A highly conserved candidate chemoreceptor expressed in both olfactory and gustatory tissues in the malaria vector *Anopheles gambiae*

R. Jason Pitts, A. Nicole Fox*, and Laurence J. Zwiebel†

Department of Biological Sciences, Center for Molecular Neuroscience and Program in Developmental Biology, Vanderbilt University, Nashville, TN 37235

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***Anopheles gambiae* is a highly anthropophilic mosquito responsible for the majority of malaria transmission in Africa. The biting and host preference behavior of this disease vector is largely influenced by its sense of smell, which is presumably facilitated by G protein-coupled receptor signaling [Takken, W. & Knols, B. (1999) *Annu. Rev. Entomol.* 44, 131–157]. Because of the importance of host preference to the mosquitoes' ability to transmit disease, we have initiated studies intended to elucidate the molecular mechanisms underlying olfaction in *An. gambiae*. In the course of these studies, we have identified a number of genes potentially involved in signal transduction, including a family of candidate odorant receptors. One of these receptors, encoded by *GPRor7* (hereafter referred to as *AgOr7*), is remarkably similar to an odorant receptor that is expressed broadly in olfactory tissues and has been identified in *Drosophila melanogaster* and other insects [Krieger, J., Klink, O., Mohl, C., Raming, K. & Breer, H. (2003) *J. Comp. Physiol. A* 189, 519–526; Vosshall, L. B., Amrein, H., Morozov, P. S., Rzhetsky, A. & Axel, R. (1999) *Cell* 96, 725–736]. We have observed *AgOr7* expression in olfactory and gustatory tissues in adult *An. gambiae* and during several stages of the mosquitoes' development. Within the female adult peripheral chemosensory system, antiserum against the AgOR7 polypeptide labels most sensilla of the antenna and maxillary palp as well as a subset of proboscis sensilla. Furthermore, AgOR7 antiserum labeling is observed within the larval antenna and maxillary palpus. These results are consistent with a role for *AgOr7* in both olfaction and gustation in *An. gambiae* and raise the possibility that *AgOr7* orthologs may also be of general importance to both modalities of chemosensation in other insects.**

Despite dramatic advances in other areas, mosquito-borne diseases still present compelling challenges for global public health. Every year millions of people suffer from diseases such as malaria, dengue fever, yellow fever, and encephalitis, according to the World Health Organization (for more information visit www.who.int). Disease transmission is in part driven by the requirement of a vertebrate blood meal by female mosquitoes to complete their gonotrophic reproductive cycle. In mosquitoes, olfaction as a sensory mode predominates the animals' host preference behavior (reviewed by ref. 1) and as such makes a significant contribution to their overall ability to transmit disease, which may be quantified as vectorial capacity (2). Similarly, olfaction contributes to the impact of many other insects of economic and medical importance (3). It is therefore anticipated that a greater understanding of olfactory processes in insects may facilitate the development of methods designed to interfere with the interactions of insect pests with their host organisms.

We have used molecular and informatics-based approaches to search for genes that are potentially active in olfactory signal transduction in *Anopheles gambiae*. To date, we and others have identified and characterized olfactory genes of different classes, including candidate receptors (4–6), arrestins (7, 8), odorant-binding proteins (9–12), and a group of Gα genes (M. Rützler and L.J.Z., unpublished data). Of these, one candidate odorant receptor (OR) in *An. gambiae*, *AgOr7*, is a member of a group of extremely well conserved, apparent single gene orthologs from insects, including *Drosophila melanogaster (DOr83b)* (13–15), *Heliothis virescens (HvirR2)* (16), *Apis mellifera* (*AmelR2*), and others (17). While still functionally uncharacterized, these genes are expressed in many OR neurons, where it may act as a dimerization partner for other ORs (14, 17). As such, this particular gene may prove useful as a target for disruption of olfactory signal transduction and, by extension, the downstream behaviors affected by it. Indeed, the study of this unique candidate OR type may lead to novel approaches designed to reduce olfactory sensitivity and therefore the vectorial capacity of mosquitoes by disrupting vector/host interactions.

In this study, we describe the initial characterization of *AgOr7 in vivo*, where it is widely expressed in olfactory organs of both adult and immature stages in *An. gambiae*. Furthermore, we show that *AgOr7* is robustly expressed in a primary adult gustatory organ. In contrast to the high degree of divergence and restricted expression patterns of other insect ORs (13, 14, 16), the extreme conservation of *AgOr7* and its broad expression profile in olfactory and gustatory tissues has raised the possibility that it plays a significant and generalized role in chemosensory signal transduction in mosquitoes and perhaps other insects.

Methods

Mosquito Rearing. *An. gambiae sensu stricto* (G3 strain) were reared as described (4). For stock propagation, 4- to 5-day-old female mosquitoes were bloodfed for 20–30 min on anesthetized mice, following guidelines set by the Vanderbilt Institutional Animal Care and Use Committee.

