Olfactory G proteins: **Simple and complex signal transduction**

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In both vertebrates and invertebrates, olfactory perception is mediated by G-protein-coupled receptors. Recent work, in both mouse and *Caenorhabditis elegans*, sheds light on the role of specific G proteins in olfactory signal transduction, neuronal morphology and axon guidance.

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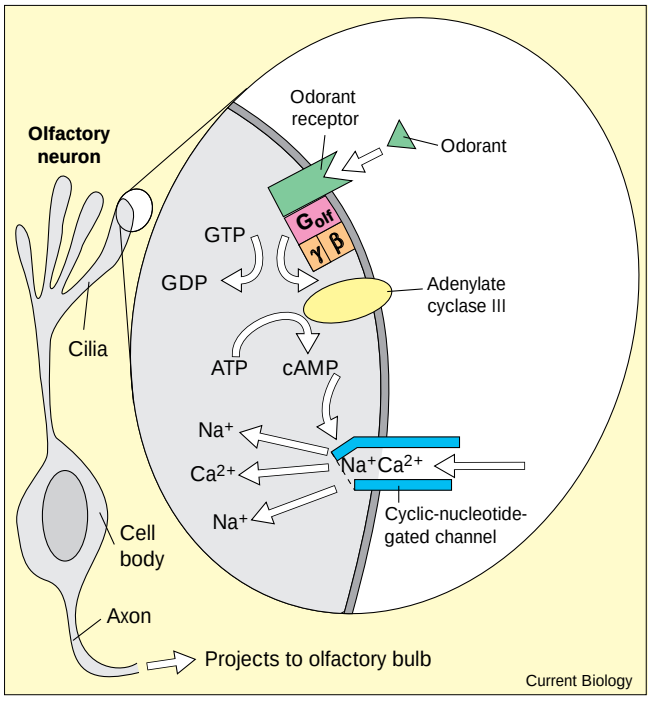
In animals as different as humans and worms, olfactory discrimination is mediated by a large, diverse family of G protein-coupled receptors [1,2]. In mammals, two anatomically distinct olfactory systems, comprising millions of primary neurons, coexist. The main olfactory system mediates responses to most olfactory cues, whereas the vomeronasal system mediates pheromone responses (whether humans have a functional vomeronasal system remains unclear). In the nematode *Caenorhabditis elegans*, a subset of the thirty-two chemosensory neurons are involved in olfaction (the detection of volatile compounds). We shall focus here on recent advances in understanding the role of G proteins in olfactory signal transduction in the main olfactory system of the mouse and in *C. elegans*.

At its most basic level, the olfactory system in any animal must allow the brain to discern which olfactory receptors have encountered odorant at any given time. In mammals, this is accomplished by regulating the approximately 1,000 diverse olfactory receptor genes, so that each neuron expresses just one of the receptor genes (reviewed in [3,4]). As each neuron expresses only one receptor, the brain can determine which receptors are activated by identifying excited neurons. Each mammalian olfactory neuron appears to use the same machinery for transducing signals from its odorant receptor molecules. Upon odorant binding, the receptor is thought to activate G_{olf}, a $G_s\alpha$-like G protein [5]. G_{olf}-mediated activation of adenylate cyclase III then raises intracellular cAMP levels, causing a cyclic-nucleotide-gated channel to open. The influx of cations through this channel ultimately leads to the formation of an action potential, which allows the primary neuron to signal to the brain (Figure 1). The inositol 1,4,5-trisphosphate (IP_3) second messenger system has also been suggested to play a role in the response to some odorants, but recent experiments indicate that it is unlikely to have a primary role [6].

How does the brain determine which neurons are active? The cell bodies of the set of neurons expressing a given receptor are randomly distributed among neurons expressing different receptors, but their projections converge to discrete loci in the olfactory bulb called glomeruli (Figure 2; reviewed in [3,4]). Thus, the brain can determine which receptors have been activated by examining the spatial pattern of activity in the olfactory bulb; individual odorants are associated with specific spatial patterns.

