CHAPTER 6

THE CHEMOSENSORY SYSTEM OF *CAENORHABDITIS ELEGANS* AND OTHER NEMATODES

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Abstract. Olfactory systems allow organisms to detect and discriminate between thousands of low molecular mass, mostly organic, compounds which we call odours. Organisms as diverse as humans and nematodes utilize the same basic mechanisms for this sensory perception. Represented in the olfactory repertoire of both vertebrates and invertebrates are aliphatic and aromatic compounds with diverse 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; successful foraging depends on the animal's ability to detect a gradient in one odorant while ignoring extraneous odours. 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. Animals modify their behaviour based on the interpretation of these environmental cues. The biochemical and physiological processes of chemosensory perception involve the recognition of small chemical molecules by specialized transduction pathways in the organism. These pathways are responsible for the transformation of information from extrinsic molecules into signals that the nervous system can interpret. The highly conserved G-protein signalling pathway is used to provide this chemosensory ability. The interaction of an odorant with an olfactory receptor results in the activation of heterotrimeric GTP-binding proteins (G proteins). G-protein signalling has been the subject of intense research over the last two decades. G proteins are present in all eukaryotic cells and signalling through G-protein-coupled receptors and heterotrimeric G proteins is one of the main means of transducing extracellular signals in the cell. *Caenorhabditis elegans* is an excellent model organism to study the molecular mechanisms behind signalling pathways in that it possesses unique traits amenable to both forward and reverse genetics. Exploiting these traits has shed much light on the mechanisms behind G-protein signalling. As molecular manipulations routinely used for *C. elegans* are becoming available for other nematodes, an increasing amount of chemosensory information is becoming available for a diverse range of nematodes from an even more diverse range of habitats.
Keywords: chemoreception; chemoreceptor genes; olfaction; nematode; G protein

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NEMATODE CHEMOSENSORY BEHAVIOUR

Nematodes are thought to have diverged early in metazoan evolution (Poinar 1983). Diversity within the phylum Nematoda is enormous; there are nearly 20,000 species classified in the phylum. Nematodes occupy a wide range of habitats including terrestrial and marine environments. The vast majority are free-living microbivores, but many species have adopted a parasitic lifestyle. Most plants and animals have at least one parasitic nematode species uniquely adapted to exploit the concentration of food and resources that the host species represents. The relationships between nematodes and their hosts are also varied, so too are the reproductive strategies employed by nematodes. The development of adaptable sensory systems is central to survival. Through evolution, chemoreception has become the primary neurosensory tool used by nematodes to detect food sources, potential hosts, noxious compounds, reproductive partners and sometimes to enable them to choose between alternative developmental states (Krieger and Breer 1999; Prasad and Reed 1999).

The chemotactic responses of the free-living soil nematode *Caenorhabditis elegans* have been extensively investigated for over thirty years. *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 (Ward 1973; Dusenbery 1974; Bargmann and Horvitz 1991). In the soil *C. elegans* feeds on a large variety of bacteria associated with decaying organic matter (Andrew and Nicholas 1976). The by-products of bacterial metabolism include various carboxylic acids, alcohols, aldehydes, esters, ketones and hydrocarbons (Zechman and Labows 1985; Schöller et al. 1997) and several of these compounds are highly attractive to *C. elegans* (Bargmann et al. 1993). 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. Carbon dioxide is a well characterized attractant which is produced as an end product of metabolism by plants, micro-organisms and animals. The plant parasitic nematode, *Meloidogyne incognita*, has been shown to respond to a gradient of carbon dioxide (Pline and Dusenbery 1987). Using cylinders of moist sand Robinson (1995) showed that *M. incognita*, *Rotylenchulus reniformis* and *Steinernema glaseri* were all attracted to a linear gradient of carbon dioxide. Numerous free-living marine nematodes aggregate in and around decaying animal bodies and plant material. Riemann and Schrage (1988) demonstrated that the free-living marine nematode *Adoncholaimus thalassophygas* was attracted to carbon dioxide, which may help it to locate sites of anaerobic decomposition as a source of food.

Unlike free-living nematodes such as *C. elegans*, which feed on a wide range of bacterial species (Andrew and Nicholas 1976; Balan 1985) as well as filaments of fungal mycelium, fungal spores and yeast (Balanova and Balan 1991), parasitic nematodes must fine-tune their chemosensory repertoire to respond more precisely to host-specific cues. Plant-parasitic nematodes respond to plant allelochemicals to ensure close synchrony between host and parasitic life cycles. The majority of plant-parasitic nematodes infect plant roots and some have evolved sophisticated interactive relationships with host cells to sustain a sedentary parasitic habit. The

root-knot nematodes, *Meloidogyne* spp., have a potential host range encompassing more than 3000 plant species. Potato root diffusates stimulate movement of hatched juveniles of *Globodera rostochiensis* (Clarke and Hennessy 1984) and may aid in host location. However, exposure of males of *G. rostochiensis* to the diffusate elicits no response (Riga et al. 1996). Males exit from the roots into the soil but probably remain in close proximity to the roots, apparently needing only sex pheromones to attract them to females. Masamune et al. (1982) isolated a natural hatching stimulus for the soybean cyst nematode. This stimulus, called glycinoeclepin A, was shown to stimulate the hatching of larvae from eggs *in vitro* from the roots of kidney beans. Although root diffusates are generally considered attractive to nematodes, several chemicals produced within the roots of some plants have been characterized that repel plant-parasitic nematodes. One such plant is the marigold (*Tagetes* spp.), which produces the compound α-terthienyl (Bakker et al. 1979; Gommers and Bakker 1988). This compound when photoactivated produces reactive oxygen species, which are highly toxic to nematodes. The compound, α-terthienyl, has been used to suppress populations of certain economically important plant-parasitic nematodes.