Genome Screening. The *An. gambiae* bacterial artificial chromosome (BAC) end sequences were transferred from Genoscope (www.genoscope.cns.fr/externe/English/Projets/Projet_AK/organisme_AK.html) to a local database and searched by a modified tBLASTn using the *DOr83b* sequence as a query. A single BAC, 04E15, was identified as containing most of the putative *AgOr7* coding sequence. Individual fragments of 2–6 kb were identified by DNA blots and subcloned into pBluescript II KS+ (Stratagene) and subsequently sequenced by using BigDye chemistry and an ABI PRISM 377 DNA Sequencer (Applied Biosystems) according to the manufacturers' protocols.

cDNA Cloning. Based on the known genomic sequence, the following oligonucleotide primers were designed and used to

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Abbreviations: OR, odorant receptor; BAC, bacterial artificial chromosome; TM, transmembrane region; SEM, scanning electron micrography.

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AY363725 and AY363726).

*Present address: Division of Biology, California Institute of Technology, Pasadena, CA 91125.

†To whom correspondence should be addressed. E-mail: l.zwiebel@vanderbilt.edu.

amplify the complete coding region of *AgOr7* from antennal cDNA in a PTC-200 thermal cycler for 45 cycles with an optimal annealing temperature of 54°C (MJ Research, Waltham, MA): AgOr75′RT, 5′-ATGCAAGTCCAGCCGACCAA-3′; AgOr73′RT, 5′- TTACTTCAGCTGCACCAGCA-3′. PCR products were cloned into the pCRII-TOPO cloning vector (Invitrogen) and sequenced as described above.

Alignments. Primary amino acid sequences of the following genes were aligned by using the CLUSTAL X software package (18). GenBank accession numbers are indicated in parentheses: *Drosophila melanogaster* Or83b (NM079511), *Heliothis virescens* R2 (AJ487477), *Anopheles gambiae* Or7 (AY363725, AY363726), *Apis mellifera* R2 (AJ555537), *Calliphora erythrocephala* R2 (AJ555538), *Tenebrio molitor* R2 (AJ555539), *Bombyx mori* R2 (AJ555487), and *Antheraea pernyi* R2 (AJ555486). Exon/intron positions for *D. melanogaster* and *An. gambiae* genes were determined on the basis of a comparison of cDNAs with genomic sequences.

RNA Expression. Total RNA was isolated from *An. gambiae* G3 tissues by using the RNeasy kit (Qiagen, Valencia, CA): embryos (morning of laying), early instar larvae (2–4 days old), late instar larvae (10–14 days old), pupae, or adult tissues (4–6 days old). First-strand cDNA synthesis was carried out by using ≈0.5 μg of RNA and SuperScript II reverse transcriptase (RNase H-negative) according to the supplier's protocol (Invitrogen). Each cDNA sample was amplified by using intron-spanning primers: Or7exon6.2, 5′-TGCTGCTACACATGCTGAC-3′; Or7exon8.1, 5′-TAGGTGACAACGGCTCCAA-3′ for 40 cycles with an optimal annealing temperature of 60°C. In each assay the ribosomal protein 7 gene (*rps7*) (19) was amplified in tandem as a control for cDNA integrity by using the primers *rps7*a, 5′-GGCGATCATCATCTACGTGC-3′; *rps7*b, 5′-GTAGCTGCTGCAAACTTCGG-3′. PCR products were gel purified by using the QIAquick gel extraction kit (Qiagen), subcloned into the pCRII-TOPO cloning vector, and sequenced as described above.

Antibodies. A synthetic peptide, Or7a, corresponding to amino acids 268–281 of *AgOr7* was coupled to keyhole limpet hemocyanin (KLH) and used to immunize white rabbits (Sigma-Genosys, The Woodlands, TX). Polyclonal antibodies (IgGs) were purified from crude serum by using protein-G Sepharose (Sigma), followed by subtraction against a KLH Affi-Gel (Bio-Rad) column according to the manufacturers' protocols.

Scanning Electron Micrography (SEM). Specimens for electron microscopy were fixed in 40% ethanol/4% paraformaldehyde for 10 min at room temperature followed by dehydration through a graded series of ethanol washes to 100%. Specimens were then attached to stubs with silver conductive paint, coated with gold during spin rotation, and examined in a Cambridge StereoScan microscope.

