Olfaction in *Drosophila*

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The fruit fly, *Drosophila melanogaster*, is equipped with a sophisticated olfactory sensory system that permits it to recognize and discriminate hundreds of discrete odorants. The perception of these odorants is essential for the animal to identify relevant food sources and suitable sites for egg-laying. Advances in the last year have begun to define the molecular basis of this insect's discriminatory power. The identification of a large multi-gene family of candidate *Drosophila* odorant receptors suggests that, as in other animals, a multitude of distinct odorants is recognized by a diversity of ligand-binding receptors. How olfactory signals are transduced and interpreted by the brain remains an important question for future analysis. The availability of genetic tools and a complete genome sequence makes *Drosophila* a particularly attractive organism for studying the molecular basis of olfaction.

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Abbreviations

acj6	*abnormal chemosensory jump 6*
amos	*absent md neurons and olfactory sensilla*
bHLH	basic helix-loop-helix
CNGC	cyclic-nucleotide-gated channel
DOR	*Drosophila* OR
OBP	odorant binding protein
OR	odorant receptor
ORN	olfactory receptor neuron
trp	*transient receptor potential*

Introduction

In insects, olfaction is a crucial sensory modality for controlling many aspects of behavior. Mate selection, food choice and navigation toward suitable oviposition sites all depend on a functioning sense of smell. Despite their small size, insects have olfactory systems of surprising sensitivity. Male moths are able to perceive inordinately small amounts of pheromone emitted by conspecific females at a great distance and initiate stereotyped flying behaviors in pursuit of the female (for a review, see [1]). The fruit fly, *Drosophila melanogaster*, has been of particular interest for the study of olfaction because of its suitability for genetic manipulation, the recent availability of a complete genome sequence [2••,3••], and its ability to learn in simple olfactory-based associative learning paradigms. Advances in the past year have begun to provide molecular insights into how these animals interact with their sensory environment. This review will focus on the recent identification of candidate *Drosophila* odorant receptors, advances in understanding signal transduction and physiology of the olfactory neuron, mechanisms of odor coding peripherally and centrally, and the link between olfaction and behavior.

Candidate odorant receptors identified

Nearly 10 years after the first report of odorant receptors (ORs) in any species [4], candidate *Drosophila* odorant receptor genes (DORs) have been identified ([5••,6••]; see also [7]). In hindsight, this time lag seems understandable: the DORs have no primary sequence identity with either vertebrate or *C. elegans* receptors and are present at exceedingly low levels in olfactory cDNA libraries. DOR genes were ultimately identified by a combination of differential screening [6••] and analysis of *Drosophila* genomic DNA databases [5••,6••]. Sixteen DOR genes, encoding proteins of approximately 400 amino acids with seven predicted membrane-spanning domains, were identified in a search of approximately 20% of the genome [5••,6••]. This suggested that the complete repertoire would consist of at least 100 DOR genes. However, subsequent analysis of the complete sequence of the eukaryotic fly genome revealed a total of only 60 genes with homology to the DORs [2••,3••] — significantly fewer than the nearly 1000 candidate chemosensory receptor genes in the *C. elegans* [8] and mouse genomes [9]. The DOR genes initially reported are extremely divergent, with an average amino acid identity of approximately 20% [5••,6••]. Analysis of the entire repertoire of receptors reveals a number of subfamilies with significantly greater sequence identity [2••,3••]. Functional evidence that the DOR genes in fact encode the *Drosophila* ORs will require either genetic loss-of-function analysis, as first shown for the nematode ODR10 diacetyl receptor [10], or functional expression in heterologous systems [11–14,15••,16]. Consistent with a role in odor recognition, some, but not all, DOR genes are expressed in small subsets of olfactory receptor neurons (ORNs) in either the antenna or maxillary palp [5••,6••], the olfactory sensory organs of the adult fly [17]. There have been no reports of DOR gene expression in the larva, in which 21 ORNs in the dorsal organ recognize olfactory cues (for a review, see [17]). There is also no detectable expression of DOR genes in taste neurons in the proboscis or contact chemoreceptors of the leg and wing (LB Vosshall, K Scott, R Axel, unpublished data). Therefore, it is likely that the DOR genes are dedicated to the perception of volatile odorants in the adult fly and that a distinct gene family recognizes pheromones and tastants. In fact, candidate *Drosophila* gustatory receptors that bear no sequence identity to the DOR genes have recently been described [18].

