

Odorant receptors: Axon contact-mediated diversity

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To make the most of a small number of neurons, the nematode olfactory system includes neurons that are bilaterally symmetrical in morphology, but differ in the sets of genes they express. An intriguing recent example is the axon contact-mediated asymmetry in expression of the *str-2* odorant receptor gene.

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Current Biology 2000, 10:R152–R154

0960-9822/00/\$ – see front matter
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In the nematode *Caenorhabditis elegans*, the major chemosensory organs are the two structures known as amphids, each of which contains eleven chemosensory neurons. The amphids are bilaterally symmetrical, so that each chemosensory neuron in the right amphid has a morphological counterpart in the left amphid. As in other parts of the *C. elegans* body plan, the cells of the amphid are stereotyped in their lineage and position. The initial detection of volatile odorants is primarily accomplished by three pairs of neurons: AWA, AWB and AWC. The left and right AWA and AWC neurons detect attractive odorants, and the left and right AWB neurons detect aversive odorants [1,2]. Recently, *C. elegans* chemosensory receptors have been identified and their expression patterns are being studied [2–4]. An exciting twist to this emerging story is that the left and right members of an amphid neuron pair display differences in gene expression, potentially increasing the number of different types of neuron by a factor of two. Moreover, in the case of the asymmetry between the two AWC neurons, axon contact plays a role in establishing asymmetrical gene expression [5].

The *C. elegans* genome contains approximately 1,000 genes encoding proteins that appear from their sequence likely to be receptors of the seven-transmembrane domain class, a substantial portion of which may be receptors involved in the detection of volatile odorants [4]. To accommodate the expression of the large gene family in a relatively small number of neurons, each neuron appears to express a large number of receptor genes. Receptor expression is restricted, so that numerous receptors mediating the same behavior are coexpressed in an individual neuron. The receptors expressed in a given neuron appear to undergo independent adaptation, thus allowing a neuron to respond to changes in one odorant's concentration even in the context of a steady high concentration of another odorant [1,6]. In the case of one of the receptor gene products, the odorant sensitivity

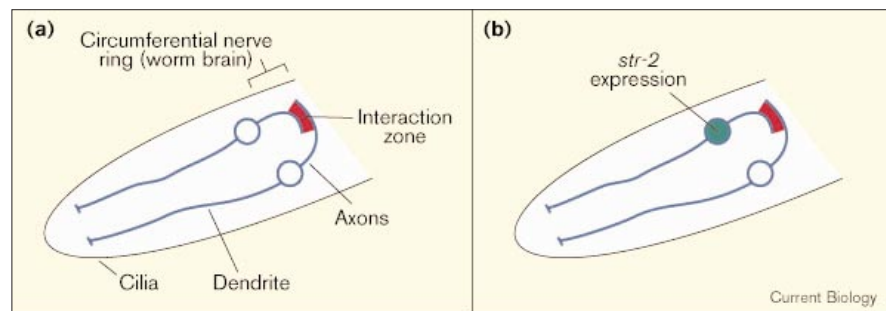
has been established — Odr-10, normally expressed in AWA neurons, has been shown to mediate detection of the attractant chemical diacetyl [3]. Importantly, when transgenic nematodes were generated in which the *ODR-10* gene was misexpressed in AWB neurons, the animals were found to avoid diacetyl [2]. This experiment indicated that the specific behavioral output of AWB neurons was not changed by the abnormal production of Odr-10 — the only apparent change was in the neuron's odorant specificity.

Given the small number of neurons involved in mediating chemotaxis to volatile odorants, it is remarkable that the *C. elegans* chemosensory system works so well. One mechanism by which the system can effectively increase its number of different neurons and hence its ability to respond to a greater number of different stimuli, is for the left and right neurons to be different from one another. Indeed, this appears to be the case for the AWC neuron with respect to the odorant receptor STR-2. This was elegantly demonstrated by Troemel *et al.* [5], who used regulatory elements from the *str-2* gene to drive expression of the green fluorescent protein (GFP). They found that the transgene was asymmetrically expressed in the morphologically identical left and right AWC neurons (Figure 1). In certain animals, expression was restricted to the right AWC neuron, and in other animals it was restricted to the left AWC neuron. It is important to note — as Troemel *et al.* do — that all the observations were made with *str-2* transgenes and not of the endogenous *str-2* gene, but as multiple transgenes gave the same results, it is highly likely that this reflects the regulation of the endogenous gene. It is worthwhile, therefore, to delve into the observations further.

One intriguing aspect of the observations made by Troemel *et al.* [5] is that *str-2* expression on one or the other side of the animal is stochastic. There appears to be a subtle skewing, however — more animals were found to express the *str-2* transgenes in the right AWC neuron than in the left AWC neuron. This skewing was observed with both a *str-2::GFP* transgene and with the similarly functioning *str-2::ODR-10-GFP* transgene, and my statistical analyses of the combined data indicate that the skewing is significant ($p < 0.01$), although it is not found with every independent integrated strain. Non-random asymmetry is not surprising, given the left–right asymmetry observed in the *C. elegans* body plan in general, and in the chemosensory neurons in particular (see below) [7]. It is important to note that, while there may be subtle skewing, there is still a stochastic aspect to the asymmetry, so that either the right or the left AWC neuron can express *str-2*.