Immunocytochemistry. Immunofluorescence was performed as described (20) with some modifications. Heads with attached appendages of 4- to 6-day-old female mosquitoes were hand-dissected and prefixed in a solution of 4% paraformaldehyde in PBS (PBS: 137 mM NaCl/2.7 mM KCl/10 mM Na_2HPO_4/2 mM KH_2PO_4) plus 0.1% Triton X-100 (PBSTx) at 4°C for 30 min. This material was rinsed three times with PBS and infused overnight in a solution of 25% sucrose in PBSTx at 4°C before embedding and freezing in Tissue-Tek OCT compound (Sakura Finetek, Torrance, CA). Samples were sectioned at 10–15 μm in a CM 1900 cryostat (Leica Microsystems, Bannockburn, IL) at −20°C. Sections were collected on Superfrost plus slides (VWR Scientific) and allowed to dry at room temperature for 30 min, then fixed in 4% paraformaldehyde in PBSTx at room temperature for 30 min. Slides were rinsed three times with PBSTx and blocked with PBSTx plus 5% normal goat serum (NGS, Vector Laboratories) at room temperature for 60 min. Primary antisera, including anti-AgOR7 and anti-horseradish peroxidase, were diluted at 1:500 in PBSTx plus 5% NGS and incubated on slides at 4°C overnight. Slides were rinsed three times with PBSTx and incubated with secondary Cy3-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch) at a 1:500 dilution in PBSTx plus 5% NGS at room temperature for 2 h then rinsed as before. Nuclei were labeled by using TOTO-3 (Molecular Probes) diluted 1:4000 in PBSTx at room temperature for 20 min followed by a brief wash in water. Slides were mounted with Vectashield fluorescent medium (Vector Laboratories). Confocal images were captured by using the LSM 510 META system attached to an Axioplan fluorescent microscope (Zeiss). Other images were captured by using a DP70 charge-coupled device camera attached to a BX-60 fluorescent microscope (Olympus).

Results

A BAC overlapping the *AgOr7* gene was identified from an *An. gambiae* genomic library (21) on the basis of its similarity to the *DOr83b* gene. Restriction fragments that contained portions of *AgOr7* were subcloned into plasmid vectors and sequenced, leading to the isolation of the majority of the gene, starting within intron 1 and ending beyond the 3′ translational stop (Fig. 1*B*). The remainder of the *AgOr7* genomic sequence was extracted from the *Anopheles* genome (6, 22). Based on the known genomic sequence, oligonucleotide primers were then designed and used to amplify the complete 1,437-bp coding region of *AgOr7* from antennal cDNA. The conceptual translation of the *AgOr7* coding region is 478 aa in length (Fig. 1*A*). Two *AgOr7* coding region clones were sequenced completely. Eight nucleotide polymorphisms were identified between the two, none of which led to a change in the translation presented in Fig. 1*A*. Two unique, silent polymorphisms were also found in the BAC subclones.

The *AgOr7* amino acid sequence was aligned with highly similar, previously identified, sequences from other insect species. The five full-length sequences share greater than 60% identity and 80% similarity. Most remarkable is the extreme conservation of the C-terminal one-third of the genes, where all nine sequences are nearly 90% identical over their final 160 aa (Fig. 1*A*). Specifically, the AgOR7 polypeptide shares 78% identity and 89% similarity with the entire DOr83b polypeptide, including 94% identity in the final 160 aa.

AgOr7 is located on *An. gambiae* chromosome 2R, spans 11 kb in length, and is composed of eight exons and seven introns, and the splice sites of the last four introns are identical in *AgOr7* and *DOr83b* (Fig. 1*B*). Furthermore, we used the completed genome sequences of *An. gambiae* (22) and *D. melanogaster* (23) to assess potential microsynteny in these regions. Fig. 1*C* shows that genes located on either side of both *AgOr7* and *DOr83b* sequences are very well conserved. For example, on the centromeric side of *AgOr7*/*DOr83b* resides a pair of uncharacterized genes, *agCP1696* in *An. gambiae* and *CG14669-PA* in *D. melanogaster*, whose translations are almost 80% identical. Further underscoring this microsynteny, in each case the homologous pair is transcribed in the same direction (Fig. 1*C*).

Hydrophobicity plots (24) and other analyses (see below) of the *AgOr7* translation were used to determine the potential TMs of the polypeptide. Similar analyses were used previously to predict potential TM helices within the *D. melanogaster* and *H. virescens* OR families, and separately for the *HvirR2* orthologs (13, 16, 17). Our placement of the first six TMs is similar to previous placements (17), but we postulate an alternative location for the seventh TM (TMVII), one that is shifted by 25 aa toward the N terminus (Fig. 1*A*). Secondary structure prediction using PSIPRED (25) indicated the high probability of an α-helix in