Odorant binding proteins – olfactory proteins of unknown function

The olfactory sensory organs of *Drosophila* are decorated with sensory hairs called sensilla. The ORNs extend dendrites into the sensilla, which are bathed in a sensillar

lymph. Present at high concentration in this fluid are members of a large family of odorant binding proteins (OBPs) of unknown function. These proteins may solubilize hydrophobic odorants in the aqueous sensillum lymph, present these odorous ligands to the receptor, or assist in terminating the odor response by removing ligands from the receptor [19–21]. There are at least 30 of these small secreted proteins in *Drosophila* [2••,3••,22,23], and there is extensive phylogenetic conservation of OBPs among insects [24–26]. The large number of OBPs and their expression in small subdomains of the *Drosophila* antenna has prompted some discussion of a direct role for OBPs in odor recognition [21–23]. The first functional evidence that OBPs indeed participate in olfactory responses came from analysis of *Drosophila* mutants that lack the LUSH OBP [21]. The LUSH protein is encoded by the *lush* gene, so named because its mutant phenotype is an abnormal attraction to high concentrations of ethanol. *Lush*$^{-/-}$ mutant flies fail to be repelled by high concentrations of ethanol [21]. However, functional studies of vertebrate and nematode ORs expressed in heterologous systems indicate that receptors can be activated by ligand in the absence of OBPs [11,13,14,15••,16]. This argues against a model of OBP function in which the odorant receptor recognizes a ligand–OBP complex.

Transduction of the olfactory signal

How olfactory signals are transduced in *Drosophila* is unknown. Olfactory neurons in other animals use cyclic nucleotides or IP_3 as second messengers [27,28]. In vertebrate and some *C. elegans* ORNs, ORs couple to G proteins to increase cAMP and cGMP, respectively; this leads to gating of a cyclic-nucleotide-gated channel (CNGC). Mice deficient in either G_{olf} (the $G\alpha_s$ subunit expressed at high levels in olfactory cilia and thought to stimulate increases in cAMP [29]) or the CNGCα subunit [30] are anosmic (i.e. lacking olfactory function). Similarly, nematodes with mutations in various chemosensory-specific G proteins [31•,32] and CNGC subunits [33,34] have chemosensory defects. A second mode of signal transduction, involving a Ca^{2+} channel related to the *Drosophila* TRP (*transient receptor potential*; *trp*) protein, operates in distinct *C. elegans* chemosensory neurons [35]. Although components of the cAMP and IP_3 signaling pathways have been identified in *Drosophila*, evidence for an essential role in olfactory signal transduction has not emerged [36–42]. IP_3 may contribute to modulation of olfactory signaling in the fly, as mutants defective in various points in the signaling pathway display subtle olfactory behavioral deficits [41,42]. However, the lack of clear anosmic phenotypes in signal-transduction mutants studied thus far suggests either that multiple second-messenger systems operate in *Drosophila* olfaction, or that the essential signaling components remain to be identified. Olfactory adaptation depends upon normal Ca^{2+} homeostasis, as animals mutant for either the *trp* channel or the IP_3 receptor have normal olfactory responsivity but show defects in adaptation [39,43]. With putative *Drosophila* ORs in hand, it should now be possible to identify downstream signaling components either through genetics or functional genomics.

