the 12-hour collection window. Data points were combined, averaged, and normalized relative to 1,000 copies of the constitutively expressed ribosomal protein gene *rpS7* (Salazar et al., 1993). The relative expression levels of *agα*-transcripts in female antennae (Fig. 3B) suggest a prominent role for *G_{as}* in female *An. gambiae* antennal tissue, where its levels almost double the relative amount of *agi*, the *agα* transcript with the nearest degree of antennal expression.

Immunolocalization of Gα-proteins in female antennae of *An. gambiae*

In order to determine specificity, a number of antisera (Table 1) were tested against Western blots of whole-protein extracts of heads including appendages from *An. gambiae* (Fig. 4, and data not shown). Of the tested sera, anti-Gq/11 serum raised against a peptide epitope of mouse *G_{α11}* specifically detected a single band that was consistent with the molecular weight of *AG_{αq}* proteins (41.6 kDa, unmodified polypeptide chain). Moreover, this immunoreactivity could be blocked by preadsorption with the Gq/11 cognate peptide listed in Table 1. Anti-DGq1 serum, which was raised against the *D. melanogaster* *G_{αq}* isoform involved in phototransduction (Scott et al., 1995), labeled a protein of similar molecular weight. However, despite the considerable similarity between the cognate peptides of the anti-Gq/11 and anti-DGq1 sera (Table 1), this immunoreactivity cannot be blocked by preadsorption with the Gq/11 cognate peptide. This suggests that anti-DGq1 serum identifies a different protein than anti-Gq/11, presumably *AG_{αq}* isoforms 1 and 4, where the anti-DGq1 antigen is well conserved, while the anti-Gq/11 peptide is more conserved in isoforms 2 and 3 (Table 1). Furthermore, anti-Go serum (Schaefer et al., 2001) specifically reacts to a single band of a molecular weight consistent with *AG_{αo}* (40.4 kDa, unmodified polypeptide chain). Importantly, this labeling was lost after preadsorption of anti-Go serum with an excess of recombinant rat *G_{αo}*, where the presumptive antigen in *AG_{αo}* is highly conserved (12/13 amino acids, Table 1).

In addition, we tested the specificity of the Gq/11, DGQ1, and Go antisera by investigating the distribution of immunoreactivity in head cryosections prepared from *An. gambiae* (Fig. 5). The distribution of *G_α*-expression in *D. melanogaster* has been well established (Wolfgang et al., 1990; Talluri et al., 1995), and it was reasonable to expect that this expression pattern would be conserved in *An. gambiae*. In these studies, anti-DGq1 serum labeling was specific to the retina (Fig. 5A,D). Furthermore, in situ hybridization utilizing an antisense RNA probe specific to *agq1* reveals exclusive expression of this transcript (and possibly *agq4* mRNA, which is 94% identical in the probe region) in the retina (Supplementary Fig. 3A,B). This accounts for all observed anti-DGq1 labeled tissues and supports the specificity of this antiserum to *AG_{αq1}* and *AG_{αq4}*. The specific expression of *agq1* transcript in head tissue (Fig. 3A) suggests that the encoded protein, in a similar fashion to *G_{αq1}* of *D. melanogaster* (Scott et al., 1995), is also involved in phototransduction. Anti-Gq/11-specific labeling was high in the retina and lamina ganglionaris, while lower levels of neuropil labeling were observed in the medulla (Fig. 5B,E). This is similar to the expression pattern described for *D. melanogaster* *G_{αq3}* (Talluri et al., 1995), which represents the analogous isoform of *AG_{αq2}*. Consistent with the expression of *G_{αo}* subunits in *D. melanogaster* heads (Wolfgang et al., 1990), *AG_{αo}*

immunoreactivity was most intense in the neuropil of the medulla and the fat body and was absent from the retina (Fig. 5C,F). Similar expression patterns were observed for ago transcripts. Here, in situ hybridization using an antisense RNA probe that was specific to both transcripts identifies expression in neuronal cell bodies of the cortical layer that surround the optic neuropil (Supplementary Fig. 3C,D). In the case of the commercially available anti-Gq/11 serum, preadsorption controls for antibody specificity were carried out using the cognate peptide and revealed no labeling in head sections (data not shown). In addition, no anti-Go immunoreactivity to head sections was observed after preadsorption of this serum (data not shown) using excess rat G_{ao} protein, as described above for Western blots.