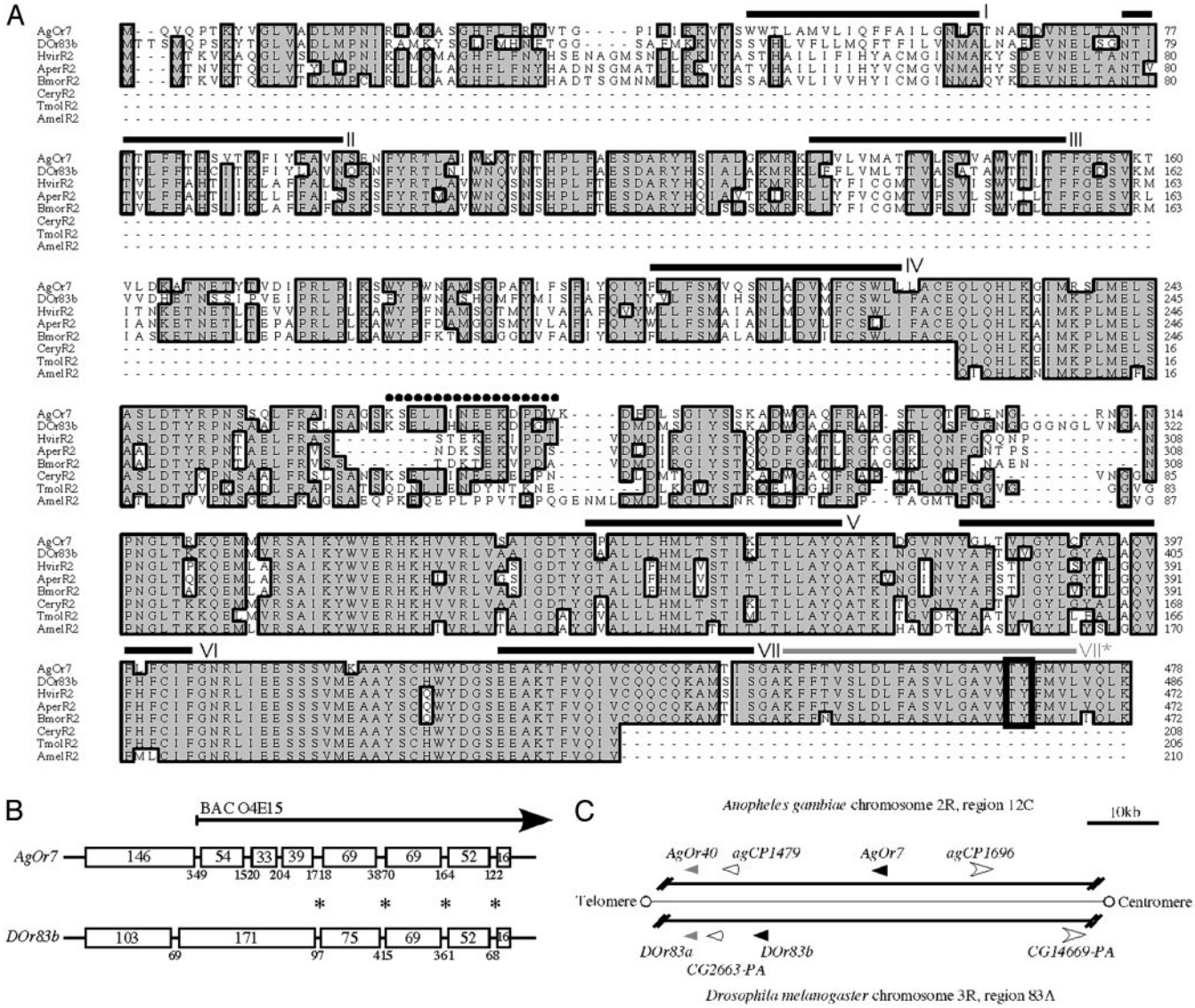

Fig. 1. (*A*) Alignment of AgOR7 ortholog peptides, displayed in the single-letter amino acid code. Identical residues are boxed and shaded. Transmembrane regions (TMs) I–VII are indicated with black bars. Previous placement of TMVII (4) is indicated with a gray line. Black dotted line shows the location of the peptide antigen used in antiserum production. Threonine (T) and tyrosine (Y) residues that are sites of potential phosphorylation are enclosed in heavy black boxes. For a list of genes and accession numbers see *Methods*. (*B*) Exon and intron structures of the *AgOr7* and *DOr83b* genes. Numbers within boxes indicate exon sizes in amino acids. Intron lengths (not drawn to scale) in base pairs are shown below each line. Asterisks highlight conserved splice sites. Heavy black line shows the position of the *An. gambiae* BAC from which the *AgOr7* gene was isolated. (*C*) Microsynteny within the genomic regions surrounding *AgOr7* and *DOr83b*. Gene names and their relative positions are listed above and below lines, respectively. Arrowheads indicate directions of transcription, and like arrowheads are apparent orthologs: gray ≈50% identical, black and white ≈80% identical at the amino acid level.

the region of amino acids 428–448, even though that region had a lower hydrophobicity index than a more downstream region (gray box, Fig. 1*A*).

