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Chemoreceptor genes: what can we learn from *Caenorhabditis elegans* and how can we apply this information to studies on other nematodes?

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Summary – Soil dwelling nematodes encounter many types of volatile and water-soluble molecules in their environment. For free-living nematodes like *Caenorhabditis elegans*, successful foraging depends on the ability to detect a gradient in one odorant while ignoring extraneous odours. The infectious stages of plant and animal parasitic nematodes also rely on chemoreception as their primary host finding cue. Using a combination of genetic, molecular and bioinformatic approaches chemoreceptor genes have been identified in *C. elegans*. These *C. elegans* chemoreceptor genes encode seven-transmembrane G-protein coupled receptors (GPCR) and comprise the largest gene family in this nematode. GPCR are also involved in olfactory signal transduction across a broad spectrum of animals including insects, crustaceans, fish and mammals, but the *C. elegans* (and *Drosophila*) chemoreceptor genes have no sequence homology to vertebrate GPCR odour receptor genes and they also differ from vertebrate odour receptor genes in their genomic structure. We review the genomic structure and diversity of odorant and chemoreceptor gene families in vertebrates and invertebrates and describe our attempts, using homology-based approaches, to isolate chemoreceptor genes in the entomopathogenic nematode *Heterorhabditis bacteriophora*.

Olfactory systems allow organisms to detect and discriminate between thousands of low molecular mass, mostly organic, compounds which we call odours. Represented in the olfactory repertoire of both vertebrates and invertebrates are aliphatic and aromatic compounds with diverse

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functional groups including aldehydes, esters, ketones, alcohols, ethers, carboxylic acids, amines, halides and sulphides. Soil dwelling nematodes encounter many types of volatile and water-soluble molecules in their environment. Water-soluble chemicals tend to diffuse slowly in the soil and may provide short range chemosensory cues, whereas volatile compounds diffuse more rapidly and thus can be used for long range chemotaxis to distant food sources. For free-living nematodes such as *Caenorhabditis elegans*, successful foraging depends on the ability to detect a gradient in one odorant while ignoring extraneous odours. The infective stages of plant and animal parasitic nematodes also rely on chemoreception as their primary host finding cue.

Odour receptors first isolated from vertebrates

The first odour receptor (OR) gene family was identified in the rat by Buck and Axel (1991). Odour induced elevation of cyclic GMP in rat olfactory epithelium had suggested that G-protein coupled receptors might be involved in olfactory signal transduction. G-protein coupled receptors have a seven-transmembrane (7-TM) spanning topology. Conserved motifs within the membrane spanning domains of vertebrate 7-TM receptors were used by Buck and Axel to design degenerate PCR primers. Using these primers, a large family of 7-TM genes, which are selectively expressed in olfactory epithelium, was identified. Subsequently OR receptors were isolated from over 20 species of vertebrates ranging from lampreys to humans. Typically chemoreceptor genes are found to be encoded by large multigene families. These receptor sequences are archived in the Olfactory Receptor Database (http://senselab.med.yale.edu/senselab/ORDB/) and the G protein-coupled receptor database (http://www.gpcr.org/). The coding region of a mammalian 7-TM OR is *ca* 1 Kb and lacks introns. 7-TM odour receptors can be distinguished from other 7-TM receptors by the conserved motifs located in the intracellular loops IC1 and IC2 and in parts of the transmembrane (TM) spanning domains TM3 and TM5. Hypervariable regions are found in parts of TM3, TM4 and TM5 of the receptors and these regions are thought to form the ligand binding domains. The human genome comprises up to 1000 OR sequences which reside in multiple clusters spread throughout the genome. Odour receptors in the model organisms *C. elegans* and *Drosophila* have been discovered

only in recent years and were found to be divergent 7-TM proteins with limited similarity to each other and to mammalian 7-TM odour receptors.

Studies on the physiology and genetics of chemoreception in *C. elegans*

While the identification of *C. elegans* odour receptor sequences was initially slow as compared to mammals, studies on the physiology and genetics of chemoreception in *C. elegans* proceeded rapidly. *C. elegans* responds to a wide spectrum of water-soluble and volatile chemicals. Na^+, Li^+, Cl^-, and OH^- ions are attractive to *C. elegans*, as are the water-soluble molecules cAMP, cGMP, lysine, histidine, cysteine and biotin. In the soil *C. elegans* feeds on a large variety of bacteria associated with decaying organic matter. The by products of bacterial metabolism include various carboxylic acids, alcohols, aldehydes, esters, ketones and hydrocarbons, and several of these compounds are highly attractive to *C. elegans*. In addition to describing the chemosensory repertoire of *C. elegans*, researchers have isolated mutants with a variety of chemosensory defects. These include the chemotaxis deficient *che* and *tax* series, the *osm* series of osmotic defective mutants and the *odr* series which are defective in their responses to volatile odorants (reviewed by Bargmann and Mori (1997)).