Figure 1

(a) Simplified diagram of the AWC pair of neurons showing axonal interactions. Odorant detection occurs in cilia present at the front of the animal, at the ends of long dendrites. The cell bodies and axonal projections to the circumferential nerve ring are shown. Red indicates the area in which the axons contact each other and can potentially interact directly. (b) In adult worms, *str-2* is expressed in either the left or right AWC neuron but never both. This is indicated by the green shading in one of the two cell bodies.



The question naturally arises as to the generality of these observations on *str-2* [5]. Are other genes – perhaps a large number — expressed asymmetrically in the AWC neurons? And is this kind of asymmetry a more general phenomenon, occurring in other chemosensory and non-chemosensory neurons? Unfortunately *str-2* is the only odorant receptor gene so far known to be expressed in AWC neurons, so the extent to which other genes show left–right asymmetric expression in these neurons is not yet known. If other receptor genes are asymmetrically expressed, it will be interesting to know if there is coordination between the different genes in their asymmetric regulation. Asymmetric expression has also been observed in the case of the guanylyl cyclases thought likely to be chemosensory receptors of the ASE neurons that detect non-volatile chemicals [7]. In this case, however, the asymmetry is fixed: two members of the guanylyl cyclase family were found to be expressed consistently in the left ASE neuron, and one member was expressed consistently in the right ASE neuron. The physiological relevance of this non-random asymmetry remains obscure.

The mechanisms that might generate the asymmetric expression of *str-2* have been explored using laser ablation and mutant analysis. The left and right AWC neurons arise from different precursors. Ablation of the precursor of an AWC neuron on either side of the animal causes the contralateral AWC neuron to remain ‘off’ as defined by expression of the *str-2* transgene, and presumably the endogenous homologue. This indicates that either the AWC neuron itself, or a closely related cell, sends a positive signal for *str-2* expression to the contralateral AWC neuron. In the absence of this signal, the default state is lack of expression of *str-2*. If an AWC neuron is killed late in development — after the choice as to which AWC neuron will express *str-2* has been made — there is no effect on the pre-established decision by the contralateral AWC neuron whether or not to express *str-2*.

Where do the interactions between the two AWC neurons occur? And are they direct or indirect? While the cell bodies of the two AWC neurons do not touch,

their axons touch at a number of points in the nematode’s circumferential nerve ring (a structure that includes many other neurons and axons). So direct communication between the two AWC axons is a possibility, though of course indirect interactions are also possible. The observation that a variety of mutations known to affect axon guidance in *C. elegans* also perturb asymmetric gene expression in the AWC neurons is consistent with the interactions between those neurons being either direct or indirect [5].

Various potential mechanisms of signaling between the AWC neurons have been explored, the starting point being a genetic screen for mutations that cause bilateral expression of *str-2* along with single and double mutant analyses of likely genes. While the initial signal remains obscure, the genetic data imply that calcium signaling, involving the Unc-2/36 calcium channel [8,9] and the calcium/calmodulin-dependent protein kinase CaMKII [10], plays a role. The cGMP signaling pathway — which is known to be involved in olfactory signal transduction — plays a role in maintaining *str-2* expression in AWC. The Notch signaling pathway appears not to be involved, despite its role in similar stochastic processes in other cell types. Three other genetic complementation groups identified in the screen may define new genes involved in establishing the asymmetry, perhaps encoding receptors or ligands involved in axonal interactions.

The regulation of neuronal gene expression by contact between neurons (or their axons) is a powerful mechanism for increasing the diversity of neurons while maintaining some coordination in a developing nervous system. One documented example of seemingly random, but coordinated gene expression involves the R7 and R8 photoreceptor neurons in the *Drosophila* eye. In this case, a random choice between two opsins in each R7 neuron leads the R7 neuron to direct a binary developmental switch between induced and default cell fates in the R8 cell [11,12]. It will be interesting to compare other examples of coordinated neuronal gene expression identified in invertebrate and vertebrate nervous systems.

In conclusion, two examples have now been described in *C. elegans* of pairs of chemosensory neurons that exhibit asymmetric gene expression: the biased stochastic asymmetric expression of *str-2* in AWC neurons, and the fixed asymmetric expression of guanylyl cyclases in ASE neurons. If asymmetric gene expression turns out to be more widely used in the *C. elegans* olfactory system, it may serve to effectively double the number of distinct types of primary chemosensory neuron and increase the effectiveness of olfactory discrimination. The coordination of gene expression in neurons that contact each other may be a critical general mechanism underlying the creation of functional neural networks.

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