Olfaction: **Scents and sensibility**

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Expression of a receptor protein has, for the first time, been definitively correlated with sensitivity to a particular odorant. This receptor, expressed in the nematode *Caenorhabditis elegans*, appears to be distinct from the putative vertebrate odorant receptors.

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Here is the problem: 1000 receptors and 10 000 ligands, match them up and find a way to make sense of the result. This is what the vertebrate olfactory system manages to accomplish by discriminating between at least this many odorous molecules. When putative odorant receptor genes were identified and cloned some five years ago, one of the big surprises was that the gene family, with as many as 1000 members, was second in size only to that devoted to the immune response [1]. What does this mean for understanding the olfactory neural code? How much of the discrimination occurs at the level of the peripheral receptors, and how much is the result of the combinatorial processing of multiple inputs in higher brain centers?

Although it was thought that answers to these critical questions would soon be forthcoming after the cloning of odorant receptor genes, a crucial piece of information remained obdurately lacking: how many odors, and of what types, do each of these receptors recognize? From physiological recordings, it has been known for some time that individual olfactory neurons typically respond to anywhere from three to ten odors [2,3]. To do this, they must express either multiple receptors or a single receptor that is promiscuous in its binding selectivity. If, as most investigators in the field believe, the latter option is more likely, what are the parameters that determine how many and which odorants a receptor will bind? Are they related chemically, and if so how? Nothing in the physiological literature gives a hint about this. The difficulty is that we know precious little about the 'pharmacology' of odorant receptor proteins. Yet without this piece of the puzzle, there is little hope of understanding the neural processing that gives rise to the exquisite discriminations made by the olfactory system.

Why is it that we know a great deal about odorant receptor genes, but very little about the proteins they encode? This is primarily because odorant receptor proteins have been frustratingly resistant to expression in a heterologous system. In only one reported case has any of the more than 100 cloned receptor genes been functionally expressed, a success achieved by Heinz Breer and colleagues using a baculovirus vector in sf9 cells [4]. And even in this lone case the response, as measured by the production of a second messenger, to the odorants lillial and lyral was comparatively small and saturated at a low concentration, suggesting that receptor expression was not robust. Adding to the difficulty of this sort of experiment are the highly improbable odds of matching a given cloned receptor with the appropriate odorant ligands. Testing 10 000 odorants on each receptor is simply not practical.

The traditional strategy for neurobiologists faced with these sorts of hopelessly complex problems has been to search for solutions in simpler, but no less interesting, systems. Following this well-worn path, Sengupta *et al.* [5] went after odorant receptors in the nematode *Caenorhabditis elegans*. *C. elegans* responds to a far smaller number of odorants than do mammals such as ourselves, and has a correspondingly smaller number of cells devoted to the task. Nevertheless, olfaction is still one of the primary sensory systems in this animal, and at least 10 % of the neurons in its entire nervous system are devoted to chemosensation [6].

By using a behavioral screen for chemosensory mutants, Sengupta *et al.* [5] isolated a line of worms with a defective ability to sense the attractant di-acetyl. From previous experiments [7], it was known that di-acetyl signalling requires the activity of a paired set of chemosensory neurons known as the AWA cells. In *C. elegans*, perhaps unlike vertebrates, a single olfactory cell expresses multiple receptors and is able to respond to multiple odors [8]. The AWA neurons, for example, also signal the presence of pyrazine and thiazole compounds. From the behavioral assay, a strain defective in di-acetyl detection was determined to have a mutation in a single gene, dubbed *odr-10*, which was mapped to the X chromosome. Using the powerful genetic techniques and partial genome map available with *C. elegans*, the *odr-10* gene was cloned and found to encode a protein of 339 amino acids.

