

Unraveling Chemosensory Diversity

Minireview

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Most animals have the ability to detect, discriminate, and react to chemicals present in their external environment. These include water-soluble molecules, volatile odors, and pheromones, molecules that are released from animals and elicit fixed behaviors and physiological responses in animals of the same species. In lower organisms, chemosensory stimuli elicit stereotyped responses. In mammals, responses to some stimuli, such as pheromones, are stereotyped while responses to other stimuli are conscious and measured. Each organism is confronted with a complex array of chemicals. How does the nervous system detect the individual components of this array and organize this information so as to achieve a high level of perceptual discrimination and generate a variety of innate responses?

Two recent papers in *Cell* provide insight into the mechanisms used by chemosensory systems to detect and encode sensory stimuli. In one, Dulac and Axel (1995) report the identification of a novel multigene family that may encode as many as 100 different types of pheromone receptors in mammals. In the second, Troemel et al. (1995) present evidence for the existence of a number of different

gene families that may code for chemoreceptors in the nematode *Caenorhabditis elegans*. Comparisons of the structural features and patterns of expression of the receptor families identified in these two studies and those of mammalian odorant receptors reveal common themes of chemosensory transduction, but emphasize that different chemosensory systems have evolved different strategies to process sensory information (Figure 1).

Candidate Pheromone Receptors in Mammals

In mammals, olfactory stimuli are detected by sensory neurons located at two distinct sites: the olfactory epithelium (OE), which is located in the posterior nasal cavity, and the vomeronasal organ (VNO), a tubular structure that opens into the nasal cavity. Whereas volatile odors are detected in the OE, the VNO is thought to be specialized to detect pheromones that provide information about gender, dominance, or reproductive status and that elicit innate social and sexual behaviors as well as profound neuroendocrine changes (Halpern, 1987; Wysocki, 1989; Shepherd, 1994). Consistent with these distinctive functions, sensory signals generated in the OE and VNO are transmitted through different neural pathways in the brain. OE-derived signals ultimately reach higher cortical centers that mediate the conscious perception of odors, while VNO-derived signals do not. Instead, VNO-derived signals are targeted to the amygdala and hypothalamus, regions implicated in innate behavioral and physiological programs associated with reproduction.

Recent studies indicate that, in the mammalian OE,

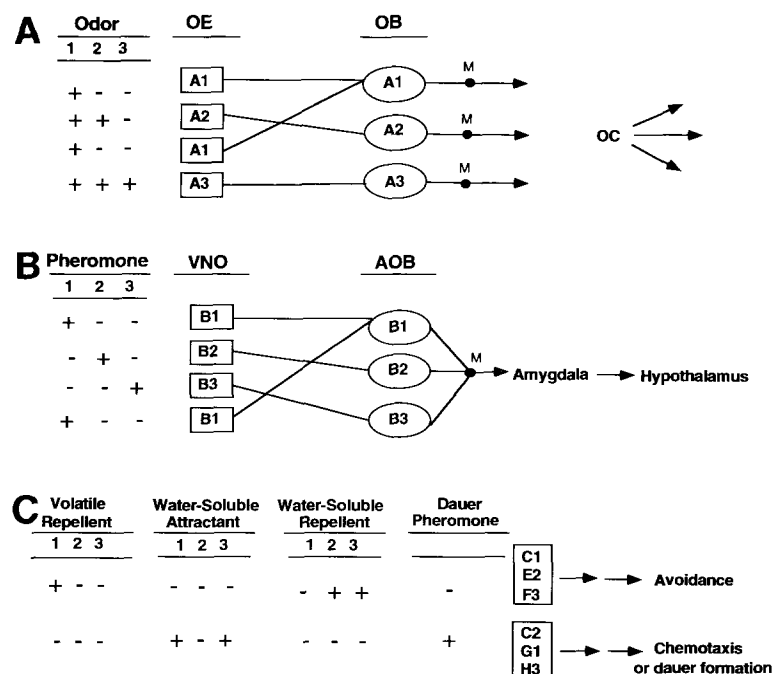


Figure 1. Possible Strategies Used to Detect and Encode Chemosensory Stimuli in Different Systems