ORs are expressed in sensory neurons in olfactory tissues of insects, including the antennae and maxillary palps (26). The expression pattern of the *AgOr7* transcript was examined by nonquantitative RT-PCR analysis of RNA isolated from several developmental stages of *An. gambiae*. Specifically, *AgOr7* transcript was clearly detected in preimago stages, including early-stage larvae, late-stage larvae, and pupae, but not in embryos (Fig. 2). The most robust expression was observed in the adult antennae and the maxillary palps (Fig. 2), organs that constitute the major OR fields in adult mosquitoes (1). Interestingly, a significant level of *AgOr7* RT-PCR product was also observed in the proboscis (Fig. 2), a mouthpart that has previously been shown to have a gustatory function in mosquitoes (27). In addition, a trace amount of *AgOr7* expression product was detected in legs, whereas bodies devoid of heads and legs yielded no observable amplification product (Fig. 2). Similar expression patterns were observed separately in both male and female adult tissues (data not shown). The ribosomal protein s7 (19) gene (*rps7*) was amplified as a measure of cDNA integrity in tandem with *AgOr7*. In each case strong *rps7* products of the expected size for cDNA were observed.

To examine the spatial expression of the AgOR7 protein, a polyclonal antiserum was used in immunolocalization studies within head structures of *An. gambiae* adult females. Similar to the antennae of other anophelines, an *An. gambiae* adult antenna is composed of a 13-segmented flagellum that houses several classes of sensory setae known as sensilla (28–30). An SEM

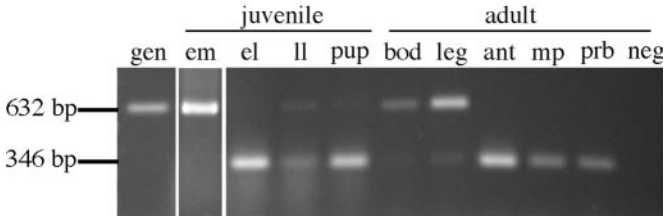

Fig. 2. Expression of the *AgOr7* gene in *An. gambiae* by RT-PCR. Lanes are as follows: gen, genomic DNA control; em, embryos; el, early-stage larvae; ll, late-stage larvae; pup, pupae; bod, bodies; leg, legs; ant, antennae; mp, maxillary palps; prb, proboscises; neg, no template. Numbers indicate expected sizes of genomic and cDNA products in base pairs (bp).

examination of a single distal antennal segment of a female reveals the presence of both sensilla trichodica (ST) and grooved peg (GP), also known as basiconic, olfactory sensilla (Fig. 3*A*). In addition, characteristic sets of very large, mechanosensory sensilla chaetica (SC) originate from whirl-like structures at the proximal segment boundaries (Fig. 3*A*). Two other types of sensilla, the large coeloconic, or sunken pegs, and the small coeloconic, are found on *An. gambiae* antennae (data not shown). Anti-AgOR7 labeling was apparent in all segments except the first, the most proximal segment that attaches to the Johnston's organ (Fig. 3*B*). Furthermore, Anti-AgOR7 labeling was observed in all ST and in many cell bodies (Fig. 3*C*). However, in this analysis, anti-AgOR7 labeling was notably absent from GPs (Fig. 3*D*) and sunken pegs (data not shown). The small coeloconic sensilla were not examined. Using an antiserum against horseradish peroxidase, which is widely used as a insect neuronal marker (31, 32), we also observed that anti-AgOR7 specifically labeled neurons (Fig. 3 *C* and *D*). Previous studies of the RNA expression of *DOr83b, HvirR2*, *AmelR2*, and *CeryR2* have also demonstrated that these genes are expressed in most, if not all, olfactory receptor neurons (16, 17, 33). In this study it was possible to visually follow anti-AgOR7 labeling originating in cell bodies and projecting into the lumen of an individual sensillum, presumably along the dendritic extension of the neuron (Fig. 3 *C* and *D*). Anti-AgOR7 labeling was also observed in sections of the maxillary palps, secondary olfactory organs (34, 35) that are sensitive to carbon dioxide and other odors in mosquitoes (27, 30). Labeling was specific to neurons within the second, third, and fourth (penultimate) segments that innervated capitate pegs (Fig. 3 *E* and *F*), the single type of sensory sensillum on mosquito maxillary palps (27). Preimmune serum controls for all studies showed very faint background fluorescence similar to untreated sections (Fig. 5, which is published as supporting information on the PNAS web site). Furthermore, the labeling could be completely blocked by preincubating the antiserum with the peptide antigen used in antiserum production, but was not affected by preincubation with a peptide from another *An. gambiae* odorant receptor (Fig. 5). Taken together, these data show that, as with *AgOr7* orthologs in other species, not only is *AgOr7* highly expressed in olfactory tissues, but the AgOR7 protein is also localized in most OR neurons.