Analysis of the distribution of hydrophobic residues in the sequence of the ODR-10 protein revealed seven putative transmembrane regions, suggestive of a G-protein-coupled receptor. A search of the Genbank database, however, revealed little similarity to any known protein, except for a low (12 %) level of sequence identity to one of the vertebrate olfactory receptors. Indeed, ODR-10 appears to lack almost all of the sequences that characterize the mammalian odorant receptor family. Furthermore, the putative third intracellular loop, notably short in the vertebrate odorant

receptors (17 residues), is twice as long in ODR-10. This region has been implicated in G-protein coupling interactions, so this difference may be useful in determining the second messenger system used by the *C. elegans* receptor [9]. The presence of introns in *odr-10* is also unusual; although a few G-protein-coupled receptor genes do have introns, most, including the vertebrate odorant receptor genes, do not [9]. What this may mean for the control of gene expression and the possibility that alternative splicing generates transcripts encoding additional receptors will undoubtedly be the subject of intense investigation. This is particularly interesting in view of the fact that multiple receptors are expressed by individual cells in *C. elegans*.

The novel sequence of the *C. elegans* receptor provides a possible explanation for the curious failure to identify candidate odorant receptor genes, using the known sequences of the vertebrate receptors, in invertebrates. This failure has been especially galling in the insect world, where olfaction is known to be a primary sense and where so much behavioral and neurophysiological data regarding the olfactory system have been compiled. In spite of the best efforts of a number of laboratories working in animals from *Drosophila* to honeybee to moth, invertebrate odorant receptors have previously gone undetected. Indeed, one of the most important consequences of the Sengupta *et al.* [5] work is likely to be the provision of potential primer sequences to search for insect odorant receptor genes using the polymerase chain reaction (PCR).

But the major immediate contribution of this work is the correlation it provides between a cloned odorant receptor and a specific odorant ligand sensitivity. ODR-10 appears to have a high affinity for di-acetyl, and the mutation that enabled the gene to be identified — a substitution of a tyrosine for a histidine in the third transmembrane domain — leaves the animal with virtually no di-acetyl sensitivity. The possible discovery of other, equally subtle mutations in this gene, and the characterization of their effects on ligand sensitivity, could provide invaluable insight into the key residues and structural features of these receptors.

The evidence that ODR-10 is indeed an odorant receptor is comprehensive: *odr-10* mutants are specifically defective in responses to di-acetyl and not to pyrazine or thiazoles; the *odr-10* phenotype can be rescued by injection of the mutant animals with a cosmid containing the normal gene; deletion of a significant portion of the *odr-10* gene results in a di-acetyl-insensitive animal; the *odr-10* gene is specifically expressed in AWA cells; and the protein is localized to the sensory cilia of those cells.

In a particularly elegant experiment, wild-type *odr-10* was placed under the control of the *odr-3* promoter, which drives expression not only in the AWA neuron, but also in AWC neurons that are not normally sensitive to di-acetyl. Animals expressing the *odr-3::odr-10* construct were able to detect di-acetyl, indicating that ODR-10 can confer di-acetyl sensitivity on either the AWA or the AWC neurons, or both. An intriguing extension of this experiment, at least theoretically possible in *C. elegans*, might be to use an alternative promoter to express wild-type *odr-10* in another class of chemosensitive cell, perhaps the ASE neurons that sense non-volatiles such as cAMP and salts. This would amount to a heterologous expression system within the animal itself, and could provide a method for exploring receptor structure–function relationships through *in vitro* mutagenesis, expression and testing.

What are the implications for the strategy used by the vertebrate olfactory system to encode a chemical environment rich in stimuli? First there is the issue of similarity, or rather the lack of it, to the vertebrate family of receptors. Many of the differences suggest different strategies are used to control gene expression: for example, the fact that chemosensitive neurons in *C. elegans* clearly express more than one receptor suggests that specific transcriptional regulatory mechanisms, distinct from those in vertebrates, are employed. ODR-10 itself clearly differs from the vertebrate receptors, but is nonetheless a member of the G-protein- coupled receptor family. This new receptor gives us a further tool to explore the critical molecular features of ligand binding and receptor activation. There is, however, still a profound need to develop a system in which many individual odorant receptors can be functionally expressed and assayed for ligand binding, and that could be used in a kind of pharmacological analysis of these receptors. The enormous odorant receptor family is a kind of gift of nature in which all the mutations that an experimenter might want to assay for different ligand sensitivities have already been produced by evolution. We just have to figure out how to unwrap the present.

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