AG $_{aq2}$ and AG $_{aq3}$ expression in female antennae

The anti-Gq/11 serum utilized in this study was raised against a peptide of 19 amino acids, 18 of which are conserved in the deduced sequences of both AG $_{aq2}$ and AG $_{aq3}$. Accordingly, localization of these two isoforms cannot be distinguished. Specific anti-Gq/11 labeling was clearly associated with the flagellar axon bundle along the antenna, as well as with most or all olfactory sensilla of the female antenna (Fig. 6) (McIver, 1982). AG $_{aq}$ was associated with trichodic sensilla (Fig. 6A – C) as immunolabeling was typically observed inside the sensilla or as a projection toward sensilla, which is consistent with AG $_{aq}$ expression within ORN dendrites. No labeling of neuronal somata was observed. In contrast, the neuronal marker anti-HRP/nervana (Sun and Salvaterra, 1995) typically shows strong labeling of neuronal somata, axons, and inner dendrites, while outer dendrites of trichodic neurons were only weakly labeled or unlabeled. Anti-Gq/11 labeling was also associated with grooved peg sensilla (Fig. 6A,D,E), where it appeared weaker and more diffuse than in trichodic sensilla. However, Gq/11 immunoreactivity was not associated with mechanosensory sensilla chaetica shafts, although specific immunoreactivity was observed at their base (Fig. 6A), where they are innervated (McIver, 1972; Boo, 1980). Additionally, large coeloconic sensilla have been implicated in olfaction (McIver, 1982). We observed that Gq/11 labeling is associated with large coeloconic sensilla, where it is confined to a small region at the base of each sensillum (Fig. 6F,G). Thereby anti-Gq/11 specific labeling was not overlapping with anti-HRP/ nervana labeling, which indicates nonneuronal localization of AG $_{aq}$ in this case. Preabsorption of anti-Gq/11 serum with its cognate peptide blocks all immunoreactivity in female antennae (Supplementary Fig. 1).

AG $_{aq1}$, AG $_{aq4}$, and AG $_{ao}$

Anti-DGq1 serum does not react to any structure along the antenna (Fig. 7A,B). This is consistent with our RT-PCR expression analysis, in which no *agq1* or *agq4* transcript was detected in olfactory tissues (Fig. 3A) and results of in situ RNA hybridization experiments to *agq1*, which did not indicate transcript expression within olfactory tissues (data not shown). *agq1* and *agq4* encode peptides similar to the anti-DGq1 antigen. Anti-Go immunoreactivity (Fig. 7C – F) was observed along the flagellar axon bundle as well as at the base of individual sensilla chaetica. Importantly, this immunoreactivity was lost when anti-Go serum was preadsorbed with full-length, recombinant rat G_{ao} protein (Supplementary Fig. 2; see Table 1 for conservation of the anti-Go epitope between *D. melanogaster*, *An. gambiae*, and rat). No labeling of neuronal cell bodies or dendrites was observed. Finally, in situ hybridization experiments with antisense RNA probe specific to *ago* clearly indicated that this transcript is expressed in antennal neurons, which were identified by colabeling with anti-HRP/nervana serum (Sun and Salvaterra, 1995).