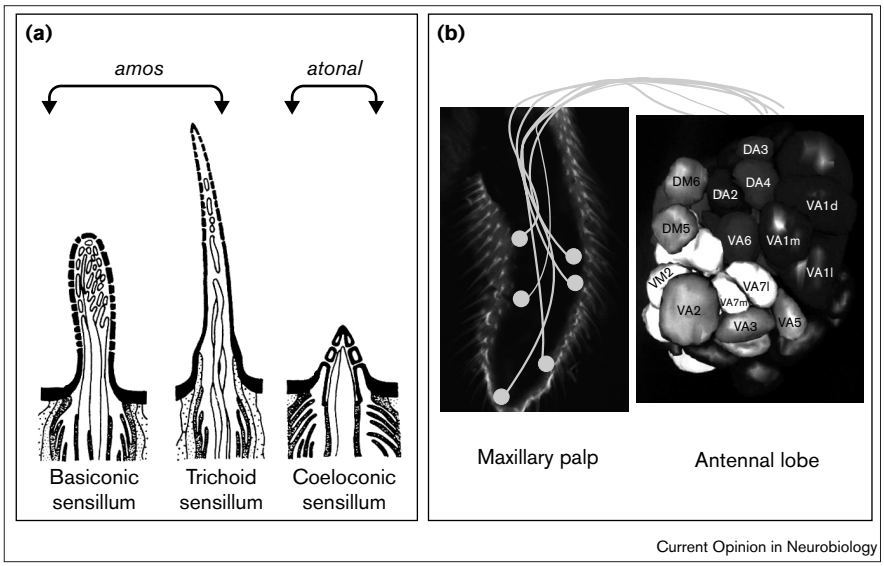
Single unit physiology reveals an odor code in peripheral olfactory sensitivity

Although we do not understand how olfactory signals are transduced, much is known about the response properties of individual sensilla to odorous ligands. *Drosophila* ORNs, one to four of which reside beneath each sensillum, have proven too small for routine physiological analysis (although see [44]). Therefore, functional studies have relied on extracellular recordings that measure changes in electrical potential in the lymph surrounding ORN dendrites. By analyzing the amplitudes of responses, activation of individual neurons can be inferred from the extracellular recordings. Such experiments demonstrate that there are regional differences in olfactory sensitivity across the fly's olfactory organs and that a diversity of response properties can be found when examining individual sensilla [45,46••,47,48••]. de Bruyne *et al.* [48••] used these single-unit recording techniques to characterize all 60 sensilla on the surface of the maxillary palp. Six functional classes of sensilla were defined and together they are believed to account for all possible responses by the 120 ORNs in this organ. The results proved to be of surprising complexity: a given odorant could activate one neuron and inhibit another, while a single neuron exhibited both excitatory and inhibitory responses to different odorants [48••].

Odor coding and the map of olfactory receptor gene expression

What renders ORNs functionally distinct, with characteristic response properties? In the mouse, there is good evidence that ORs are the primary determinants of the functional properties of the ORN. In the mouse, each ORN is thought to express a single OR, whose ligand-specificity controls the responsivity of the cell [11,15••,16]. In contrast, in *C. elegans*, each neuron expresses multiple chemosensory receptors [49]. The activation of one class of ORNs through any of its resident receptors produces an attractive response (i.e. the nematode moves toward the chemical source), whereas a second class mediates repulsion [50]. Therefore in the nematode, the ORN is the determinant of the behavioral response, not the receptor. Whether the fly olfactory system uses the organizational logic of the mouse or the nematode is still a subject of debate. Clyne *et al.* [5••] argue for the expression of multiple DOR genes per ORN on the basis of a projected total of 18 DOR genes in the maxillary palp, an organ described to have only six functional types of cells [48••]. Vosshall *et al.* used fluorescent RNA *in situ* hybridization to rule out co-expression of several antennal DOR genes [6••] and statistical estimates to argue for expression of one or a small number of DOR genes per ORN. The limited number of DOR genes in the complete *Drosophila* genome [2••,3••] should permit direct experimental proof of either model in the near future.

Figure 1



The anatomical specializations of the *Drosophila* olfactory system: three types of sensory hair and 43 glomeruli. **(a)** Three morphologically distinct sensory hairs cover the surface of the *Drosophila* antenna (adapted from [17]). There are between one and four ORNs and associated support cells underneath each sensillum. The neurogenic genes *amos* and *atonal* are required for the formation of trichoid/basiconic and coeloconic sensilla, respectively [53,54•,55•]. The expression of *amos*, and the development of the basiconic and trichoid sensilla, is dependent on the Runt-domain transcription factor encoded by the *lozenge* gene [54•]. **(b)** How ORN projections from antennal and maxillary palp cells expressing a given DOR gene are organized in the antennal lobe is not known. The availability of discrete molecular markers of subsets of olfactory neurons, the DOR genes, and a complete map of antennal lobe glomeruli [58••], will greatly facilitate the analysis of ORN projections. In this illustration, hypothetical neurons expressing a given receptor in the maxillary palp are represented. The antennal lobe diagram is derived from Figure 2 of Laissue *et al.* [58••]. Whether ORNs expressing a given DOR gene converge upon a limited number of antennal lobe glomeruli is currently unknown.