Entomopathogenic nematodes (EPNs) are a ubiquitous group of obligate and lethal parasites of insects. They are characterized by their ability to carry and transmit a specific insect-pathogenic symbiont bacterium. Two EPN families are currently recognized: the Steinernematidae and the Heterorhabditidae. Analysis of small-subunit ribosomal DNA reveals that these families are not closely related phylogenetically (Blaxter et al. 1998), but appear to have evolved similar morphological and ecological traits through convergent evolution (Poinar 1983). As parasitic nematodes have a more focused life cycle than free-living nematodes it is not surprising that the insect-parasitic nematode, *Heterorhabditis bacteriophora* has a similar but more restricted chemosensory repertoire than that of the free-living nematode, *C. elegans* (O'Halloran and Burnell 2003). The most notable difference in the chemotactic responses of these two nematode species is that *H. bacteriophora* infective juveniles are unresponsive to a large number of compounds which *C. elegans* finds highly attractive. The latter compounds are typical by-products of bacterial metabolism and include aldehydes, esters, ketones and short-chain alcohols (Bargmann et al. 1993), which would not provide helpful cues to assist a parasitic nematode find its host. Rasmann et al. (2005) reported the first identification of an insect-induced below-ground plant signal, (*E*)-β-caryophyllene, which strongly attracts the EPN, *Heterorhabditis megidis*. This plant signal is a sesquiterpene released by maize roots in response to feeding by the larvae of the beetle *Diabrotica virgifera virgifera*. (*E*)-β-caryophyllene is only detected from maize leaves and roots after herbivory and so is probably not the only long-range attractant for *H. megidis*, as Rasmann et al. (2005) also demonstrated that nematodes were moderately attracted to healthy and mechanically damaged plants. Therefore, what we see is that many species of nematode are adapted to a very specific repertoire of odours, which are used to exploit the concentration of food and resources that the host or food source represents.

THE CHEMOSENSORY SYSTEM OF *C. ELEGANS* AND OTHER NEMATODES

The nematode nervous system is designed to integrate many distinct environmental stimuli so that the organism can respond appropriately. A great deal is known about the properties of the neuronal circuits and the specialized neurons that encode sensory information in *C. elegans* (White et al. 1978; 1986; 1991). The *C. elegans* brain consists of a circumpharyngeal nerve ring containing 302 neurons comprising 118 morphologically distinct cell types, all of which interconnect in a reproducible manner to form a variety of neural circuits and pathways. Gap junctions occur between neurons and between muscle cells. *C. elegans* neurons have a simple (mostly monopolar or dipolar), relatively unbranched morphology and nerve processes are generally organized into ordered bundles, which, in the majority of classes, run longitudinally (e.g. ventral and dorsal nerve cords) or circumferentially (commissures).

In *C. elegans* processes from the circumpharyngeal nerve ring run anteriorly as six cephalic (head region) nerve bundles forming the inner and outer labial neurons. The dendrites in four of these nerve bundles have their cell bodies just anterior to the nerve ring, in a region loosely referred to as the 'anterior ganglion' (Chalfie and White 1988). Axons from these cell bodies synapse with the nerve ring. Two other cephalic nerve bundles contain processes from the lateral ganglia, from which amphid neuronal axons run into the nerve ring. Two bilaterally symmetric amphids in the *C. elegans*' head each contain the dendritic endings of 12 types of sensory neurons.

The nematode chemosensory organs are the amphids (Figure 1), located near the head, and the phasmids (Figure 2), located at the nematode's posterior. Nematodes are subdivided into two classes by presence or absence of phasmids, the Class Secernentea which has phasmids and the Class Adenophorea which does not possess phasmids. Phasmids are similar in general structure to the amphids, both consisting of a group of neurons opening to the exterior. In *C. elegans*, chemosensory cells within the phasmid negatively modulate reversals to repellents (Hilliard et al. 2002). The amphid neurons responsible for chemosensory and thermosensory behaviours have been identified in *C. elegans* (Secernentea) through behavioural analysis of animals in which defined neurons were ablated using a laser microbeam (Bargmann and Mori 1997). Eight types of neurons (ADF, ADL, ASE, ASG, ASH, ASI, ASJ, ASK) have one or two long slender cilia that are directly exposed to the environment through the amphid pore (Figure 1). These neurons detect mostly water-soluble chemicals (Table 2). Three types of neurons (AWA, AWB, AWC) have flattened, branched cilia that terminate near the amphid pore, but enclosed by a support cell called the amphid sheath cell. These neurons detect volatile odorants (Table 2). One type of neuron that detects thermal cues (AFD) has a complex, brush-like dendritic membrane structure at the sensory ending which is embedded in the amphid sheath cell (Figure 1).

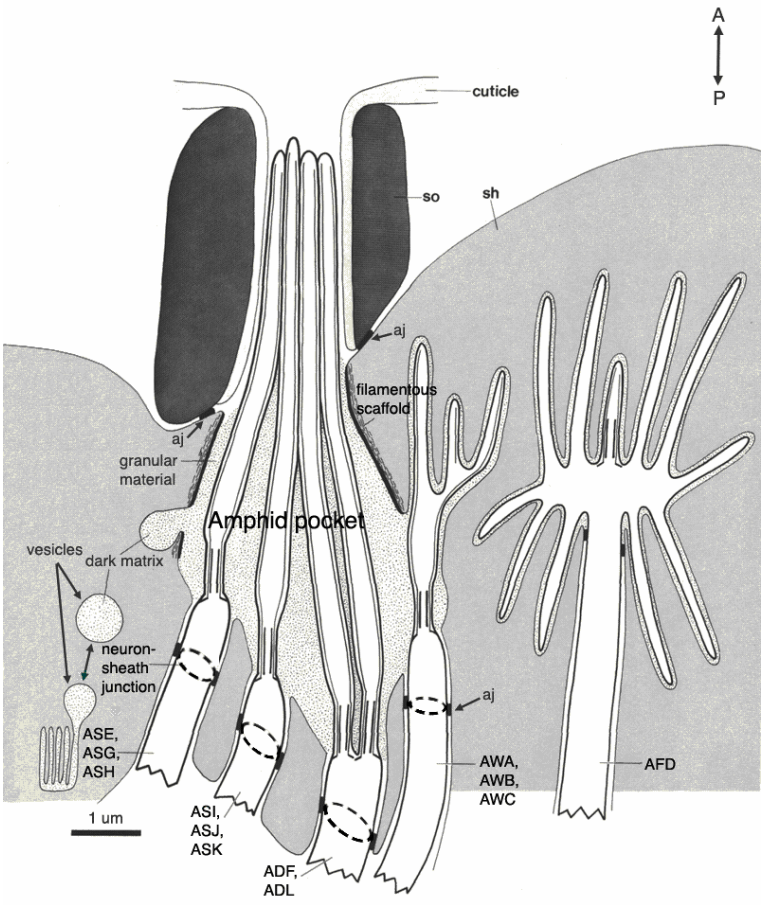

Figure 1. *Schematic longitudinal section through an amphid of C. elegans*
The amphid channel is formed from a socket cell (so) and a sheath cell (sh). The socket cell is joined by belt junctions to surrounding hypodermal cells. The socket channel is lined with cuticle that is continuous with the external cuticle. The anterior sheath channel has a dark, non-cuticular lining surrounded by a filamentous scaffold. The sheath and socket cells are joined together by belt junctions encircling the channel. The space between the cilia in the posterior sheath channel is filled with a dark matrix that appears to be packaged into vesicles further posterior, transported forward, and deposited around the cilia. The dendrites of three channel neurons and one wing neuron (AWA) are shown. The distal segment of the AWA cilium leaves the fascicle of channel cilia to re-invaginate the sheath cell. The AFD dendrite remains separate from the fascicle of wing and channel cilia. All of the dendrites form belt-shaped junctions with the sheath cell near their point of invagination. Main scale bar is 1.0 micrometer and A P arrows refer to anterior and posterior direction. (Reproduced with permission from www.wormatlas.org).