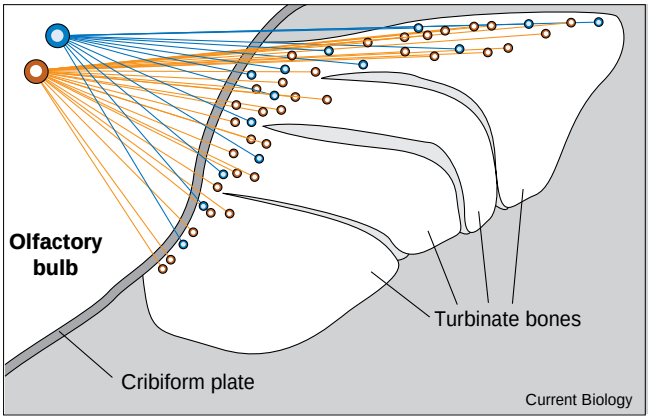
In *C. elegans*, as many as 200–400 genes encode receptors involved in the detection of volatile odorants. Unlike the mouse, *C. elegans* has far fewer receptor-expressing neurons than receptors. Receptor expression is restricted such that numerous receptors mediating the same behaviour are co-expressed in an individual neuron. Distinct receptors in each neuron adapt independently, thus allowing a neuron to respond to changes in one odorant's concentration even

Figure 1



The mouse olfactory signal transduction cascade. Odorant binding to the olfactory receptor is thought to activate G_{olf}. Activated G_{olf} then dissociates from $G\beta\gamma$ and activates adenylate cyclase III, leading to an increase in the intracellular cAMP concentration, which in turn opens the cyclic-nucleotide-gated channels. The consequent influx of Na^+ and Ca^{2+} ions ultimately leads to an action potential, which travels down the axon of the primary neuron to signal to the brain.

Figure 2



Olfactory-receptor-expressing neurons and their projections to distinct glomeruli in the olfactory bulb. The diagram shows the projection patterns of neurons expressing two distinct olfactory receptors, coloured orange and blue respectively. (There are actually four zones of receptor expression in the murine olfactory epithelium; the two types of neuron depicted both reside in the most dorsal of the four zones.) Although neurons expressing these two receptors occur in the same zone of the olfactory epithelium, they project to distinct glomeruli in the olfactory bulb. Thus, the brain can determine which receptor is activated by examining the spatial pattern of activated glomeruli in the olfactory bulb.

in the context of a steady high concentration of another odorant. In most cases, the neuron does not direct different responses to the different odorants it detects. There are examples, however, where receptors whose activation leads to different responses are coexpressed in the same neuron. In these instances, the receptors for these distinct odorants must couple to distinct signal transduction pathways in order to direct distinct behaviors (reviewed in [7]).

Signal transduction in *C. elegans* olfactory neurons is clearly very complex: individual neurons integrate information from multiple receptor types and, in some cases, direct different responses to different odorants. Roayaie *et al.* [8] have recently reported evidence that ODR-3 [9] is a G_i/G_o-like Gα protein involved in many olfactory responses in olfactory neurons (and in mechanosensory neurons). In certain neurons, ODR-3 is thought to signal through a cyclic-nucleotide-gated channel; in other neurons, other channels may be involved. ODR-3 is one of a group of G proteins implicated in *C. elegans* olfactory sensation [10]. The expression patterns of these different G proteins partially overlap, allowing different receptors in the same neuron potentially to couple to different signal transduction pathways [7]. *C. elegans* primary olfactory neurons thus accomplish some of the processing of olfactory information that, in mammals, occurs only in higher order neurons.

G_{olf} mutant mice have olfactory and non-olfactory defects

To examine the role of G_{olf} in olfactory perception, Belluscio *et al.* [11] recently generated G_{olf} knockout mice by targeted gene inactivation. G_{olf}-deficient animals appear to be anosmic: an electrophysiological test, the electro-olfactogram (EOG), showed a severe diminution in response to a wide variety of odors in newborn mice. This diminution was even more pronounced in G_{olf}-deficient animals that survived beyond three weeks. The more pronounced deficiency in these slightly older animals may be explained by the decrease in the expression of the ubiquitous G protein $G_s\alpha$, which can probably couple to the olfactory receptors as its amino-acid sequence is 80% identical to that of G_{olf}. The resting physiological properties of the olfactory neurons in the G_{olf}-deficient mice appear normal, indicating that the diminished EOG reflects direct involvement of G_{olf} in odor-evoked signal transduction. A similar phenotype — anosmia to a wide variety of odorants — is exhibited by mice deficient in the olfactory cyclic-nucleotide-gated channel [6].

The G_{olf}-deficient mice also exhibit behavioral defects that are consistent with anosmia [11]. The newborn animals do not feed properly, and by two days after birth 75% of them die without milk in their stomachs. As feeding is mediated by olfactory cues, this phenotype is consistent with an olfactory defect. Moreover, rare surviving G_{olf}-deficient mice, although fertile, do not display appropriate maternal behavior (which has been shown to be mediated in part by the main olfactory system). It is interesting to note that G_{olf}-deficient animals also have a non-olfactory phenotype: locomotor defects manifested by hyperactivity. Thus, in addition to its critical role in olfactory perception, G_{olf} appears also to play a role in the central nervous system.