(A) In this model of the rat OE, each neuron (box) expresses one type of odorant receptor (A1–A3). Each odor binds to numerous receptor types, and each receptor interacts with numerous odors. Neurons expressing the same receptor are randomly arrayed in one zone in the OE, but transmit signals to the same glomerulus (oval) in the olfactory bulb (OB). Mitral relay neurons (M) transmit signals from individual glomeruli to the olfactory cortex (OC), which relays information to many other brain regions. (B) In one model of the rat VNO, each neuron expresses one type of pheromone receptor (B1–B3). Conceivably, each receptor might be highly specific for a particular pheromone. Neurons expressing the same receptor are scattered in the VNO, but send signals to the same glomerulus in the accessory olfactory bulb (AOB). Individual mitral cells (M) might integrate signals from different glomeruli receiving input from different receptors. Information derived from the VNO is targeted to the hypothalamus, which mediates innate responses to pheromones. (C) In one model of chemosensation in *C. elegans*, each sensory neuron uses numerous dif-

ferent receptors (C1, C2, E2, F3, G1, H3) to recognize different chemicals, some of which may generate different responses. The receptors can belong to different receptor families. This scheme could provide for the existence of parallel, noninteracting pathways of sensory signaling, or signal integration and processing, in a single neuron.

odorants are detected by as many as 1000 different G protein-coupled odorant receptors that are encoded by a multigene family (Buck and Axel, 1991). Each neuron appears to express a single receptor type. Neurons expressing the same receptor are randomly distributed in one of four spatial zones in the OE, but, in the olfactory bulb, the axons of these neurons converge on only a few stereotyped glomeruli (Figure 1; Ressler et al., 1993, 1994; Vassar et al., 1993, 1994). Together with previous functional studies (Shepherd, 1994), these findings have provided insight into the mechanisms underlying olfactory information coding. In contrast, virtually nothing is known about the mechanisms by which pheromonal signals are transduced in the VNO or the strategies used to encode the identities of pheromones. In addition, although the VNO has been implicated in a variety of different pheromone-mediated effects, the chemical nature of most pheromones remains obscure.

In the study by Dulac and Axel (1995), an ingenious approach was devised to search for genes encoding pheromone receptors in the rat. Making the assumption that different VNO neurons would express different receptors, they prepared cDNA libraries from single VNO neurons and then looked for cDNAs present in one library, but not another. Comparing two different neurons, they detected only one difference. The protein encoded by the unique cDNA bore no resemblance to odorant receptors or to any other known protein. However, it did exhibit seven hydrophobic stretches, the signature feature of seven transmembrane domain, G protein-coupled receptors. Sequence analyses of six related cDNAs showed that this protein belongs to a family of proteins whose members share sequence motifs, but each of which is unique. On the basis of genomic library screens and Southern blotting experiments, Dulac and Axel predict that the VNO receptor family may be composed of about 100 members.

Perhaps the most compelling evidence that the multigene family identified by Dulac and Axel encodes sensory receptors comes from *in situ* hybridization studies. They found that each receptor probe hybridized exclusively to VNO neurons. Furthermore, each probe recognized only a small fraction (1%–4%) of VNO neurons, and different probes were found to recognize different sets of neurons. This suggests that in the VNO, as in the OE, each neuron may express only a single receptor type.

Interestingly, neurons that express the same VNO receptor appear to be randomly dispersed throughout the dorsal-ventral and anterior-posterior extent of the VNO neuroepithelium. This random interspersion of neurons expressing different receptors, which is reminiscent of that seen in the OE, has two implications. First, it suggests that the developing cell may use a stochastic mechanism to select a single receptor gene for expression. Second, it indicates that information about a particular ligand is highly distributed across the epithelial sheet rather than spatially mapped onto a specific site.