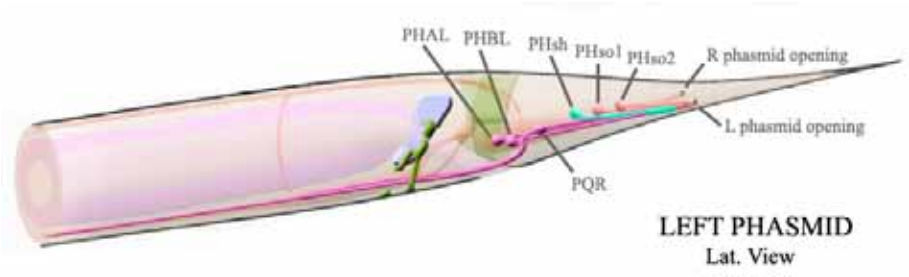

Figure 2. *Illustration of the lateral view of the left phasmid of C. elegans*
The phasmids are similar in their structure to amphid sensilla, but smaller. They are located at the lateral sides of the tail and enclose the ciliated dendrites of PHA, PHB and, on the left side, PQR neurons as well as one sheath (sh) and two socket cells (so1 and so2). The cilia of the PHA and PHB neurons extend into the external medium through the channel created by the socket cells. The ending of posterior process of PQR is wrapped by PHso2L. Phasmid sheath cells extend short processes posteriorly into tail tip which swell to form a protective pocket near the phasmid openings for PHA and PHB cilia. (Reproduced with permission from www.wormatlas.org)

There is considerable variation in size and form of the amphids between the Secernentea and the Adenophorea. Typically, paired amphids are situated laterally, but in some Adenophorea and in many Secernentea the amphids are more dorsal. The Adenophorea display much variation in their amphid organs and adenophorean amphids are usually larger and often present in greater numbers than are secernentean amphids. The microbivore *Leptonemella* spp. is a member of the Adenophorea, with large amphids (18-30 μm long) that display sexual dimorphism in their morphology, being spiral in females and loop-shaped in males (Hoschitz et al. 1999). In several animal-parasitic nematodes belonging to the Secernentea the positions of the amphidial neuronal cell bodies in the lateral ganglia are analogous to that observed in *C. elegans* (Ashton et al. 1999). Because positional homologies are conserved between these nematodes species it is likely that many functional homologies are also conserved. Ashton et al. (1999) investigated two neuron classes (ASF and ASI) in the parasitic nematode *Strongyloides stercoralis*. They found that these neurons control the decision whether to become an infective larva directly (homogonic development) or to become a free-living adult worm. This developmental switch parallels the decision in *C. elegans* whether to become a dauer larva (when conditions are adverse) or to continue normal development to adulthood. In the same study Ashton et al. (1999) noted that the ASE class of amphidial neurons in *S. stercoralis* had a chemosensory function, as in *C. elegans*, but unlike *C. elegans* this same neuron also has a thermosensory function.

Table 1. *Responses of H. bacteriophora to volatile and water-soluble compounds (O'Halloran and Burnell 2003).*

Attractants	
Alcohols	1-pentanol*, 1-hexanol*, 1-heptanol, 2-heptanol, 1-octanol, 2-octanol, 1-nonanol, 2-nonanol, 3-nonanol
Thiazole/Pyrazine	4,5-dimethylthiazole, 2-isobutylthiazole, 2-methylpyrazine, benzothiazole, 2-acetylthiazole
Organic acids	caproic acid, caprylic acid, methylvaleric acid
Others	carbon dioxide, dry-ice
Weak attractants	
Alcohols	2-mercaptoethanol, 1-butanol, 1-propanol, 1-ethanol, 3-heptanol
Others	carbonated water, uric acid¶, host assay, hexanal
Neutral compounds	
Alcohols	isobutanol, isoamyl alcohol
Ketones	acetone, 2-butanone, 2-pentanone, 2-hexanone, 2-heptanone, diacetyl
Aldehydes	benzaldehyde, valeraldehyde
Pyrazines	acetylpyrazine
Amines	butylamine
Esters	ammonium acetate, isopropyl acetate, isoamyl acetate, ethyl acetate
Others	copper sulphate¶, L-cysteine¶, dimethyl sulphoxide, paraffin, formamide, zinc sulphate¶, diethyl ether
Repellents	
Alcohols	methanol, 1-hexanol*, 1-pentanol*
Pyrazines	2,6-dimethylpyrazine, pyrazinamide
Others	L-lysine¶, d-biotin¶

*Some molecules listed with an asterisk are attractive at high concentrations and repellent at low concentrations.

¶These compounds were applied to the agar 120 minutes before the infective juveniles were added.

Table 2. *Neuronal functions in C. elegans as defined by laser ablation (Bargmann and Mori 1997)*

	Neuron	Function
Sensory neurons	AWA	volatile chemotaxis; diacetyl, pyrazine, thiazole
	AWB	volatile avoidance
	AWC	volatile chemotaxis; benzaldehyde, butanone, isoamyl alcohol, thiazole
	AFD	thermotaxis
	ASE	Na^+, Cl^-, cAMP, biotin, lysine chemotaxis, egglaying
	ADF	dauer pheromone; Na^+, Cl^-, cAMP, biotin chemotaxis
	ASG	dauer pheromone; Na^+, Cl^-, cAMP, biotin, lysine chemotaxis
	ASH	osmotic avoidance, nose-touch avoidance, volatile avoidance
	ASI	dauer pheromone; Na^+, Cl^-, cAMP, biotin, lysine chemotaxis
	ASJ	dauer pheromone (recovery)
	ASK	lysine chemotaxis, egg-laying
	ADL	octanol avoidance, water-soluble avoidance

MOLECULAR MECHANISMS OF CHEMOTAXIS

Chemoreceptor genes in Caenorhabditis

A variety of behavioural screens have been developed in *C. elegans* to identify mutant nematodes with defects in their chemosensory behaviours. These include direct screens for chemotaxis-defective mutants (che and tax – Ward 1973; Dusenbery 1974) as well as nematodes with defective responses to volatile odorants, but not to water-soluble attractants (*odr* mutants – Bargmann et al. 1993). Some chemosensory neurons are involved in dauer formation and so some chemosensory mutants were first isolated based upon defects in their ability to form dauer larvae (daf mutants, e.g., daf-11 and daf-21 – Riddle et al. 1981; Thomas et al. 1993).