Consistent with a role in odorant recognition, DOR genes are expressed in small numbers of ORNs distributed in spatially invariant patterns on the antenna or maxillary palp [5••,6••], reminiscent of the maps of odor coding defined by electrophysiology [45,46••,47,48••]. In the case of the maxillary palp, the number of cells expressing a given DOR gene is in rough accord with the number of cells in each functional class of neuron defined electrophysiologically. The implication is that if DOR genes encode ligand-binding odorant receptors then there should be a correlation between receptor expression and the functional physiological properties of the neuron expressing a particular receptor [5••,48••]. Interestingly, flies mutant for the POU homeodomain protein encoded by the *acj6* (*abnormal chemosensory jump 6*) gene have altered maxillary palp responses [46••]. Two of the six classes of palp neurons are lost and another two are transformed into a neuron with novel chemical specificity [46••]. This phenotype may be due in part to changes in DOR gene expression, as two maxillary palp receptors are missing in *acj6* mutants [5••]. Therefore, DOR gene expression may be regulated in part through the action of a combinatorial of transcription factors that include *acj6* [5••,46••]. Regulatory sequences responsible for directing proper spatial expression of DOR genes have been shown in one study to lie immediately upstream of the DOR coding region [6••].

Specification of olfactory organs: the missing neurogenic gene revealed

Underlying the molecular and behavioral complexity of the fly olfactory system is a diversity of cell types. The surface of the antenna is covered with three morphologically distinct sensory hairs, the basiconic, trichoid, and coeloconic sensilla [17] (see Figure 1a). Neurons associated with all three types have recently been shown to respond to olfactory stimuli [47], although DOR gene expression has not been confirmed in all sensillar types [5••,6••]. These three sensillar types arise through diverse mechanisms during development [51,52]. Rodrigues and colleagues have described two stages in the development of olfactory sense organs: the determination of a founder cell in the antennal imaginal disc, followed by recruitment of additional cells to form a pre-sensillum cluster, from which the mature sensillum develops [51,52]. Activity of the proneural basic helix-loop-helix (bHLH) protein coded by *atonal* is required only for the formation of coeloconic sensilla, but not for the other two types of sensilla [53]. (Mutants of the *atonal* gene lack the chordotonal organ, the internal stretch receptors of the embryo, and hence are 'atonal'.) A series of experiments by Rodrigues and co-workers suggest that the proneural gene required for the determination of basiconic and trichoid sensilla is an unidentified bHLH protein [53]. Fulfilling this prediction, two groups recently succeeded in identifying a candidate for this missing proneural gene, called *amos* (*absent md* [*multiple dendritic*] *neurons and olfactory sensilla*), using degenerate PCR against conserved residues in the *atonal* branch of the bHLH family [54•] and yeast two-hybrid screening [55•]. *amos* is expressed in proneural clusters in the developing embryo [54•,55•] and in domains of the antennal imaginal disc that give rise to olfactory sensilla [54•]. Gain-of-function and synthetic loss-of-function experiments have confirmed the proneural activity of this bHLH protein in patterning the embryonic nervous system and the antenna [54•,55•]. Previously, the Runt-type transcription factor, *lozenge*, was shown to be required for the dose-dependent formation of all basiconic

sensilla and a subset of trichoid sensilla [56]. (These genes are also named descriptively after their mutant phenotype. The *runt* mutation produces a shortened embryo. The *lozenge* mutation affects the adult eye by making it smaller, of a novel oval shape, and glossy.) The mechanism of this effect of *lozenge* on sensillar patterning is nicely provided by new data showing that *lozenge* regulates levels of *amos* expression [54•]. Therefore, the precise interplay between the proneural genes *atonal* and *amos* and the prepattern gene *lozenge* results in the correct patterning of the fly's olfactory organs (Figure 1a).

