

Insect Odorant Receptors: Channeling Scent

Tal Soo Ha¹ and Dean P. Smith^{1,*}

¹Departments of Pharmacology and Neuroscience, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-9111, USA

*Correspondence: dean.smith@utsouthwestern.edu

DOI 10.1016/j.cell.2008.05.007

Odorant detection in insects involves heterodimers between an odorant receptor (OR) and a conserved seven-transmembrane protein called Or83b, but the exact mechanism of OR signal transduction is unclear. Two recent studies in *Nature* (Sato et al., 2008; Wicher et al., 2008) now reveal that these OR-Or83b heterodimers form odorant-gated ion channels, revealing a surprising new mode of olfactory transduction.

The ability to detect and respond appropriately to chemical cues in the environment is essential for the survival of most animals. Indeed, large fractions of the coding capacities of animal genomes are dedicated to the expression of odorant receptors (ORs) for chemical detection. From nematodes to vertebrates, ORs are seven-transmembrane G protein-coupled receptors (GPCRs) that are activated through the binding of specific odor molecules. Binding of the appropriate ligand to classical GPCRs is thought to induce conformational changes in the GPCR that trigger G protein activation and second messenger generation. It seemed reasonable to assume that insect ORs would follow this signal transduction mechanism. Two recent studies published in *Nature* now show that far from following the classical GPCR pathway, ORs of the fruit fly *Drosophila melanogaster*, when bound to the common receptor subunit Or83b, may actually form odorant-gated cation channels (Sato et al., 2008; Wicher et al., 2008).

There were early hints that insect chemical transduction may be distinct from that of other olfactory model systems. The first was the lack of homology between insect ORs and those of other species. Nearly a decade after the first vertebrate ORs were identified, extensive homology-based cloning efforts in *Drosophila* still failed to identify any of the elusive insect chemoreceptors. Success was finally achieved through two approaches. First, a bioinformatics scan of the recently sequenced *Drosophila* genome revealed multiple

transmembrane protein candidates that were then tested for expression restricted to olfactory organs (Clyne et al., 1999; Gao and Chess, 1999). Second, a differential cDNA expression screen and large-scale sequencing effort identified putative fly ORs (Vosshall et al., 1999). Unexpectedly, once identified, the sequences of the *Drosophila* chemoreceptors were found to be as similar to those of ion channels as to those of the chemoreceptor proteins of other species. Characterization of the *Drosophila* ORs hinted that olfactory signaling was distinct in insects. Unlike classical GPCRs from most species, which share a common membrane topology with the protein N terminus at the cell surface and the C terminus within the cell, *Drosophila* ORs appeared to have the reverse membrane topology (Benton et al., 2006). This raised the question of how fly ORs could transduce signals after binding their odorant ligands.

The *Drosophila* OR family is encoded by 60 genes. Most members are “tuning” ORs that define the odorant specificity of the olfactory neuron in which they are expressed. As in vertebrates, *Drosophila* olfactory neurons expressing the same tuning OR innervate a common target (glomerulus) in the brain, suggesting that a similar logic underlies odor discrimination in vertebrates and insects. Also in the fly OR family is a protein called Or83b that forms heterodimeric complexes with the tuning ORs but is clearly distinct from them. Or83b is not an OR per se and, in the absence of a tuning OR, cannot confer odorant sensitivity (Elmore et

al., 2003). Or83b is widely expressed in most but not all olfactory neurons and is the only highly conserved OR protein among divergent insect species. This suggests that Or83b is under different selection pressure than other members of the OR gene family. Flies lacking Or83b have widespread defects in odorant detection because tuning ORs cannot be transported to the dendrites of olfactory neurons (Larsson et al., 2004). Interestingly, this observation is reminiscent of trafficking defects caused by mutations in multisubunit ion channels in cultured mammalian cells (Margeta-Mitrovic et al., 2000). The question remained whether Or83b is merely a chaperone for the tuning ORs or if it plays a more direct role in OR signaling.

Using patch-clamping to measure the ion currents in cultured vertebrate cells expressing fly ORs and Or83b, Wicher et al. (2008) and Sato et al. (2008) reveal a new mode of odorant signaling in insects. Both groups demonstrate that only coexpression of Or83b with a tuning OR confers odor-sensitive ion currents in the cultured cells (indicated by an inward flow of current), implying that the OR-Or83b heterodimer acts as a ligand-gated ion channel. Sato and colleagues used outside-out excised membrane patches to determine a single channel conductance of 20–30 picosiemens (pS) for several OR-Or83b heterodimer combinations in response to odor ligand, indicating that the OR complexes possess ligand-gated channel properties. Meanwhile, Wicher and coworkers found that mutations in Or83b could alter the permeability of

the odor-evoked currents. Both studies concluded that the OR-Or83b heterodimers form nonselective cation channels permeable to sodium ions, potassium ions, and calcium ions.