The first chemoreceptor genes in C. elegans were isolated using a bioinformatics approach (Troemel et al. 1995). A cluster of 9 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 6 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, eleven were expressed only in small subsets of chemosensory neurons. The low levels of similarity within these 7-TM sub-families explain the small number of genes identified via this approach. For example, the three largest families of genes identified by Troemel et al. (1995) were the sra, srb and srg genes. The sra family shared only about 35% amino-acid identity overall, the eleven srb genes were distantly related from the sra genes and shared only 10-15% amino-acid similarity. The thirteen srg genes identified were essentially unrelated to the sra or srb genes by sequence, but were between 10 and 30% similar to one another. 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 similarity to vertebrate olfactory receptors (~10% amino-acid identity). Odr-10 mutants were isolated from C. elegans in behavioural screens for animals that failed to respond to the odorant diacetyl (Sengupta et al. 1996). The odr-10 gene is expressed only in the cilia of the AWA olfactory neurons in each amphid. Mutations in the odr-10 gene lead to a selective loss in the nematodes' ability to sense diacetyl, however the nematodes exhibit normal chemotaxis to other odorants recognized by the AWA olfactory neurons, and thus are not completely defective in AWA function. odr-10 cDNA also specifically restores diacetyl sensitivity to mutants that have lost their ability to respond to several odorants (such as odr-7, which have defective expression of a transcription factor controlling odr-10 expression, Sengupta et al. 1996). The function of ODR-10 as a chemoreceptor was further confirmed when odr-10 was transformed into mammalian cells where it functioned as a diacetyl-activated chemoreceptor (Zhang et al. 1997).

Unlike vertebrate genes encoding olfactory receptors, the *odr-10* gene contains introns (Robertson 1998). The sequence similarity between ODR-10 and the vertebrate olfactory receptors is limited to a few residues in the predicted proteins; however, these two receptor families do share more similarity with each other than with other G-protein-linked receptors. Nevertheless it is difficult to discern whether vertebrate and invertebrate olfactory receptors are derived from a common ancestor (Robertson 2000; 2001). Analysis of the *C. elegans* genome by Robertson (Robertson 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* (Robertson 2001). Another point of interest is the significant reduction in chemoreceptor genes in the *C. briggsae* genome. The *srz* chemoreceptor family has 60 representatives in the *C. elegans* genome compared with only 28 members within the *C. briggsae* genome (Thomas et al. 2005). The *srz* family also displays frequent gene duplication and deletion events as well as possessing sites undergoing positive selection (Thomas et al. 2005). The chemoreceptor subfamily five has 311 members in *C. elegans* and only 151 representatives in *C. briggsae.* Also, the *sra* family of chemoreceptors has 36 and 18 members in *C. elegans* and *C. briggsae*, respectively (Stein et al. 2003). Overall, *C. briggsae* has over 40% fewer chemoreceptor genes than *C. elegans*, highlighting the rapid rate of evolution of the chemoreceptor gene family in these nematodes.

Heterotrimeric G protein subunits

The heterotrimeric guanine nucleotide-binding proteins (G proteins) act as switches that regulate information-processing circuits connecting cell-surface receptors to a variety of effectors such as nucleotide cyclases and ion channels. The G proteins are present in all eukaryotic cells and control metabolic, humoral, neural and

developmental functions. In animals as different as humans and worms, G proteins mediate olfactory discrimination (Prasad and Reed 1999). G proteins are comprised of three peptides: an α subunit that binds and hydrolyses guanosine triphosphate (GTP), a β subunit and a γ subunit. The β and γ subunits form a dimer that only dissociates when it is denatured, thus representing a functional monomer.

When GDP is bound, the α subunit associates with the βγ subunit to form an inactive heterotrimer that binds to the receptor (Figure 3). Both α and βγ subunits can bind to the receptor. Monomeric, GDP-liganded α subunits can interact with receptors, but the association is greatly enhanced in the αβγ heterotrimer. When a chemical or physical signal stimulates the receptor, the receptor becomes activated and changes its conformation. The GDP-liganded α subunit responds with a conformational change that decreases GDP affinity, so that GDP comes off the active site of the α subunit (Figure 3). Because the concentration of GTP in cells is much higher than that of GDP, the outgoing GDP is replaced with GTP. Once GTP is bound, the α subunit assumes its activated conformation and dissociates both from the receptor and from βγ. The activated state lasts until the GTP is hydrolysed to GDP by the intrinsic GTPase activity of the α subunit. All isoforms of α subunits are GTPases, although the intrinsic state of GTP hydrolysis varies greatly from one type of Gα subunit to another (Carty et al. 1990; Linder et al. 1990). Once GTP is cleaved to GDP, the α and βγ subunits reassociate, the heterotrimer becomes inactive and returns to the receptor. The free α and βγ subunits each activate target effectors. Figure 3 illustrates the cycle of G-protein activation and deactivation that transmits a signal from receptor to effector. Six Gβ and twelve Gγ gene products have been identified in mammals (Hamm 1998). In the *C. elegans* genome two Gβ genes and two Gγ genes have been identified (Van der Voorn et al. 1990; Jansen et al. 1999).

GTP-binding α subunits have been divided on the basis of amino-acid similarity into four classes in mammals; $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$. Each grouping has been shown to function differently. Subunits of the $G\alpha_{12}$ class were originally isolated from a mouse-brain cDNA library (Strathmann and Simon 1991) and since have been shown to be expressed ubiquitously in diverse cell lines and tissues from different species (Dhanasekaran and Dermott 1996). Similarly, members of the $G\alpha_s$ and $G\alpha_{i/o}$ classes have been shown to be expressed in a wide range of tissue types. In *C. elegans*, *gsa-1* and *goa-1* (homologues of the $G\alpha_s$ and $G\alpha_{i/o}$ classes, respectively) were expressed in all cells examined (Jansen et al. 1999). The members of the $G\alpha_q$ class are often co-expressed in a variety of cell types (Milligan et al. 1993). The $G\alpha_s$ class stimulates cAMP production (Graziano et al. 1987), in contrast to $G\alpha_i$ proteins, which inhibit cAMP production and are sensitive to the Pertussis toxin (PTX) (Simon et al. 1991). $G\alpha_q$ proteins have been shown to be refractory to PTX modification (Simon et al. 1991) and the $G\alpha_{12}$ class represents yet another class of PTX-insensitive Gα proteins (Parks and Wieschaus 1991).