A new map of the antennal lobe and the logic of central olfactory coding

How is information from functionally distinct ORNs in the antenna and maxillary palp relayed to the brain in a meaningful way? Approximately 1500 ORNs, expressing a combinatorial of about 60 DOR genes, extend axons and make synapses with central projection neurons in the antennal lobe of the brain. In the fly, as in all arthropods and vertebrates, these synapses are arranged into discrete structures called glomeruli [28]. In the mouse, ORNs expressing a given OR project with precision to two of a possible 1800 glomeruli in the olfactory bulb, the vertebrate equivalent of the antennal lobe [9,57]. This creates a map of olfactory receptor activation in the brain, such that the activation of randomly dispersed olfactory neurons in the peripheral sensory epithelium is translated into a focal point of activation in the olfactory bulb. We do not know if similar logic of convergence is employed in the organization of the *Drosophila* olfactory system (Figure 1b), but the number of DOR genes [2••,3••] approximates the numbers of glomeruli, as it does in vertebrates [9]. The total number of olfactory glomeruli in *Drosophila* has recently been determined in a study that generated a high-resolution three-dimensional map of the antennal lobe [58••]. There are 43 morphologically distinct glomeruli in *Drosophila* and these are largely conserved between individuals and are not sexually dimorphic. Recent functional studies in insects and rodents suggest that small combinatorials of glomeruli are activated in response to a given odorant, and that these patterns of glomerular activation are invariant between individuals [59•,60]. Studies performed at the level of a single vertebrate ORN suggest that a given OR can bind multiple odorants and that a single odorant interacts with multiple receptors [15••]. Therefore, odor discrimination may be accomplished by activation, by a given odorant, of a distinct subset of ORs that project to a unique combinatorial of glomeruli. Whether the decoding of olfactory information by higher brain centers relies strictly upon spatial information encoded in the glomerular map, or whether temporal coding is used, is unknown and remains a point of controversy in the field (see [61]). The prospects for distinguishing between these models of olfactory perception are excellent in *Drosophila* because it will soon be possible both to describe the complete molecular and cellular components of the olfactory system and to manipulate them genetically.

Smell and behavior

Behavioral genetic screens have yielded a number of interesting mutations that affect olfactory behavior in *Drosophila* (reviewed in [62]), but in many cases the underlying genes responsible for the behavioral deficits are still unknown. Now that the complete sequence of the *Drosophila* genome is available [2••,3••] it will be possible to unite the genetics of olfaction with the new map of the genome. This process will undoubtedly expand our knowledge of the development and function of the olfactory system in *Drosophila*.

Behavioral experiments conducted by Hardin's group have yielded the surprising result that olfactory sensitivity in *Drosophila* varies with the time of day [63••]. Antennal responsivity to ethyl acetate was found to be strongest during the period of subjective night [63••], a period when flies exhibit reduced locomotor activity and a sleep-like resting state [64,65]. This rhythm in olfactory response was self-sustained in periods of constant darkness and abolished by mutations that disrupt the circadian clock [63••]. The cellular mechanism for this electrophysiological rhythm is currently unknown. Enhanced olfactory function during periods of rest may have adaptive value. Arousal at the approach of a predator during a vulnerable period of rest or exploitation of food sources during a period of low competition are two scenarios where this phenomenon may be of value.

Conclusions

Fruit flies identify and interpret volatile odorants in their environment with a complement of approximately 1500 ORNs and central olfactory circuits far simpler than those of vertebrates. The long-awaited identification of candidate ORs in *Drosophila* opens up this system to a fuller functional characterization. The functional relationship between diverse sensilla, determined by a cascade of proneural genes, and the DOR genes expressed in the underlying neurons remains to be understood. Ligand–receptor interactions will need to be defined, as will our understanding of how receptor activation is transduced. The wiring of functionally distinct subpopulations of ORNs to the newly defined 43 olfactory glomeruli of the antennal lobe will be of interest. Finally, the complete genome sequence of this insect will be a valuable resource in all future endeavors that seek to relate cellular and organismal behaviors to underlying molecular and genetic mechanisms.

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