Dulac and Axel's findings, together with previous functional studies of the VNO, strongly suggest that the molecules that they have identified may be the VNO pheromone

receptors. The structures of these molecules suggest that, like odorant receptors, the VNO receptors may be G protein coupled. Furthermore, their patterns of expression in the VNO resemble patterns of odorant receptor expression in the OE. Why would the VNO and OE utilize entirely different receptor families to detect sensory stimuli? One possibility is that the two receptor families are uniquely suited to the different functional needs of the VNO and OE. In the OE, it appears that each odorant receptor may recognize a particular structural feature shared by many odorants and that each odorant may be recognized by many different receptors. It is thought that the identity of an odorant is encoded by the unique combination of receptors with which it interacts. Although the identities of different pheromones might be similarly encoded by overlapping combinations of VNO receptors, this need not be the case. First, the VNO may not need a coding strategy that maximizes the number of different ligands that can be discriminated. Second, given the functional role of the VNO in eliciting innate behaviors, the VNO might, in fact, employ a coding scheme that utilizes receptors that specifically recognize only those ligands relevant to its function, presumably pheromones.

Another possible explanation for the existence of two different receptor families is that signaling through a VNO receptor might require binding to both a pheromone and a carrier protein bound to that pheromone. The carrier protein might identify the pheromone as being derived from a particular species or from a particular part of the animal. Interestingly, aphrodisin, a hamster pheromone, is composed of two components, one of which is a carrier protein. On the other hand, the expression of different G proteins in the VNO and OE and the common embryologic derivation of the VNO and OE from the olfactory placode suggest the possibility that the two receptor types might have been coexpressed in the same cell in a primitive ancestor. As may be the case in *C. elegans* neurons (see below), linkage of the two receptor types to different transduction pathways might have allowed for qualitatively different responses to environmental stimuli interacting with the two receptor types or permitted independent adaptation to those stimuli.

The identification by Dulac and Axel of candidate pheromone receptors should now permit the exploration of a variety of questions concerning the mechanisms by which animals communicate via chemical signals. How many pheromones are there and what are they? Are they completely different for different species, or are there overlapping sets or combinations that distinguish different species? And perhaps most importantly, how is information about pheromones encoded and processed to generate species-specific and circumstance-specific social and sexual behaviors?

Candidate Chemosensory Receptors in *C. elegans*

Studies of chemoreception in invertebrate species have suggested that there may be both striking parallels and interesting differences between the mechanisms used to encode and process sensory information in vertebrate and invertebrate systems. One of the simplest chemosensory

systems studied is that of the nematode *C. elegans*. The adult hermaphrodite of this species has 302 neurons whose positions, morphology, and synaptic connections are identical in every individual (White et al., 1986). Of these, 32 appear to be chemosensory neurons. Grouped at several distinct, bilaterally symmetrical locations, these neurons have ciliated endings that are exposed to the environment.

Like mammals, *C. elegans* is able to detect both water-soluble and volatile chemicals as well as pheromones derived from members of the same species. These molecules generally elicit one of two responses: chemotaxis (attraction) or avoidance. However, the responses to at least some pheromones are more complex. For example, one pheromone, the dauer pheromone, can prevent normal development by inducing development into an alternative form, the dauer larva.

By studying the responses to various chemicals and pheromones following laser ablation of individual chemosensory neurons, researchers have been able to characterize the molecules to which individual neurons respond and the types of responses mediated by these neurons (Bargmann and Horvitz, 1991a, 1991b). In general, each chemosensory neuron can respond to a variety of different molecules. However, volatile attractants and water-soluble molecules are generally detected by different neurons, and different neurons also mediate chemotaxis and avoidance. Individual neurons are able to sort out different kinds of sensory information. For example, a single neuron can mediate both chemotaxis and dauer larva formation. Another remarkable feature of chemosensation in *C. elegans* is that it can adapt independently to two different chemicals detected by the same neuron (Colbert and Bargmann, 1995). Furthermore, the response of a neuron to a chemical can be saturated without blocking the response of that neuron to a second chemical (Ward et al., 1973; Bargmann et al., 1993). How can this be explained? Does each neuron express multiple different receptors for different chemicals? Or, does each neuron express a single receptor that recognizes many odorants and the results of cross-adaptation and cross-saturation studies reflect the downstream integration of information provided by different cells that detect the same chemical?

To search for genes encoding chemosensory receptors in *C. elegans*, Troemel et al. (1995) designed a clever strategy that took advantage of the rapidly growing database of information provided by the *C. elegans* Genome Project. Their initial strategy was based on observations that *C. elegans* genes with related functions are frequently clustered in operons. Focusing on regions around genes encoding potential chemosensory transduction molecules, they first identified a cluster of nine genes that encoded a family of related proteins whose structures suggested that they were G protein-coupled receptors.