In *C. elegans* a representative of each of the four main mammalian Gα classes is present, as well as 17 additional Gα subunits, giving a total of 21 GTP-binding Gα subunit genes (Jansen et al. 1999). Fourteen of the additional Gα subunits are expressed almost exclusively in a small subset of the chemosensory neurons in *C.*

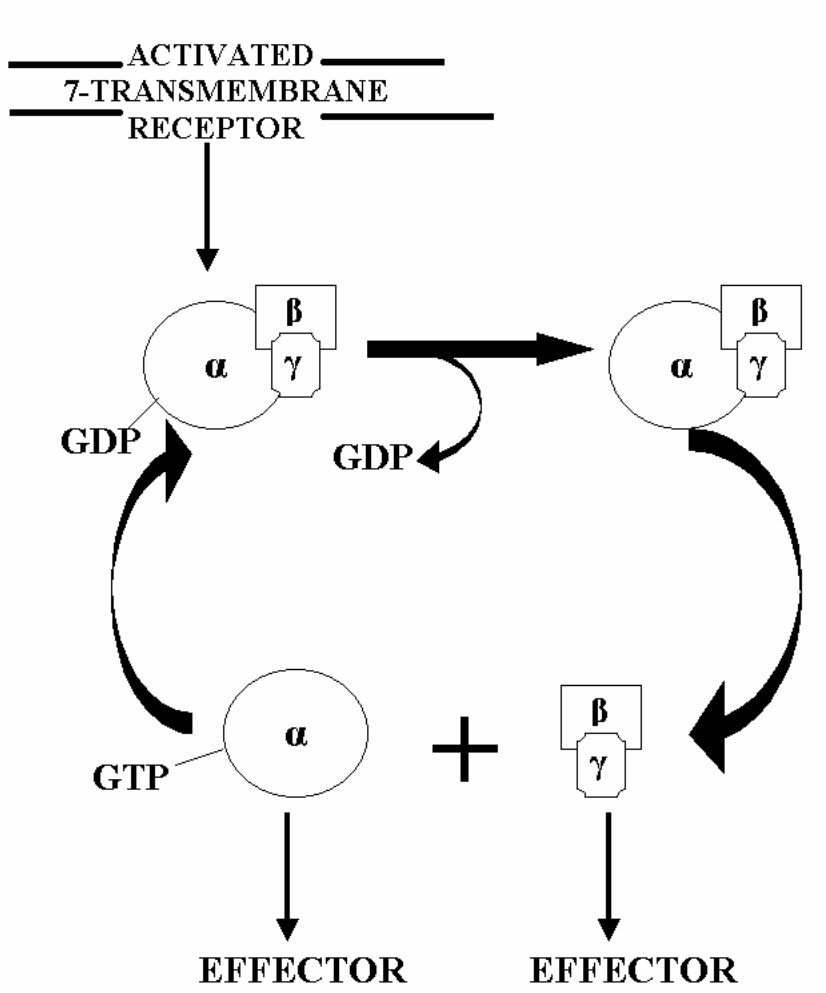

Figure 3. *The regulatory cycle of heterotrimeric G proteins subunits. When a chemical or physical signal stimulates the receptor, the receptor becomes activated and changes its conformation. The GDP-liganded α subunit responds with a conformational change that decreases GDP affinity, so that GDP comes off the active site of the α subunit and is replaced with GTP. Once GTP is bound, the α subunit assumes its activated conformation and dissociates both from the receptor and from βγ. The activated state lasts until the GTP is hydrolysed to GDP by the intrinsic GTPase activity of the α subunit. Once GTP is cleaved to GDP, the α and βγ subunits reassociate, the heterotrimer becomes inactive and returns to the receptor. The free α and βγ subunits each activate target effectors. Black lines indicate the neuronal membrane.*

elegans (Jansen et al. 1999). Although none of the Gα genes expressed in *C. elegans* amphids are essential for viability, their expression pattern clearly indicates a role for them in chemoreception. Similarly to chemosensory receptors, multiple Gα subunit genes are used in each cell (Jansen et al. 1999). We have constructed a data set containing homologues of putative Gα genes from a variety of metazoa, protistans and fungi. The final alignment contained 146 taxa and 751 aligned amino-acid positions. Our analysis reveals that nematodes have evolved multiple novel Gα subunit genes through a series of duplication events early in nematode evolution (O'Halloran et al. unpublished data). A single *C. elegans* olfactory neuron expresses multiple chemoreceptors and multiple heterotrimeric G proteins (Troemel et al. 1995; Jansen et al. 1999). The novel nematode-specific Gα genes increase the functional complexity of individual chemosensory neurons and facilitate the integration of signals from different odorant molecules within a single neuron.

Downstream signalling from chemoreceptors and G proteins

G-protein-mediated signalling is intrinsically kinetic. Signal amplitude is determined by the balance of the rates of GDP/GTP exchange (activation) and of the rates of GTP hydrolysis (deactivation). Downstream of G proteins, several novel proteins implicated in the deactivation and activation processes of Gα proteins have come into light in recent years (Ross and Wilkie 2000). Proteins involved in the deactivation process have been termed GTPase-activating proteins (GAPs) and include the G_q-stimulated phospholipase C-β (PLC-β) and the mammalian G_{13}-stimulated p115RhoGEF, a guanine nucleotide exchange factor for Rho GTPase (Chen et al. 2001). The most recently identified regulators of G-protein-signalling (RGS) proteins are found throughout most eukaryotes and are also G-protein GAPs (Watson et al. 1996).