DISCUSSION

In order to identify additional genetic elements that might contribute to olfactory and other types of sensory and nonsensory signal transduction in *An. gambiae*, we identified the presumptive genes encoding AG_α subunits and investigated their developmental and tissue-specific expression patterns. In many instances, temporally and/or spatially restricted expression patterns are important indicators in assessing the biological function of molecules in specific processes and, in that context, we note that AG_{aq1} (encoded by *agq1*) is exclusively expressed in head tissue and thus is likely to function in head-specific processes such as phototransduction. Indeed, AG_{aq1} displayed a transcript structure similar to DG_{aq1} , which has been shown to function in phototransduction in *D. melanogaster* (Scott et al., 1995), and anti- DG_{aq1} serum, which was raised against a peptide that shares high homology with the carboxyl termini of AG_{aq1} and AG_{aq4} , reacted specifically to epitopes expressed in retina from *An. gambiae*.

Quantification of G_α subunit mRNA levels in female antennae revealed that G_{as} and G_{ai} are expressed at considerably higher levels than other *agq*-transcripts, consistent with a prominent role for these subunits in *An. gambiae* olfactory organs. Their cellular localization in these tissues, however, remains to be investigated in order to gain understanding of their function. Furthermore, we found significant levels of *agc*-transcript in female antennae encoding an AG_{ac} subunit homologous to the *D. melanogaster concertina* gene product and $G_{a12/13}$ proteins. Several effectors for $G_{a12/13}$ proteins have been identified (Hart et al., 1998; Lopez et al., 2001; Niu et al., 2001; Yamaguchi et al., 2002; Suzuki et al., 2003; Zhu et al., 2004) that typically act on transcription or actin cytoskeleton structure via small GTP binding proteins (reviewed in Neves et al., 2002). However, no ion channel targets have been described that would suggest $G_{a12/13}$ can function to stimulate action potentials.

AG_{ao} and the two AG_{aq} isoforms that had been identified in the initial PCR screen in *An. gambiae* olfactory tissue were expressed at low levels when compared with $AG_{as/i}$. This result was surprising in the case of AG_{aq} , since G_{aq} proteins have previously been localized in sensory neurons of several insect species (Talluri et al., 1995; Laue et al., 1997; Jacquin-Joly et al., 2002; Miura et al., 2005). While immunolocalization studies with G_{ao} - and G_{aq} -specific antibodies revealed that both G_α proteins are expressed along the flagellar axon bundle, only AG_{aq} was localized to *An. gambiae* ORN dendrites, and thus is likely to participate in olfactory signal transduction in the mosquito. G_α -proteins are membrane-bound and the amount of membranes is comparably small in the receptive areas of ORNs. By contrast, dendritic membranes in photosensory neurons are more densely packed and, consequently, a higher intensity of immunolabeling was observed in the retina than in olfactory sensilla (Fig. 5, Fig. 6).

The axonal localization of G_α proteins is similar to *B. mori* G_{aq} that is localized to the axon-glia interface, where it is hypothesized to facilitate signaling between neurons and glia cells (Laue et al., 1997). AG_α localization along the axon bundle may also indicate a function in communication between neurons and adjacent cells. In situ hybridization experiments indicated that *ago* transcript in antennae is expressed in neurons only (Fig. 7E,F). This suggests that AG_{ao} protein is indeed enriched within the axonal portion of these neurons and not expressed in support cells. It is noteworthy that AG_{aq} and AG_{ao} subunits were also observed at the base of sensilla chaetica, which are believed to function in mosquito mechanosensation (McIver, 1972; Boo, 1980).

AG_{aq} localization to *An. gambiae* sensilla trichodea and grooved peg sensilla is similar to previously observed G_{aq} localization at the dendritic membrane of *B. mori* ORNs (Laue et al., 1997). However, the anti- $G_{q/11}$ immunoreactivity associated with *An. gambiae* grooved

peg sensilla is more diffuse than the neuronal integral membrane protein marker HRP/nervana (Sun and Salvaterra, 1995). In the case of *B. mori*, specific anti-Gq/11 labeling was cryptically localized to the sensillum lymph (Laue et al., 1997), which was interpreted as an artifactual extraction of the antigen during the tissue-embedding process. Similarly, specific anti-Gq/11 labeling in *An. gambiae* grooved peg sensilla may also reflect a modest degree of antigen extraction. Moreover, the Gq/11-specific immunoreactivity in large coeloconic sensilla was associated with a small region at the base of each sensillum. This suggests that AG_{aq} may be localized in the apical region of the tormogen support cell rather than in the ORN. The anti-Gq/11 serum used here was raised against a peptide epitope that is well conserved in AG_{aq2} and AG_{aq3}, although we cannot distinguish whether this serum also reacts to AG_{aq1&4}, peptide subunits in which 13 of 19 amino acids are conserved.