In addition to adult sensory structures, clear anti-AgOR7 labeling of neurons within the antenna and maxillary palpus was seen in head cryosections of *An. gambiae* larvae (Fig. 6 *B* and *D*, which is published as supporting information on the PNAS web site). Both organs house terminal sensilla in mosquitoes that are thought to be chemosensory in function on the basis of their fine structures (27, 36). In the case of the maxillary palpus, labeling was clear in a small number of cell bodies and in projections into the sensillar cone (Fig. 6*D*).

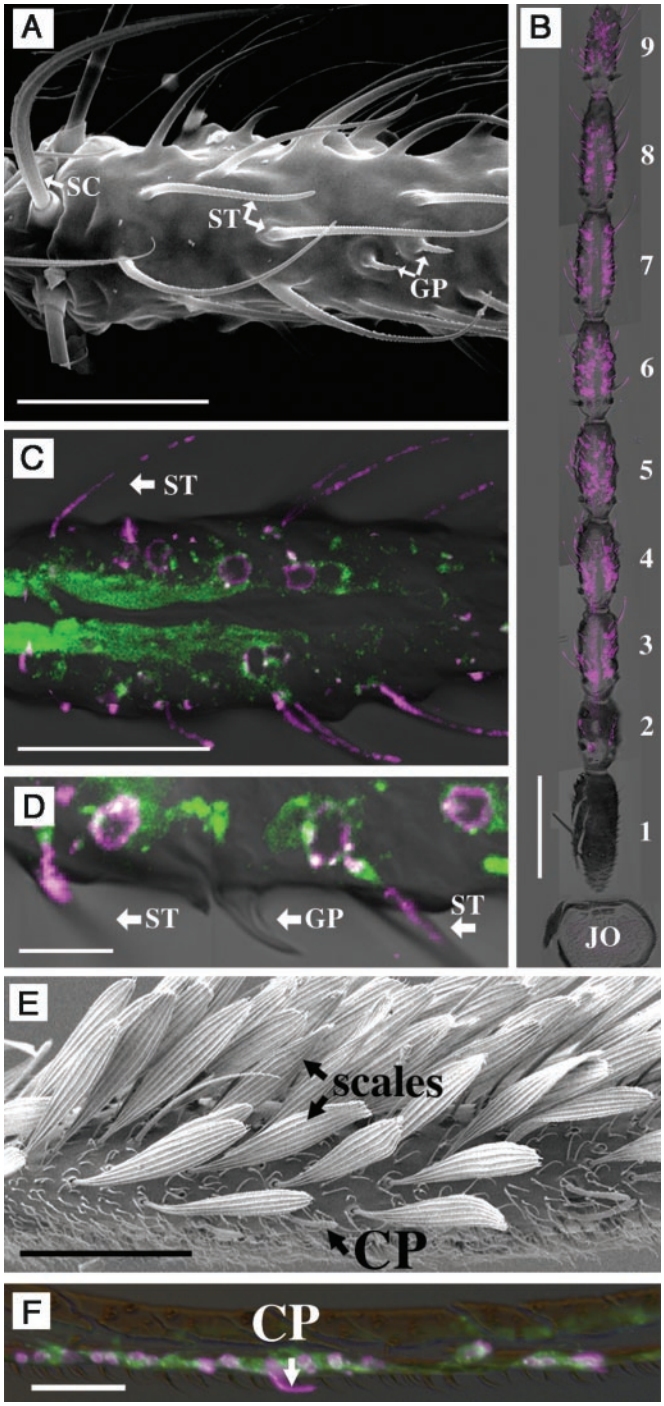

Fig. 3. Localization of the AgOR7 protein in *An. gambiae* female antenna and maxillary palp. Magenta is anti-AgOR7 marked with Cy3-labeled secondary antibody. Green is anti-horseradish peroxidase conjugated with FITC. (*A*) SEM of the 10th flagellar segment. SC, sensilla chaetica; ST, sensilla trichodica; GP, grooved peg. (Scale bar is 25 μm.) (*B*) Antenna AgOR7 labeling in flagellar segments 2–9. JO, Johnston's organ. (Scale bar is 100 μm.) (*C*) AgOR7 labeling in the trichodic sensilla of a single distal antennal segment. (Scale bar is 25 μm.) (*D*) AgOR7 labeling is absent from grooved peg sensilla. (Scale bar is $\approx$4 μm.) (*E*) SEM of a palp segment showing nonsensory scales and capitate peg (CP) sensilla. (Scale bar is 40 μm.) (*F*) AgOR7 labeling in a capitate peg of the third palpal segment. (Scale bar is 40 μm.)