RGS proteins accelerate the GTPase activity of G-protein α-subunits, thus driving them to their native inactive state. Mammals have ~20 proteins containing the ~120 amino-acid RGS domain that defines RGS proteins. The RGS domain folds into a nine-helix structure that binds to the Gα subunit, thereby stimulating its GTPase activity (Tesmer et al. 1997). Although many RGS proteins consist of little more than an RGS domain, a subset of them also contain a large amino-terminal conserved region of unknown function, as well as a G gamma-like (GGL) domain that is able to bind a specific G-protein β subunit (Snow et al. 1998; Chase et al. 2001). Thirteen RGS genes have been identified in *C. elegans*. Two of these have been analysed and shown to act on the homologues of the G proteins G_o and G_q (known as GOA-1 and EGL-30, respectively). The RGS protein EGL-10 inhibits signalling by G_o, which in turn inhibits egg-laying and locomotion behaviours (Mendel et al. 1995), whereas the RGS protein EAT-16 inhibits signalling by G_q, which has effects that are the opposite of those caused by G_o (Brundage et al. 1996; Miller et al. 1999). EGL-10 and EAT-16 are the only two RGS proteins in nematodes with GGL domains and have been shown to bind Gβ *in vivo*, although it is still unclear how this might influence RGS activity.

Other downstream components of the chemosensory network of *C. elegans* have

been described, such as the *odr-1* and *daf-11* genes, which code for guanylyl cyclase, an effector enzyme responsible for producing the secondary messenger (cGMP) via heterotrimeric G-proteins. Guanylyl cyclase expression is essential for all AWC-sensed odorants (L'Etoile and Bargmann 2000). The heteromeric TAX-2/TAX-4 cyclic-nucleotide gated cation channel is sensitive to cGMP and insensitive to cAMP, suggesting that *C. elegans* uses cGMP as a second messenger in olfaction, unlike mammals, which have been shown to utilize cAMP as a secondary messenger in olfactory neurons (Prasad and Reed 1999). Another novel protein required for olfaction, mechanosensation and olfactory adaptation in *C. elegans* is OSM-9, a multiple transmembrane domain protein required for the activity of ODR-10 (Colbert et al. 1997). Bioinformatic analyses of *osm-9* revealed a previously unsuspected diversity of mammalian and invertebrate genes in this family. Cyclic-nucleotide gated-channel mutants such as *tax-2* or *tax-4* respond normally to some olfactory stimuli suggesting an alternative pathway of chemosensation which may involve *osm-9* (Colbert et al. 1997). Other olfactory effectors downstream of the receptor include various kinases. EGL-4 is a cGMP-dependent kinase which regulates multiple developmental and behavioural processes (Fujiwara et al. 2002; L'Etoile et al. 2002). The classical Ras-MAPK (mitogen-activated protein kinase) signal transduction pathway was also shown to be activated in *C. elegans* upon application of the attractant isoamyl alcohol (Hirotsu et al. 2000). Thus it is clear that G-protein-coupled odour transduction pathways are complex in mammalian systems, but are more complex still in nematodes in which multiple signal transduction mechanisms in the same cell are used to distinguish between odorants.

FUTURE PROSPECTS

The molecular tools that have been used to investigate the chemosensory system of *C. elegans* are now being developed and applied to studies on other nematodes. This technology transfer of research methodology from *C. elegans* is a slow process because of the diversity of nematodes studied by researchers and the lack of resources devoted to individual systems. Many groups have exploited the molecular knowledge of *C. elegans* to study other nematode systems. Kwa et al. (1995) were one of the earliest groups to demonstrate the use of *C. elegans* to study parasitic-nematode genes. They designed a mutant rescue assay to show that the β-tubulin genes from *Haemonchus contortus* could modulate drug resistance in *C. elegans*. Another more recent study demonstrated the ectopic expression of an *H. contortus* GATA transcription factor (*elt-2*) in *C. elegans* (Couthier et al. 2004). This factor is a central regulator of endoderm development. This study showed that the development of the *H. contortus* lineage is strikingly similar to that of *C. elegans*. Transformation of *C. elegans* with promoter/reporter gene constructs for the pepsinogen gene, *pep-1*, from *H. contortus* and the cysteine protease gene (*ac-2*) in *Ostertagia circumcinta* has also been demonstrated by Britton et al. (1999), revealing good spatial agreement with the localization of the native proteins encoded by these genes in the parasites. Hashmi et al. (1998) had some success at

transforming *H. bacteriophora* by microinjection of reporter constructs. This transformation resulted in approximately 7% of the F1 generation exhibiting *lacZ* expression. Urwin et al. (2002) demonstrated that ingestion of dsRNA by preparasitic juvenile cyst nematodes leads to RNA interference of cysteine proteinases, major sperm proteins and a novel *Heterodera glycines* gene. Taken together, these studies suggest a high degree of conservation of the post-transcriptional and post-translational gene regulatory mechanisms between parasitic nematodes and *C. elegans*. As research methods from *C. elegans* and indeed other model organisms too are utilized by nematode researchers, a substantial amount of genetic, phylogenetic and pharmacogenomic knowledge pertaining to olfaction is gradually coming to light.

It seems that genes implicated in the nematode nervous system often have peculiarities associated with them. Along with the expansion and diversification of some neuronal gene families there has been a selective reduction and/or loss of certain others. For example, the largest and most diverse nicotinic acetylcholine receptor (nACHR) gene family is that of *C. elegans* (Mongan et al. 1998). nACHRs mediate the fast actions of the neurotransmitter acetylcholine at nerve muscle junctions and in the nervous system. The molecular diversity within this family includes very distinct groups, which are thought to have diverged early in nematode evolution (Treinin and Chalfie 1995). Lineage-specific expansion of neural Gα genes also appears to have occurred in nematodes. The NGF family of neurotrophins are protein growth factors with crucial roles in the determination of neuronal survival and regulation of neuronal numbers throughout vertebrate development. Completion of the *C. elegans* genome sequence has confirmed that the distinct 'hard wired' nematode nervous system does not require neurotrophins or their receptors (Ruvkun and Hobert 1998). *C. elegans* lacks voltage-dependent sodium-channel genes, which are present in the more primitive jellyfish (Bargmann 1998), suggesting the ability to generate a sodium-based action potential was lost during nematode evolution. Conversely a novel *osm*-related transient receptor potential (TRP) ion channel, OSM-9, was identified in *C. elegans* (Colbert et al. 1997), revealing the existence of an alternative chemosensory pathway within the nematode. Whole genome analysis of *C. elegans* and *C. briggsae*, in conjunction with previous studies on the *C. elegans* nervous system, indicate that the *Caenorhabditis* nervous system has some very specialized features and there are several examples of neuronal gene families that appear to have undergone nematode-specific gene expansion. Functional analysis of the *C. elegans* and *C. briggsae* gene sequences may possibly identify other novel gene families involved in nematode chemoreception. As additional nematode-sequencing projects are completed they will provide further whole-genome windows into the level of complexity associated with chemosensory signalling, as well as providing a platform of comparative genomics between a variety of divergent nematodes.