In the course of this study we never observed specific anti-DGq1 or anti-Go immunoreactivity associated with olfactory sensilla. While we cannot exclude the possibility that very low levels of AG_{ao} are present in ORN dendrites in *An. gambiae*, all confocal images were captured in the dynamic range of emitted fluorescence signal (from zero to maximum for each viewed section) and are therefore expected to faithfully reflect quantitative differences in AG_a distribution.

The anti-Go immunoreactivity associated with the axon bundle most probably represents a stronger AG_{ao} requirement at this region. This is in contrast to AG_{aq} localization in olfactory sensilla and therefore argues against a major requirement of AG_{ao} and in favor of a primary role for AG_{aq2} in *An. gambiae* peripheral olfactory signal transduction.

The presence of very low but consistently detectable copy numbers of AG_{aq3} (5–10% compared with AG_{aq2} levels) raises the possibility that AG_{aq3} subunits also potentially play a role in olfactory processing in this system. These two isoforms differ in 15 amino acid positions of the presumptive GTPase-activating domains. In this domain, Arg177, which is conserved in all known G_a proteins and contributes to catalysis (Markby et al., 1993), is differentially flanked by V residues in AG_{aq2} and A residues in AG_{aq3}; this might conceivably result in altered GTPase activity.

Both conceptual AG_{aq2} and AG_{aq3} proteins identified in *An. gambiae* share identical carboxyl termini. This region is encoded by G- and H-type exons and there is considerable evidence for the importance of this domain in receptor • G_a interactions (Hamm et al., 1988). Indeed, minor changes of the carboxyl terminus can dramatically alter receptor specificity of G_a proteins (Conklin et al., 1993). If G_{aq} is the primary G protein that underlies olfactory signal transduction in this system, characterization of AgOR agonist and antagonist spectra in heterologous systems would conceivably benefit from coexpression of the cognate AG_{aq} isoform since alteration of receptor • G_a interaction domains can alter efficacy ranking of the receptor ligand spectrum (Kenakin, 1995). Interestingly, a G_{aq} transcript isolated from *Mamestra brassicae* antennae (Jacquin-Joly et al., 2002) includes an H exon, encoding a carboxyl terminus homologous to AG_{aq1}. This suggests that G_a expression patterns should be examined carefully before incorporating a specific G_a into heterologous OR deorphanization protocols. The precise functional significance of AG_{aq2} and AG_{aq3} expression in the olfactory system of *An. gambiae* remains unexplored and potentially offers important insight toward the development of novel approaches for anti-malarial programs targeting olfactory-driven behaviors. The fact that these experiments will be extremely challenging owing to a lack of robust genetic tools that incorporate transgenic methodology should not deter such efforts in this, and other, disease vector mosquitoes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Phylogeny of *D. melanogaster* G_{α} -proteins compared with that of *An. gambiae* homologs. Scale bar denotes 5% sequence-divergence. Protein sequences for *An. gambiae* were deduced from sequence information available through the mosquito genome project and at TIGR, which was combined with sequence information of RT-PCR products. For bootstrap analysis, alignment-gaps were excluded. The *Saccharomyces cerevisiae* G_{α} -protein of the pheromone response pathway, Gpa1p was included in bootstrap analysis as an outgroup. DmGq2 was not included because no protein corresponding to this mRNA has been detected (Scott et al., 1995).

Fig. 2.