The role of G_{olf} in axon targeting

As mentioned above, although the cell bodies of neurons expressing a given receptor are randomly distributed among neurons expressing different receptors, their axonal projections converge on defined glomeruli in the olfactory bulb (Figure 2). Recent results indicate that the olfactory receptor itself plays an instructive role in axon guidance [12,13]. To test the possibility that the same olfactory-receptor-initiated signal transduction machinery is used to mediate both olfactory perception and axon targeting, Belluscio *et al.* [11] analyzed axon guidance in G_{olf}-deficient newborn mice. To do this, they crossed the G_{olf}-deficient mice with mice whose P2 olfactory receptor gene had been modified so that neurons choosing this receptor also express the *lacZ* reporter. The latter mice had been used previously to visualize the projection of P2-expressing neurons to a few distinct glomeruli in the olfactory bulb. In G_{olf}-deficient, P2-marked newborn animals, neurons expressing the P2 receptor were found to converge with a similar pattern to that seen in G_{olf}-wild-type mice.

One straightforward conclusion from this result is that G_{olf}-mediated signal transduction is not required for axon guidance. It is also possible, however, that the closely

related protein $G_s\alpha$, which is present at relatively high levels early in development, can substitute for G_{olf} and transduce the signal from the olfactory receptor that determines axon guidance. Further experiments are needed to clarify this issue, including analyses of older animals and of mice deficient in the cyclic-nucleotide-gated channel or other signal transduction components. These caveats notwithstanding, the currently available data certainly raise the interesting possibility that olfactory receptor molecules can couple to two distinct pathways, one for olfactory perception and one for axon guidance.

C. elegans odr-3 mutants

In addition to demonstrating that the *C. elegans* protein ODR-3 is a G protein, Roayaie *et al.* [8] also examined its role in *C. elegans* olfaction by determining its localization in chemosensory neurons and by analysing a number of *odr-3* genetic variants. The former issue was resolved by expressing green fluorescent protein from the *ODR-3* promoter in transgenic nematodes, which showed that ODR-3 is expressed in the chemoattractant neurons AWA and AWC, and also in neurons AWB, ASH and ADF. The use of antibodies against ODR-3 showed that the protein is concentrated in cilia of chemosensory neurons. These localization studies strongly suggest that ODR-3 is directly involved in signal transduction.

Mutants deficient in ODR-3 were found to be impaired in their ability to detect all volatile odorants, as well as being defective in osmotic and mechanosensory avoidance. This clearly implicates ODR-3 in signal transduction. ODR-3 is one of several G proteins with partially overlapping expression patterns, so receptors coexpressed in an individual neuron could potentially couple to different signal transduction pathways [7]. Indeed, Roayaie *et al.* [8] observed that the impairments of ODR-3-deficient worms are more pronounced for some odorants than for others, which might reflect activities of the other, overlapping G proteins. They also found that overexpression of ODR-3 causes more severe olfactory defects than ODR-3 deficiency, but no osmotic avoidance defects. Together with data on other *odr-3* mutations, this is clear evidence that appropriate activity of ODR-3 is important for chemoattractant responses mediated by the AWA and AWC neurons, and also for osmotic avoidance responses mediated by the ASH neurons.

The role of ODR-3 in cilia morphology

Roayaie *et al.* [8] also found that ODR-3 has an influence on the cilia morphology in AWA and AWC neurons. In wild-type worms, the AWA cilia have a filamentous structure, whereas the AWC cilia have a fan-like structure. In the ODR-3-deficient worms, the AWC cilia have long filamentous branches like those on wild-type AWA neurons, and the AWA cilia are unchanged. In worms overexpressing ODR-3, on the other hand, AWA neurons have fan-like cilia, similar to those on AWC neurons, and the cilia on AWC neurons are unchanged. These results, along with analyses of other *odr-3* mutations, clearly implicate ODR-3 in the specification of the distinct morphology of the AWA and AWC neurons. Roayaie *et al.* [8] propose that low ODR-3 levels specify the AWA cilia morphology, and high ODR-3 levels specify the AWC cilia morphology. It is important to note that the severity of the olfactory deficit does not correlate with the severity of the change in cilia morphology. Abnormal cilia morphology is thus unlikely to account for the defects in chemosensation caused by ODR-3 perturbation; the chemosensory defect is more likely to reflect ODR-3's role in olfactory receptor signal transduction.

The recent studies of G_{olf}-deficient mice and ODR-3-perturbed *C. elegans* have thus clarified the roles that these G proteins play in olfactory sensory neurons. Signal transduction appears to be more complicated in *C. elegans* than in the mouse, though in both cases the results raise interesting questions about other olfactory and extra-olfactory functions of these G proteins.

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