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REFERENCES

Andrew, P.A. and Nicholas, W.L., 1976. Effect of bacteria on dispersal of *Caenorhabditis elegans* (Rhabditidae). *Nematologica,* 22 (4), 451-461.

Ashton, F.T., Li, J. and Schad, G.A., 1999. Chemo- and thermosensory neurons: structure and function in animal parasitic nematodes. *Veterinary Parasitology,* 84 (3/4), 297-316.

Bakker, J., Gommers, F.J., Nieuwenhuis, I., et al., 1979. Photoactivation of the nematocidal compound alpha-terthienyl from roots of marigolds (*Tagetes* spp): a possible singlet oxygen role. *Journal of Biological Chemistry,* 254 (6), 1841-1844.

Balan, J., 1985. Measuring minimal concentrations of attractants detected by the nematode *Panagrellus redivivus*. *Journal of Chemical Ecology,* 11 (1), 105-112.

Balanova, J. and Balan, J., 1991. Chemotaxis-controlled search for food by the nematode *Panagrellus redivivus*. *Biologia,* 46 (3), 257-263.

Bargmann, C.I., 1998. Neurobiology of the *Caenorhabditis elegans* genome. *Science,* 282 (5396), 2028-2033.

Bargmann, C.I., Hartweig, E. and Horvitz, H.R., 1993. Odorant-selective genes and neurons mediate olfaction in *C. elegans*. *Cell,* 74 (3), 515-527.

Bargmann, C.I. and Horvitz, H.R., 1991. Chemosensory neurons with overlapping functions direct chemotaxis to multiple chemicals in *C. elegans*. *Neuron,* 7 (5), 729-742.

Bargmann, C.I. and Mori, I., 1997. Chemotaxis and thermotaxis. *In:* Riddle, D.L., Blumenthal, T., Meyer, B.J., et al. eds. *C. elegans II*. Cold Spring Harbor Press, New York, 717-738.

Blaxter, M.L., De Ley, P., Garey, J.R., et al., 1998. A molecular evolutionary framework for the phylum Nematoda. *Nature,* 392 (6671), 71-75.

Britton, C., Redmond, D.L., Knox, D.P., et al., 1999. Identification of promoter elements of parasite nematode genes in transgenic *Caenorhabditis elegans*. *Molecular and Biochemical Parasitology,* 103 (2), 171-181.

Brundage, L., Avery, L., Katz, A., et al., 1996. Mutations in a *C. elegans* Gq alpha gene disrupt movement, egg laying, and viability. *Neuron,* 16 (5), 999-1009.

Carty, D.J., Padrell, E., Codina, J., et al., 1990. Distinct guanine nucleotide binding and release properties of the three g(i) proteins. *Journal of Biological Chemistry,* 265 (11), 6268-6273.

Chalfie, M. and White, J., 1988. The nervous system. *In:* Wood, W.B. ed. *The nematode Caenorhabditis elegans*. Cold Spring Harbour Press, New York, 337-391.

Chase, D.L., Patikoglou, G.A. and Koelle, M.R., 2001. Two RGS proteins that inhibit G[alpha](o) and G[alpha](q) signaling in *C. elegans* neurons require a G[beta](5)-like subunit for function. *Current Biology,* 11 (4), 222-231.

Chen, Z., Wells, C.D., Sternweis, P.C., et al., 2001. Structure of the rgRGS domain of p115RhoGEF: p115RhoGEF, a guanine nucleotide exchange factor for Rho GTPase, is also a GTPase. *Nature Structural Biology,* 8 (9), 805-809.

Clarke, A.J. and Hennessy, J., 1984. Movement of *Globodera rostochiensis* juveniles stimulated by potato-root exudate. *Nematologica,* 30 (2), 206-212.

Colbert, H.A., Smith, T.L. and Bargmann, C.I., 1997. OSM-9, a novel protein with structural similarity to channels, is required for olfaction, mechanosensation, and olfactory adaptation in *Caenorhabditis elegans*. *Journal of Neuroscience,* 17 (21), 8259-8269.

Couthier, A., Smith, J., McGarr, P., et al., 2004. Ectopic expression of a *Haemonchus contortus* GATA transcription factor in *Caenorhabditis elegans* reveals conserved function in spite of extensive sequence divergence. *Molecular and Biochemical Parasitology,* 133 (2), 241-253.

Dhanasekaran, N. and Dermott, J.M., 1996. Signaling by the G_{12} class of G proteins. *Cellular Signalling,* 8 (4), 235-245.

Dusenbery, D.B., 1974. Analysis of chemotaxis in the nematode *Caenorhabditis elegans* by counter current separation. *Journal of Experimental Zoology,* 188 (1), 41-47.

Fujiwara, M., Sengupta, P. and McIntire, S.L., 2002. Regulation of body size and behavioral state of *C. elegans* by sensory perception and the EGL-4 cGMP-dependent protein kinase. *Neuron,* 36 (6), 1091-1102.

Gommers, F.J. and Bakker, J., 1988. Physiological diseases induced by plant responses or products. *In:* Poinar, G.O. and Jansson, H.B. eds. *Diseases of nematodes*, Vol. 1. CRC Press, Boca Raton, 3-22.

Graziano, M.P., Casey, P.J. and Gilman, A.G., 1987. Expression of complementary DNA species for G proteins in *Escherichia coli* two forms of Gs alpha stimulate adenylate cyclase. *Journal of Biological Chemistry,* 262 (23), 11375-11381.

Hamm, H.E., 1998. The many faces of G protein signaling. *Journal of Biological Chemistry,* 273 (2), 669-672.

Hashmi, S., Hashmi, G., Glazer, I., et al., 1998. Thermal response of *Heterorhabditis bacteriophora* transformed with the *Caenorhabditis elegans hsp70*-encoding gene. *Journal of Experimental Zoology,* 281 (3), 164-170.

Hilliard, M.A., Bargmann, C.I. and Bazzicalupo, P., 2002. *C. elegans* responds to chemical repellents by integrating sensory inputs from the head and the tail. *Current Biology,* 12 (9), 730-734.

Hirotsu, T., Saeki, S., Yamamoto, M., et al., 2000. The Ras-MAPK pathway is important for olfaction in *Caenorhabditis elegans*. *Nature,* 404 (6775), 289-293.