Given the observation that *AgOr7* mRNA is expressed in the proboscis (Fig. 2), we sought to define the localization of the AgOR7 protein in this apparent gustatory organ. The proboscis

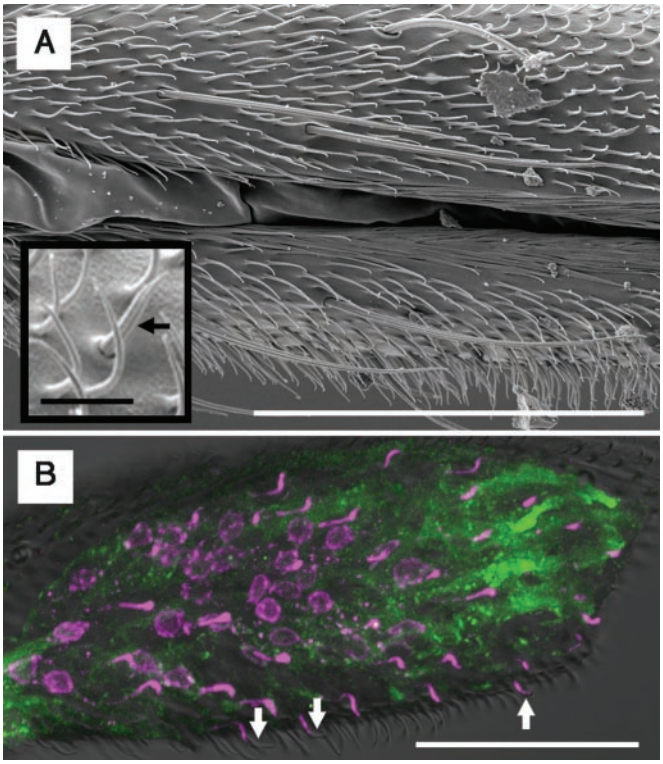

Fig. 4. Localization of the AgOR7 protein in *An. gambiae* female proboscis. Magenta is anti-AgOR7 marked with Cy3-labeled secondary antibody. Green is anti-horseradish peroxidase conjugated with FITC. (*A*) SEM of the labellum. (*Inset*) A higher-magnification SEM of the sensillum type that is labeled by anti-AgOR7 in *B*. (*Inset* scale bar is 5 μm.) (*B*) AgOR7 labeling in a distinct sensillar type (arrows) in one labellar lobe of the proboscis. (Scale bars are 50 μm.)

is a long slender organ housing the feeding stylets and extending anteriorally from the midline of the head and ending in a bulbous tip, the labellum, which is split into two halves called the labellar lobes (27) (Fig. 4*A*). As shown in Fig. 4*B*, a number of sensilla and cell bodies were labeled with anti-AgOR7 in female *An. gambiae*. The labeling was confined to an apparent single type of sensillum on the outer surface of the labellar lobes that has a wide base located in a socket and a short hair shaft ending in a point (Fig. 4*A Inset*). About 25 such sensilla could be counted on each lobe. These sensilla are reminiscent of the type 2 (T2) sensilla found on the proboscis of the mosquito *Culiseta inornata* (37). No labeling was observed along the proboscis shaft, and none of the numerous thin setae on the labellar lobes were labeled (Fig. 4*B*). This result confirms the observed expression of the *AgOr7* transcript in the proboscis and further defines the precise localization of the AgOR7 protein in a subset of proboscis sensilla.

Discussion

In this report we present a series of experiments designed to characterize *AgOr7*, a unique member of the OR gene family in a medically important mosquito species, *An. gambiae*. The striking conservation of this single receptor across a wide sampling of insect taxa (14, 17) is an important feature to consider when proposing a role for these genes in olfactory signaling in insects. Indeed, we conclude that these genes are orthologs on the basis of their sequence conservation, gene structure similarities, and microsynteny within the regions surrounding the genes in *An. gambiae* and *D. melanogaster* (Fig. 1 *A*, *B*, and *C*, respectively). It seems that considerable selective pressure has been placed on these organisms, which are separated by millions of years of evolutionary distance (38), to maintain the primary sequence of this gene. This pressure is especially apparent in the C terminus, where residue identity is highest. On the basis of its overall similarity to other ORs and its expression in olfactory tissues, the function of *AgOr7* is likely to be critical to olfaction, although, importantly, this point has yet to be supported experimentally.

In contrast to another study focused on *AgOr7* orthologs from other insect orders (16, 17), we have placed the TMVII domain slightly further upstream in the primary amino acid sequence, which allows for a longer C-terminal cytoplasmic tail. This placement is in better agreement with the predicted placement of TMVII in the *D. melanogaster* OR family (13). The hydrophobic stretch within the C terminus of AgOR7 may form an eighth helix that lies perpendicular to the plasma membrane, as was discovered in the crystal structure of the bovine rod rhodopsin protein (39–41). Importantly, C-terminal residues of G protein-coupled receptors (GPCRs) have been shown to interact with G proteins, G protein receptor kinases (GRKs), and arrestins required for downstream signaling (42, 43). A group of serines, threonines, and tyrosines are extremely well conserved (Fig. 1*A*, box) in this region and constitute potential phosphorylation sites that may be important for regulating AgOR7 protein function. Inferring the structure of an OR solely on the basis of the rhodopsin model, which is a highly specialized GPCR, is problematic (44, 45), and a better understanding of OR functional topology awaits both biochemical studies and the elucidation of one or more OR crystal structures.