Using these sequences to search the database for related genes and then performing additional searches with the newly identified genes, Troemel et al. identified 41 different genes that appeared to encode members of the G protein-coupled receptor superfamily. Each of the

proteins exhibited the seven hydrophobic domains characteristic of this superfamily as well as key residues commonly found in superfamily members. On the basis of sequence similarities, the 41 genes belong to six distinct receptor families, which are designated *sra* (for serpentine receptor class a), *srb*, *srg*, *srd*, *sre*, and *sro*. Members of the same receptor family are generally clustered in the genome, but the different families are not linked to one another.

The 41 receptor genes identified could encode chemosensory receptors or receptors for other molecules used for intercellular communication. To explore this question, Troemel et al. prepared transgenic animals carrying constructs in which the upstream regions of a number of these genes were fused to a green fluorescent protein reporter gene. Surprisingly, of 14 genes that were expressed in adults, eight were expressed only in subsets of chemosensory neurons and three others were expressed predominantly in chemosensory neurons. Neurons that expressed the various fusion genes included those previously implicated in the detection of water-soluble attractants, repellents, pheromones, and the regulation of egg-laying. In addition, examination of adult males revealed interesting patterns of male-specific expression for three genes, two of which were expressed in neurons that may detect sex pheromones.

The patterns of expression observed by Troemel et al. are strikingly different from those observed for candidate sensory receptors in the rat VNO and OE. First, some chemosensory neurons expressed multiple receptor genes, including members of the same family and members of different families. Second, some genes were expressed in multiple chemosensory neurons. Third, some genes were expressed in both chemosensory neurons and other cell types. Fourth, while some members of a particular receptor family were expressed exclusively in chemosensory neurons, other members of the same family were expressed exclusively in other cell types.

Troemel et al. note that while, theoretically, many or even all of the receptors identified could serve other functions, several observations are consistent with a role for at least some, and possibly many, of these receptors in chemoreception. One is that they are likely to couple to G proteins. This is consistent with observations that G proteins are expressed in chemosensory neurons and that mutations in G proteins produce defects in chemosensory responses. A second suggestive finding is that a surprisingly large proportion (8 of 14 receptor genes tested) were expressed exclusively or predominantly in chemosensory neurons. Moreover, none of the genes was expressed in all chemosensory neurons or even in all sensory neurons that mediate a particular behavioral response. In fact, many of the genes were expressed in only a single type of chemosensory neuron.

These studies suggest that while chemoreception in *C. elegans* may resemble vertebrate olfactory reception in its use of G protein-mediated mechanisms of sensory transduction, it may employ an entirely different strategy to detect and encode information about the chemicals in

its environment. First, consistent with previous cross-adaptation and cross-saturation studies, each neuron may express multiple receptor types, as opposed to one receptor type, as appears to be the case in rat OE and VNO neurons. A logical reason for this is that it is to the animal's advantage to sense many more molecules than it has chemosensory neurons or different combinations of responsive neurons. The 41 candidate receptor genes were found in sequenced DNA encompassing only about 15% of the genome. Troemel et al. note that even if only half of the fusion genes expressed in chemosensory neurons are sensory receptors, the approximate number of receptors expected in the genome would be about 100, suggesting that many receptors could be found in one cell type.

The studies of Troemel et al. also suggest that there may not be a specific receptor family in *C. elegans*, comparable to the mammalian VNO and OE receptor families, that is dedicated to chemoreception. Instead, chemoreceptors in *C. elegans* may belong to families whose other members serve other functions. In addition, chemoreceptors in *C. elegans* may belong to many different receptor families. This scheme might serve an important function: if different receptors expressed by the same cell transduce signals via different transduction pathways, they may function independently such that the animal could stop responding to one chemical while maintaining its ability to respond to another. On the other hand, this scheme might also allow for cross-talk among different pathways that might provide a basis for the integration and processing of sensory information within an individual chemosensory neuron. The studies by Troemel et al. now provide the means of addressing these and many other questions that should enlighten us as to the mechanisms by which a simple organism solves the problem of generating appropriate responses to an enormously complex environment.

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