The paired amphids located on either side of a nematode head are its primary chemosensory and thermosensory organs. The functions of each of the 12 amphidial neurons in *C. elegans* have been identified by laser ablation studies: eight neurons which have cilia exposed to the environment through the amphid pore detect water-soluble chemicals; three neurons which are indirectly exposed to the external environment detect volatile odorants, and a single enclosed neuron detects thermal cues (reviewed by Bargmann and Mori (1997)). Ashton *et al.* (1999) have found that the positions of the amphidial neuronal cell bodies in several vertebrate nematode parasites are very similar to the positions of these neuronal cell bodies in *C. elegans*, and they have also found that functional homologies have been conserved between certain amphidial neurons in *Strongyloides stercoralis* and *C. elegans*.

Chemoreception in the entomopathogenic nematode *Heterorhabditis bacteriophora*

Much of our research at Maynooth is focused on *Heterorhabditis bacteriophora*, an entomopathogenic nematode which belongs to the same zoological family (the Rhabditidae) as *C. elegans*. In the aroma-rich soil environment, the infective stages of animal- and plant-parasitic nematodes need to be able to detect diagnostic host specific odours to enable them to locate and infect appropriate hosts. Unlike free-living nematodes such as *C. elegans*, which feed on a wide range of bacterial species and probably also feed on filaments of fungal mycelium, fungal spores and yeast, insect-parasitic nematodes must fine tune their chemosensory repertoire to respond more precisely to host specific cues. *Caenorhabditis elegans* has been shown to respond to a very wide spectrum of chemicals varying from alcohols to aromatic compounds. Chemotaxis experiments and saturation studies have identified seven different classes of volatile molecules to which *C. elegans* shows a positive chemotactic response (Bargmann *et al.*, 1993). When *H. bacteriophora* dauer juveniles (DJ) were tested with a similar panel of odorants, our data indicate that just three classes of volatile molecules were recognised: 1-heptanol; 4,5-dimethylthiazole and CO_2. While *C. elegans* finds long chain alcohols (*e.g.*, 1-heptanol and 1-octanol) repellent and short chain alcohols highly attractive, *H. bacteriophora* DJ are strongly attracted to 1-heptanol, 1-octanol and 1-nonanol and find the short chain alcholols ethanol 1-propanol and 1-butanol to be only slightly attractive. Our data indicate that associated with the adoption of a parasitic mode of life by *Heterorhabditis* there was an adaptive change in chemotactic behaviour resulting in a decreased sensitivity to volatile by-products of bacterial metabolism and an increased sensitivity towards long chain alcohols and other insect specific volatiles.

Chemoreceptor genes in *C. elegans*

The first chemoreceptor genes in *C. elegans* were isolated using a bioinformatics approach (Troemel *et al.*, 1995). A cluster of nine related genes were found adjacent to a transmembrane guanylyl cyclase, and these genes encoded proteins with multiple predicted transmembrane domains. These sequences were then used to search the *C. elegans*

genome for related genes, and a total of 41 putative receptor genes representing six families *sra, srb, srd, sre, srg* and *sro* were identified (*sr* = serpentine receptor, a term sometimes used for 7-TM receptors). Of 14 genes for which expression data were obtained, 11 were expressed only in small subsets of chemosensory neurons. *odr-10* mutants of *C. elegans* are unable to chemotax to diacetyl. When the *odr-10* gene was cloned (Sengupta *et al.*, 1996) it was found to be a divergent 7-TM receptor with a weak homology to the *srd* genes identified by Troemel *et al.* (1995) and it also had a weak homology to vertebrate olfactory receptors *ca* 10% amino acid identity. Unlike vertebrate olfactory receptors, the *odr-10* gene contains introns and is comprised of 1 Kb of cDNA and 3.3 Kb of genomic DNA. This gene is expressed only in a single olfactory neuron in each amphid. Analysis of the *C. elegans* genome by Roberston (1998, 2001) suggests that it may encode ~550 functional chemoreceptor genes and ~250 pseudogenes which together represent ~6% of the genome. There is an ongoing and rapid process of gene duplication, deletion, diversification and movement in nematode chemoreceptor genes. For example, comparison with the *C. briggsae* genome indicates that ~28% of the *C. elegans srh* 7-TM family have been newly formed since the split with *C. briggsae* (Roberston, 2001).