A: Genomic organization at the *An. gambiae ago*-locus: the protein coding region is distributed over more than 56 kbp; coding exons are displayed as bars and sizes of selected subfragments are indicated below the schematic. Exons 1N and 1A are respective parts of two transcripts, *agon* and *agoa*, that originate from alternative splicing. Exon 2 is encoded in a region of ~5 kbp that was not sequenced during the course of the genome project. **B:** Structure of the *An. gambiae agq*-locus: three homologous pairs of exons D and D*, G and G*, as well as H and H*, respectively, are spliced alternatively as shown (transcripts *agq1*-4). Transcript structure is depicted for the regions analyzed by sequencing of RT-PCR products. Additionally, an RT-PCR product consistent with the expected complete *agq2* ORF A-H* was cloned and sequenced. Hatched boxes: exons encoding translation-STOP.

Fig. 3.

Expression analysis of *aga* transcripts in adult tissues (**A**) and preimago developmental stages as well as female adult tissues prior to and 20 hours after a blood meal (**C**) using RT-PCR. Target transcripts are denoted on the left margin. The *rps7* transcript was amplified as a control for cDNA integrity. All PCR products that originated from cDNA, as judged by size, were cloned and sequenced. Asterisks indicate sequenced RT-PCR products that may not be translated (see text) and PCR product sizes are denoted on the right, investigated tissues at the top margin. RNA was isolated from the following mosquito tissues in (**A**): m.ant., male antennae; m.p.p., male palps and proboscides; f.ant., female antennae; f.p.p., female palps and proboscides; heads, heads without appendages; bodies, thoraces and

abdomens including wings, without legs; gen, genomic DNA control. C: h.+app., heads including appendages; th., thorax including wings and legs; abd., abdomens; bf., 20 hours post blood meal; e. larv., 1-week-old larvae; l.larv., 2-week-old larvae. Left and right-most lanes in (A) and left lane in (C): 100 bp-ladder (New England Biolabs, Beverly, MA). All RT-PCR results are shown for 34 PCR cycles. Note that *ago* and *agq* primer binding sites are flanking large introns and *agi*, *agm*, and *agc* primers target exon–exon boundaries, thus obviating amplification of genomic fragments. **B:** Relative expression levels of *aga* transcripts in female *An. gambiae* antennae, determined by real-time RT-PCR are described as copies/1,000 copies *rpS7*. Error bars represent 1 standard deviation (n = 3). Note that primers for real-time analysis were placed close to the 3' end of the respective reading frames. Hence, *agoa* and *agon* could not be distinguished.

Fig. 4.

Immunoblots of *An. gambiae* heads plus appendages. Each lane represents ~ 0.5 head equivalents. Anti-Gq/11, anti-DGq1, and anti-Go sera label protein bands consistent with the expected molecular weights for AG_{aq} and AG_{ao} isoforms (arrowhead; unmodified $AG_{aq} = 41.6$ kDa, unmodified $AG_{ao} = 40.4$ kDa), respectively. Anti-Gq/11 specific labeling is inhibited by preadsorption with its cognate peptide (q11P), which does not reduce anti-DGq1 immunoreactivity consistent with its specificity to AG_{aq} -isoforms 1 and 4. Anti-Go serum reactivity (arrowhead) is blocked by preincubation along with recombinant rat G_{ao} .

Fig. 5.

Immunolocalization of G α proteins in frozen sections of adult female *An. gambiae* heads. Anti-DGq1 serum-dependent labeling (**A,D**) is specific to the retina (R). Anti-Gq/11 dependent labeling (**B,E**) is strong in the retina of the compound eye and the lamina ganglionaris (L). More moderate anti-Gq/11 labeling was also observed in the neuropil of the medulla and central nervous system (CNS) and in adjacent neuronal cell bodies. Anti-Go labeling (**C,F**) was observed in the CNS neuropil and the fat body (FB), while neuronal cell bodies and the lamina ganglionaris were labeled to a lesser extent. No labeling was observed in the retina. G α , magenta; neurons (anti-HRP (Sun and Salvaterra, 1995), green; DNA, blue; magenta/green overlap, white. Top row: composite images; bottom row: G α -specific channel. Scale bars = 50 μ m. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 6.