Several properties of the ion currents observed by the investigators could explain features of insect olfactory neurons in vivo. At high odorant concentrations, the ion current response is characterized by a short lag time between the addition of ligand and the observation of current flow (latency), consistent with a direct ligand-gated mechanism (Figure 1). Interestingly, even in the absence of ligand, these channels appear to be open a large fraction of the time. This could account for the relatively high spontaneous activity present in most insect olfactory neurons that allows them to use both increases and decreases in neuronal firing frequencies to encode odorant information (Hallem et al., 2004). Although both studies concluded that Or83b, together with an associated tuning OR, forms a ligand-gated cation channel, controversy lingers regarding exactly how the channel may function.

Wicher et al. showed that odorant stimulation triggers a cyclic nucleotide second messenger system, but Sato et al., undertaking similar experiments, came to the opposite conclusion. Wicher and colleagues observed a G protein-mediated rise in cyclic nucleotides upon odorant stimulation of the OR-Or83b heterodimer, and that the cyclic nucleotides appear to mediate gating of the heterodimer via Or83b. This increase in cyclic nucleotide concentration was dependent on expression of the tuning OR but not expression of Or83b (although Or83b was required for cyclic nucleotides to induce ion currents in the absence of other cyclic nucleotide-gated channels). The odor-induced production of cyclic nucleotides was blocked by the G protein inhibitor, GDP- β -S, suggesting that fly

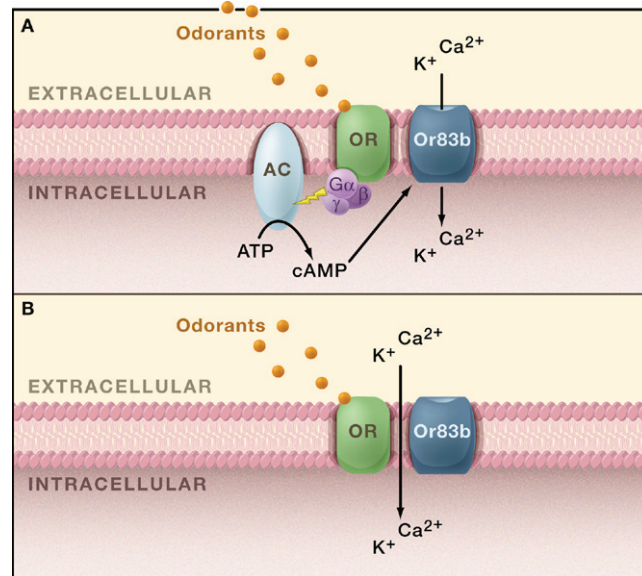


Figure 1. Two Models for Odor-Activated Cation Channels in Fly Olfactory Neurons

(A) Dual mode activation of a heterodimeric complex between the odorant receptor (OR) and Or83b (Wicher et al., 2008). At low odorant concentrations, OR activation triggers a relatively slow increase in cyclic nucleotide production that activates Or83b and allows the inward flow of current (increased membrane potential). At high odor concentrations, OR activation triggers rapid and direct ligand gating of the Or83b channel.

(B) Direct ligand-gated cation channel (Sato et al., 2008). Odor molecules activate the OR-Or83b complex directly without the need for a second messenger system. (G α , stimulatory G protein α subunit; AC, adenylyl cyclase.)

ORs might activate a cyclase (either adenylyl or guanylyl) and trigger production of cyclic nucleotides through G protein activation. Strikingly, GDP- β -S caused the odorant dose-response conductance curve to shift dramatically to the right. Wicher et al. propose a dual mode of OR activation with the second messenger pathway mediating sensitivity to low odorant concentrations and the direct gating mechanism operating only at higher odorant concentrations. These investigators suggest that cyclic nucleotides may directly gate Or83b, as a membrane-permeable cAMP mimic induced a membrane current similar to that induced by odorants. Furthermore, this induced current required Or83b but not the coexpression of an OR. The Wicher et al. study, however, does not show direct binding of cyclic nucleotides to membranes expressing Or83b. In their study, Sato et al. did observe cyclic nucleotide sensitivity in some OR-Or83b complexes, but they were unable to demonstrate odorant dependence despite performing similar experiments to Wicher et al. Obviously,

more work is necessary to elucidate the potential role of second messengers in this system. Both groups do agree, however, in the major finding that ORs are heteromeric ligand-gated cation channels, implying a distinct olfactory signal transduction strategy in insects. It will now be important to establish that this mechanism contributes to odor transduction in insects in vivo.

Not all fly olfactory neurons express Or83b. For example, the ab1c neurons found in the large basiconic sensilla (which are important for CO₂ detection) are unaffected by loss of Or83b. Also, ectopic expression of tuning ORs in *Xenopus* oocytes results in odor-specific ion currents in the absence of Or83b (Wetzel et al., 2001). Lastly, olfactory neurons lacking Or83b still have spontaneous activity in vivo. Taken together, these data suggest that Or83b-independent conductances also may be important in odorant signal transduction. As ab1c neurons express two gustatory receptors, Gr21a and Gr63a, could the theme of receptors as channels be more widespread? For example, it will be interesting to determine whether these two gustatory receptors form ion channels that are gated by CO₂ in the absence of Or83b, and whether a second messenger pathway is involved. These two new provocative studies raise many questions. It will be very interesting to see where this story of insect olfactory signal transduction goes. On the practical side, if insects use these unique ligand-gated cation channels for odor reception, then inhibitors of these channels may prove valuable as a new class of insect repellents.