AgOr7, not surprisingly, is highly expressed in olfactory organs in *An. gambiae*. This expression has now been examined at the levels of both the mRNA and the protein. Our findings indicate that AgOR7 is localized in sensilla and neuronal cell bodies in both the antennae and maxillary palps of mature adult females. This expression is limited to trichodic sensilla beginning in the second flagellar segment and continuing through the terminal 13th segment. Interestingly, AgOR7 was not observed in grooved peg or sunken peg sensilla. The grooved pegs of *An. gambiae* and *Aedes aegypti* are sensitive to a variety of odors, some of which are known kairomones for host seeking (46, 47). Therefore at this level of analysis, AgOR7 does not seem to be required for the olfactory sensitivity of grooved pegs in *An. gambiae*. We cannot exclude the possibility that AgOR7 is expressed in grooved pegs at a level below the sensitivity of our method. In any case, AgOR7 would not be excluded from participating in kairomone sensing on the basis of a lack of expression in grooved pegs because trichodic sensilla of *An. gambiae* are also sensitive to a wide variety of odors, including potential human kairomones (47, 48).

The observation reported here that *AgOr7* is robustly expressed in larval stages in *An. gambiae* (Figs. 2 and 6) is in agreement with Vosshall *et al.* (14), who observed that *DOr83b* was expressed in *D. melanogaster* larvae. The distal end of the larval antenna and maxillary palpus of mosquitoes contain several innervated, sensory-type structures that potentially function in both olfaction and gustation (27, 36). Specific expression of the AgOR7 protein in these tissues is consistent with their having a chemosensory function, although it remains unclear whether that role may be olfactory, gustatory, or both. A detailed study of these organs will be required to elucidate the specific contributions of each of these sensilla to chemosensory processes.

The demonstration in this report and others that orthologs of *AgOr7* are expressed beyond the known principal olfactory tissues has potential functional implications (17). We have used RT-PCR and immunolocalization studies to show that *AgOr7* mRNA and, more importantly, the AgOR7 protein is expressed in sensory neurons found on the proboscis of *An. gambiae* (Figs.

2 and 4). Morphological and electrophysiological studies of the proboscis of *A. aegypti* have shown that the sensilla residing in the labellum are of the contact chemosensory type (27, 30), whereas the T2 sensilla of *C. inornata* respond to salt and mechanical stimuli and are morphologically very similar to the AgOR7-expressing type in *An. gambiae* (37). Furthermore, the labellum of *D. melanogaster* and other dipterans has a gustatory function (49, 50) and is the morphological analog of the *An. gambiae* labellum. By extension, we propose that the proboscis of *An. gambiae* is a gustatory organ and therefore *AgOr7* may act broadly in chemosensory tissues, playing a similar role in both olfaction and some aspects of gustation. This gustatory function may not be unique to mosquitoes because an *AgOr7* ortholog, *HvirR2*, is also expressed in the proboscis of the noctuid moth (17).

In *An. gambiae*, a small subset of OR genes, including *AgOr7*, are expressed in legs (6), further supporting a role for *AgOr7* in gustatory signal transduction. To date, gustatory receptors (GRs) remain the only chemosensory receptor molecules whose expression has been shown in the legs of *D. melanogaster* (51, 52), where the tarsi house gustatory sensilla. Although GR expression patterns have yet to be described in *An. gambiae*, it seems likely that GRs would be expressed in tarsi and that the ORs in legs might function in a contact chemosensory pathway. Perhaps the *AgOr7* orthologs act independently of odorant/tastant binding *per se*, but are generally required for both OR and GR function, possibly as a general partner for their heterodimerization, as has been suggested previously (17, 26). In fact, *D. melanogaster* antennal neurons that express *DOr83b* in the absence of any other candidate OR do not respond to a large panel of tested odors (53). Further supporting a gustatory role for *DOr83b* is the finding that a *DOr83b* promoter (54) drives expression of a GFP reporter in sensilla in tarsi and wing margin sensilla of *D. melanogaster* in addition to its primary expression in the third antennal segment and maxillary palps (R.J.P. and L.J.Z, unpublished observations). In a general chemosensory model, orthologs of *AgOr7* and *DOr83b* would be used in both modalities of insect chemosensing, providing a further rationale for their extraordinary sequence conservation. Future study will be needed to determine whether these genes indeed have a gustatory role in insects. Defining the function of this and other ORs will provide a greater general understanding of olfaction in mosquitoes. This knowledge may, in time, facilitate the development of more sophisticated insect control strategies that rely specifically on olfactory methods.

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