Anti-Gq/11 serum (magenta) labeling is associated with most or all trichodetic (examples labeled with asterisk), grooved peg (examples labeled with arrowhead), and large coeloconic sensilla (open arrows), the flagellar axon bundle, and the base of sensilla chaetica (open arrowheads). **A:** Intermediate segments of antenna. **B,C:** Enhancement of panel A as indicated by asterisk. Note that anti-HRP serum predominantly labels neuronal somata and inner dendrites of trichodetic ORNs, while anti-Gq/11-dependent labeling is confined to the sensory hair, which contains the outer dendritic region of ORNs. **D,E:** Anti-Gq/11 labeling in grooved peg sensilla is weaker and more diffuse than in trichodetic sensilla. **F,G:** Labeling of large coeloconic sensilla is associated with the sensillum base. **A,B,D,F:** Composite

images. C,E,G: Respective Anti-Gq/11 (magenta) specific channel. Neurons (anti-HRP/nervana), green; DNA, blue; magenta/green overlap, white. Stack sizes: (A) 4.1 μm (B,C) 0.51 μm (D,E) 0.51 μm (F,G) 3.6 μm . Note that the brightfield component is not confocal and thus represents the entire 15- μm section. Scale bars = 50 μm in A; 5 μm in B–G. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 7.

Anti-DGQ1 dependent labeling was not observed along the antenna (**A,B**). Anti-Go-specific labeling (**C,D**) is associated with the flagellar axon bundle and the base of sensilla chaetica (asterisk). In situ hybridization utilizing specific antisense RNA probe (**E,F**) identifies *ago* expression in neurons (arrowhead) that are distinguished by anti-HRP serum (**E**) (Sun and Salvaterra, 1995), while neurons in control sections hybridized to sense RNA probe remain unlabeled (data not shown). *Ga*, *ago*, magenta; neurons, green; DNA, blue; magenta/green overlap, white (note: overlap may not generate white color, if one signal is significantly stronger, within the dynamic range of detection). Stack sizes are (**A,B**) 4.2 μm (**C,D**) 5.1 μm (**E,F**) 2 μm . Scale bars = 20 μm in **B,D**; 5 μm in **F**. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Table 1

Summary of Antiserum and Corresponding Antigen Epitopes Used throughout This Study

Antiserum	Antigen/putative <i>A. gambiae</i> antigen	Source/reference
anti-Gq/11	FAAVKDTILQLNLKEYNLV (mouse) FAAVKDTILQSNLKEYNLV (AGaq2 and 3)	Santa Cruz Biotechnology (Ratnaparkhi et al., 2002)
anti-DGq1	KDTIMQNALKEFNLG (D. mel. Gq1) KDTIMQTALKEFNLA (AGaq1 and 4)	C. Zuker (Scott et al., 1995)
anti-Go	VIIANNLRGCGLY (D. mel. Gao) VIIANNLRGCGLY (AGao)	P. Copenhaver (Schaefer et al., 2001)
Recombinant rat Gao (Calbiochem), which was utilized to block anti-Go immunoreactivity, shares the following epitope with AGao: IIIANNLRGCGLY		

The following antisera were tested but were not applicable to this study (data not shown): rabbit anti-Gs (Oncogene research, PC58, raised against peptide CRMHLRQYELL), rabbit anti-Gs/olf (Santa Cruz, sc-383, raised against peptide NDCRDIIQRMHLRQYELL), rabbit anti-Gs (Chemicon International, AB1639, raised against peptide RMHL-RQYELL), rabbit anti-Gi1-3 (Santa Cruz, sc-262, raised against peptide KNNLKECGLY), rabbit anti *D. melanogaster* Gi, a generous gift of Jürgen Knoblich (Schaefer et al., 2001) (raised against peptide ATDTNNVKFVFDAVTDVIIKNNLKQIGLF). In addition, a serum raised against recombinant *An. gambiae* Gi amino acids 52–194 was not applicable.