REFERENCES

- Benton, R., Sachse, S., Michnick, S.W., and Vosshall, L.B. (2006). PLoS Biol. 4, e2010.1371/journal.pbio.0040020.
- Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing,

D., Kim, J., and Carlson, J.R. (1999). *Neuron* 22, 327–338.

Elmore, T., Ignell, R., Carlson, J.R., and Smith, D.P. (2003). *J. Neurosci.* 23, 9906–9912.

Gao, Q., and Chess, A. (1999). *Genomics* 60, 31–39.

Halle, E.A., Ho, M.G., and Carlson, J.R. (2004). *Cell* 117, 965–979.

Larsson, M.C., Domingos, A.I., Jones, W.D., Chiappe, M.E., Amrein, H., and Vosshall, L.B. (2004). *Neuron* 43, 703–714.

Margeta-Mitrovic, M., Jan, Y.N., and Jan, L.Y. (2000). *Neuron* 27, 97–106.

Sato, K., Pellegrino, M., Nakagawa, T., Nakagawa, T., Vosshall, L.B., and Touhara, K. (2008). *Nature* 452, 1002–1006.

Vosshall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., and Axel, R. (1999). *Cell* 96, 725–736.

Wetzel, C.H., Behrendt, H.J., Gisselmann, G., Stortkuhl, K.F., Hovemann, B., and Hatt, H. (2001). *Proc. Natl. Acad. Sci. USA* 98, 9377–9380.

Wicher, D., Schafer, R., Bauernfeind, R., Stensmyr, M.C., Heller, R., Heinemann, S.H., and Hansson, B.S. (2008). *Nature* 452, 1007–1011.

Chromatin Proteins Do Double Duty

Sevinc Ercan¹ and Jason D. Lieb^{1,*}

¹Department of Biology and Carolina Center for the Genome Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599, USA

*Correspondence: jlieb@bio.unc.edu

DOI 10.1016/j.cell.2008.05.011

The histone acetyltransferase MOF (males-absent-on-the-first) is required for the regulation of X chromosome gene dosage compensation in *Drosophila* males. In this issue, Kind et al. (2008) show that MOF is also found on autosomes and that it has two modes of binding: one in males for X chromosome dosage compensation and the other in both sexes for X chromosome and autosomal gene regulation independent of dosage compensation.

The information encoded in genomes is interpreted by proteins that associate with specific chromosomal locations. In dissecting the function of these chromosome-associated proteins, a common assumption has been that a given protein performs a single function in the genome. However, there is a growing realization that a single protein can often be directed to separate regions of the genome by independent mechanisms to perform distinct biological functions. In this issue of *Cell*, Kind et al. (2008) uncover an example of just such a system in the fruit fly *Drosophila melanogaster* by examining the genomic binding distribution of several proteins, including the histone acetyltransferase MOF (males-absent-on-the-first), that are important for X chromosome gene dosage compensation.

MOF, an enzyme that acetylates histone H4 at lysine 16 (H4K16), is a component of the *Drosophila* dosage compensation complex. This complex also includes the male-specific lethal proteins 1–3 (MSL1, MSL2, MSL3), the maleless (MLE) protein, and two noncoding RNAs

called *roX1* and *roX2* (Straub and Becker, 2007). This complex binds to the single X chromosome of male flies, increasing expression of many X-linked transcripts 2-fold such that expression is equivalent to that in female flies, which have two X chromosomes. Although the detailed mechanism of how the dosage compensation complex acts on the male X chromosome is not clear, it has been proposed that it may promote more efficient transcriptional elongation, possibly by boosting acetylation of H4K16. Indeed, H4K16 acetylation has been shown to “loosen” the conformation of chromatin *in vitro* (Shogren-Knaak et al., 2006).

Previous studies have found that MSL1 and MSL3 are associated with the transcribed regions of genes, with a binding pattern that is skewed toward the 3′ ends of genes. Therefore, it was perhaps not surprising that Kind et al. (2008) observed that MOF, like the MSL proteins, was also enriched at the 3′ ends of X-linked genes in a dosage compensation-dependent manner. More surprising, however, was their observation of a second mode of MOF binding

that was completely independent of dosage compensation, and was found at gene promoters on autosomes and the X chromosome. Previously, MOF had been singled out as an especially interesting member of the dosage compensation complex because it was shown to associate with several components of the nuclear pore (Mendjan et al., 2006). This finding raised the intriguing possibility that MOF performs functions other than dosage compensation at other chromosomal locations. Kind and colleagues now show that MOF does indeed have two functions: a general role in both sexes at the gene promoters located on either autosomes or the X chromosome, and a male-specific role at the 3′ regions of transcribed X-linked genes (Figure 1).

The authors performed several experiments in fly tissue culture cells to build their case that MOF has two different functions. In addition to mapping MOF localization, they determined the binding sites of MSL1 and MSL3, as well as the locations of H4K16 acetylated nucleosomes using ChIP-chip. The analysis of male cells containing a single X