Commentary

Functional expression of a *Drosophila* odor receptor

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Insect olfaction has been a field of intense interest for two reasons. First, insect olfactory systems are well suited for investigating basic principles of olfactory system function and development, which are remarkably well conserved across phylogeny (1). Second, insects cause enormous losses to world agriculture and carry some of the world's most devastating diseases. Because many insects locate their plant and human hosts via olfactory cues, understanding the molecular basis of insect olfaction may lead to new approaches to insect control.

Odorant receptors are central to an understanding of odor sensitivity and discrimination. After many years of effort, a large gene family encoding candidate odor receptors was identified in *Drosophila melanogaster* (2–4) by using a novel computer search algorithm (5) or other methods. This family, the *Or* family, fulfills many of the criteria expected of odor receptor genes. It encodes a large family of seven-transmembrane domain proteins; individual members are expressed in small subsets of olfactory receptor neurons (2–4, 6); the number and distribution of neurons expressing a particular *Or* gene resemble the number and distribution of neurons exhibiting a particular odor response spectrum (7, 8); a mutation that alters the expression of *Or* genes also alters the odor response profiles of neurons (2, 9); neurons expressing an individual gene converge on common glomeruli in the antennal lobes of the brain (6, 10). What has been missing, however, to establish definitively these genes as odor receptor genes is a direct demonstration of function. This critical advance is now provided in two complementary papers in this issue of PNAS (11, 12).

Stortkuhl and Kettler overexpressed the *Or43a* gene in the fly antenna and tested for an increase in odor response *in vivo* (11). *Or43a* is normally expressed in ≈15 olfactory receptor neurons (ORNs) of the antenna, but the authors were able to drive its expression in a high fraction of the ≈1,200 antennal neurons by using the GAL4/UAS system. They then found a concomitant elevation in antennal response to a subset of odors, as measured by electroantennograms (EAGs), which are extracellular recordings of the receptor potentials of populations of neurons. Stortkuhl and Kettler found that overexpression of the *Or43a* gene conferred increased response to cyclohexanol, cyclohexanone, benzaldehyde, and benzyl alcohol, each of which contains a six-member carbon ring with a single attached polar group. Responses to several other tested odorants, including some others containing six-member rings, were unaffected. The logic of this experiment follows that of Stuart Firestein and colleagues, who used an adenovirus vector system to overexpress a mammalian odor receptor in the rat olfactory epithelium and measured elevated physiological responses to octanal and some related odorants (13, 14).

In Hans Hatt's laboratory, Wetzel *et al.* (12) found results that nicely complement those of Stortkuhl and Kettler by expressing the *Or43a* receptor in a heterologous system. *Or43a* was expressed in *Xenopus* oocytes, and responses were measured by two-electrode voltage–clamp recordings. Again, cyclohexanone, cyclohexanol, benzaldehyde, and benzyl alcohol elicited responses, with cyclohexanone and cyclohexanol inducing currents at concentrations as low as 500 nM. Six structurally related odorants and two unrelated odorants had no effect.

Taken together, these results provide direct functional evidence that an *Or* gene does, in fact, encode a *bona fide* odorant receptor. Moreover, the results are interesting in a number of other respects. The *Xenopus* oocytes responded to odors in the absence of an insect odorant-binding protein (OBP). OBPs are another large family of divergent proteins that have been identified in the olfactory systems of a variety of insects (15), including *Drosophila* (16, 17). They are present at high concentrations in the aqueous lymph surrounding the dendrites of ORNs (18, 19), and a mutation of one has been found to affect odor response in *Drosophila* (20, 21). OBPs are widely believed to play a role in the delivery of hydrophobic odorants through the hydrophilic lymph to odor receptors. However, there is little evidence to support a specific mechanism, and alternative models, including a role in the termination of odor response, have been proposed (22). The results of Wetzel *et al.* (12) indicate that an odor receptor expressed in *Xenopus* oocytes is capable of responding to odorants with a substantial degree of sensitivity and specificity in the absence of insect OBPs. Whether the presence of OBPs affects the kinetics or other parameters of the ligand–receptor interaction *in vivo* remains to be seen.

In a similar vein, one member of the *Or* family, *Or83b*, was found to be expressed in all or most ORNs (6), suggesting that it might encode a heterodimerization partner of all other members of the family. However, the results of Wetzel *et al.* (12) indicate that *Or43b* can respond to odors in the absence of *Or83b* expression.

A related implication of the results of Stortkuhl and Kettler (11) is that an odor receptor apparently is able to function in ORNs that normally express a different receptor. Thus *Or43a* is evidently able to couple with the G protein and other signaling components present in at least some other neurons, as has also been found in *Caenorhabditis elegans* (23). Unless the expression of *Or43a* represses expression or function of the endogenous receptor, the results also suggest that at least some ORNs can support the function of two receptors in the same cell. Moreover, the *Or43a* receptor can function in a sensillum that likely contains OBPs different from those normally in proximity to it.

The degree of increase in odor response observed by Stortkuhl and Kettler (11) in the fly antenna after overexpression of *Or43b* is interesting. Although the number of neurons expressing *Or43a* is increased dramatically (by one to two orders of magnitude, apparently), the amplitude of the EAG response is increased only mod-

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estly (2-fold at high concentrations and not at all at lower concentrations). How can this discrepancy be explained? The EAG represents a local response and not the response of the entire antenna. The response to a particular odor depends on the location on the antenna from which the recording was taken and is believed to represent the summed receptor potentials of a limited number of neurons in the vicinity of the recording electrode (24). In the wild type, *Or43a* is expressed only at the distal edge of the antenna, and because the recordings of Stortkuhl and Kettler were evidently made in a different location, it seems likely that their recordings in wild type reflect the activity of receptors other than *Or43a*. By contrast, the recordings from antennae that overexpress *Or43a* reflect the summed activity of these other receptors and the ectopically expressed *Or43a*. That the response is elevated only at high odor concentra-

tions suggests that *Or43a* is a low-affinity receptor for the tested odorants. ORNs in *Drosophila*—and presumably the receptors that they express—vary a great deal in their dose–response curves for a particular odorant, with some showing much greater sensitivities than others (7, 8). It seems likely that *Or43a* has a higher affinity for an odorant other than those tested to date.

The results of these papers provide a foundation for a great deal of future work, including a more detailed examination of the specificity of *Or43a* and other receptors. The work may also set the stage for a developmental analysis: does the functional expression of a *Drosophila* odor receptor have any effect on the pattern of axonal projections (25)? Finally, the results invite behavioral investigation. Does increasing the expression of *Or43a* have an effect on the animal's response to this receptor's ligands?

The demonstration of function for an *Or* gene is a major advance in the field. It may now seem, in retrospect, that the identity of the *Or* genes as odor receptors was already clear, given the abundant circumstantial evidence that had been collected previously, and the prior demonstrations of function for odor receptors in other species (13, 26, 27). However, this misapprehension calls to mind the "illusion of retroactive determinism" described by French philosopher Henri Bergson. Only after functional tests, such as those of Stortkuhl, Wetzel, and colleagues, can the identity of receptor genes be clearly established. Moreover, the results of these studies provide an important foundation for further exploration of olfaction in the model insect *Drosophila*.

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