Significant progress has been made in unravelling the steps involved in olfactory signalling in *C. elegans* (see Fig. 1). Many of these steps have been identified when genes responsible for various chemotaxis deficient mutants were cloned and characterised. The downstream effectors of the *odr-10* and other 7-TM chemoreceptors are heterotrimeric G proteins, comprised of α, β and γ subunits, each subunit encoded by a different gene. There are 21 *G*α, 2 *G*β and 2 *G*γ genes in *C. elegans* (Jansen *et al.*, 1999). The activated 7-TM receptor catalyses the exchange of GDP for GTP at a specific binding site on the Gα protein. The Gα protein (with its bound GTP) diffuses from the receptor G protein complex and interacts with ODR-1, a guanylyl cyclase which catalyses the conversion of GTP to cyclic GMP. cGMP is released from the Gα protein activates a cation channel consisting of two subunits, TAX-2 and TAX-4, encoded by the *tax-2* and *tax-4* genes (Coburn & Bargmann, 1996). Thus the coupling of chemoreceptor activation to electrical activity in sensory neurons of *C. elegans* is mediated *via* cGMP. In vertebrates, by contrast, olfactory signalling *via* G-protein coupled receptors results in the opening of cAMP gated channels (Ronnett & Moon, 2002). Another gene, *osm-9* is required for the activity of the *odr-10* pathway. *osm-9*

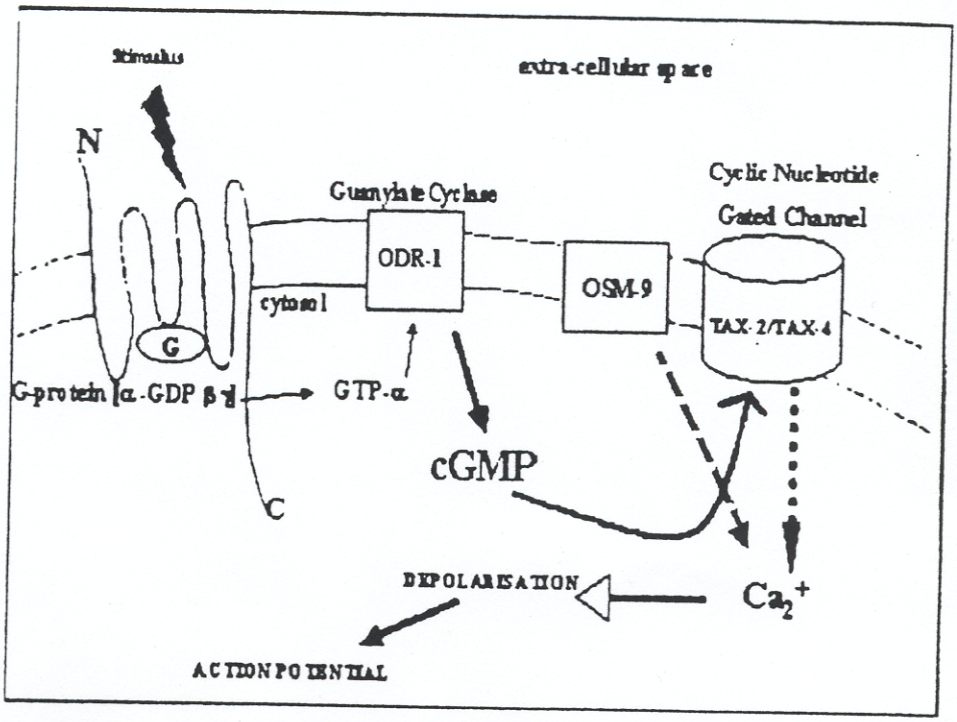

Fig. 1. *An overview of the* odr-10 *G-protein coupled seven-transmembrane receptor olfactory signalling pathway of* Caenorhabditis elegans.

encodes a predicted six-transmembrane domain protein with similarity to the transient receptor potential (TRP) receptor in *Drosophila*.

Homology-based approaches to isolate chemoreceptor genes in *Heterorhabditis bacteriophora*

Using degenerate PCR primers we have successfully cloned eight candidate G protein α subunit gene fragments and a homologue of *odr-10* gene from *H. bacteriophora*. Degenerate primers were designed based on conserved motifs identified from multiple alignments of Gα amino acid sequences from a variety of organisms. In the case of *odr-10*, degenerate primers were designed from alignments of candidate G protein coupled receptors from *C. elegans*. 5′ and 3′ RACE (Rapid Amplification of cDNA Ends) has been employed to obtain the full length coding region from one of the Gα genes. This method has also been used with the *H. bacteriophora odr-10* gene fragment; however, due to the complexity of this gene family isolating the unique 5′ end of this gene has proved to be difficult.

Future prospects

The availability of the completed *C. briggsae* genome will provide a very valuable opportunity to study the comparative evolution of chemoreceptor genes and chemosensory transduction pathways in nematodes. Since chemoreception and olfaction are critical components of the infection process for parasitic nematodes, chemoreceptor genes and signal transduction pathways have the potential to be important antihelminthic targets. Because the olfactory repertoire of parasitic nematodes is likely to be more targeted and more specific than in free-living nematodes, it is possible that the olfactory receptors will comprise smaller gene families in parasitic nematodes. While it is possible to use a comparative genomics approach to isolate individual chemoreceptor genes in parasitic nematodes, a complete understanding of chemoreceptor genes in a parasitic nematode will require access to the full genomic DNA sequence. At the moment we are unaware of any such genome sequencing initiative for any parasitic nematode.

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