Hoschitz, M., Buchholz, T.G. and Ott, J.A., 1999. *Leptonemella juliae* sp.n. and *Leptonemella vestari* sp.n. (Stilbonematinae), two new free-living marine nematodes from a subtidal sand bottom. *Annalen des Naturhistorischen Museums in Wien. Serie B. Botanik und Zoologie* (101), 423-435.

Jansen, G., Thijssen, K.L., Werner, P., et al., 1999. The complete family of genes encoding G proteins of *Caenorhabditis elegans*. *Nature Genetics,* 21 (4), 414-419.

Krieger, J. and Breer, H., 1999. Olfactory reception in invertebrates. *Science,* 286 (5440), 720-723.

Kwa, M.S.G., Veenstra, J.G., Dijk, M.V., et al., 1995. [beta]-Tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. *Journal of Molecular Biology,* 246 (4), 500-510.

L'Etoile, N.D. and Bargmann, C.I., 2000. Olfaction and odor discrimination are mediated by the *C. elegans* guanylyl cyclase ODR-1. *Neuron,* 25 (3), 575-586.

L'Etoile, N.D., Coburn, C.M., Eastham, J., et al., 2002. The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in *C. elegans*. *Neuron,* 36 (6), 1079-1089.

Linder, M.E., Ewald, D.A., Miller, R.J., et al., 1990. Purification and characterization of Go alpha and three types of Gi alpha after expression in *Escherichia coli*. *Journal of Biological Chemistry,* 265 (14), 8243-8251.

Masamune, T., Anetai, M., Takasugi, M., et al., 1982. Isolation of a natural hatching stimulus, glycinoeclepin A, for the soybean cyst nematode. *Nature,* 297 (5866), 495-496.

Mendel, J.E., Korswagen, H.C., Liu, K.S., et al., 1995. Participation of the protein Go in multiple aspects of behavior in *C. elegans*. *Science,* 267 (5204), 1652-1655.

Miller, K.G., Emerson, M.D. and Rand, J.B., 1999. Go[alpha] and diacylglycerol kinase negatively regulate the Gq[alpha] pathway in *C. elegans*. *Neuron,* 24 (2), 323-333.

Milligan, G., Mullaney, I. and McCallum, J.F., 1993. Distribution and relative levels of expression of the phosphoinositidase-C-linked G-proteins G_q alpha and G_{11} alpha: absence of G_{11} alpha in human platelets and haemopoietically derived cell lines. *Biochimica et Biophysica Acta,* 1179 (2), 208-212.

Mongan, N.P., Baylis, H.A., Adcock, C., et al., 1998. An extensive and diverse gene family of nicotinic acetylcholine receptor alpha subunits in *Caenorhabditis elegans*. *Receptors Channels,* 6 (3), 213-228.

O'Halloran, D.M. and Burnell, A.M., 2003. An investigation of chemotaxis in the insect parasitic nematode *Heterorhabditis bacteriophora*. *Parasitology,* 127 (4), 375-385.

Parks, S. and Wieschaus, E., 1991. The *Drosophila* gastrulation gene *concertina* encodes a G alpha-like protein. *Cell,* 64 (2), 447-458.

Pline, M. and Dusenbery, D.B., 1987. Responses of plant-parasitic nematode *Meloidogyne incognita* to carbon dioxide determined by video camera-computer tracking. *Journal of Chemical Ecology,* 13 (4), 873-888.

Poinar, G.O., 1983. *The natural history of nematodes*. Prentice-Hall, Englewood Cliffs.

Prasad, B.C. and Reed, R.R., 1999. Chemosensation: molecular mechanisms in worms and mammals. *Trends in Genetics,* 15 (4), 150-153.

Rasmann, S., Kollner, T.G., Degenhardt, J., et al., 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature,* 434 (7034), 732-737.

Riddle, D.L., Swanson, M.M. and Albert, P.S., 1981. Interacting genes in nematode dauer larva formation. *Nature,* 290 (5808), 668-671.

Riemann, F. and Schrage, M., 1988. Carbon dioxide as an attractant for the free-living marine nematode *Adoncholaimus thalassophygas*. *Marine Biology,* 98 (1), 81-85.

Riga, E., Perry, R.N., Barrett, J., et al., 1996. Electrophysiological responses of males of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, to their sex pheromones. *Parasitology,* 112 (2), 239-246.

Robertson, H.M., 1998. Two large families of chemoreceptor genes in the nematodes *Caenorhabditis elegans* and *Caenorhabditis briggsae* reveal extensive gene duplication, diversification, movement, and intron loss. *Genome Research,* 8 (5), 449-463.

Robertson, H.M., 2000. The large *srh* family of chemoreceptor genes in *Caenorhabditis* nematodes reveals processes of genome evolution involving large duplications and deletions and intron gains and losses. *Genome Research,* 10 (2), 192-203.

Robertson, H.M., 2001. Updating the *str* and *srj* (*stl*) families of chemoreceptors in *Caenorhabditis* nematodes reveals frequent gene movement within and between chromosomes. *Chemical Senses,* 26 (2), 151-159.

Robinson, A.F., 1995. Optimal release rates for attracting *Meloidogyne incognita*, *Rotylenchulus reniformis*, and other nematodes to carbon dioxide in sand. *Journal of Nematology,* 27 (1), 42-50.

Ross, E.M. and Wilkie, T.M., 2000. GTPase-activating proteins for heterotrimeric G proteins: regulators of G protein signaling (RGS) and RGS-like proteins. *Annual Review Biochemistry,* 69, 795-827.

Ruvkun, G. and Hobert, O., 1998. The taxonomy of developmental control in *Caenorhabditis elegans*. *Science,* 282 (5396), 2033-2041.

Schöller, C., Molin, S. and Wilkins, K., 1997. Volatile metabolites from some gram-negative bacteria. *Chemosphere,* 35 (7), 1487-1495.

Sengupta, P., Chou, J.H. and Bargmann, C.I., 1996. odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. *Cell,* 84 (6), 899-909.

Simon, M.I., Strathmann, M.P. and Gautam, N., 1991. Diversity of G proteins in signal transduction. *Science,* 252 (5007), 802-808.

Snow, B.E., Hall, R.A., Krumins, A.M., et al., 1998. GTPase activating specificity of RGS12 and binding specificity of an alternatively spliced PDZ (PSD-95/Dlg/ZO-1) domain. *Journal of Biological Chemistry,* 273 (28), 11749-17755.

Stein, L.D., Bao, Z.R., Blasiar, D., et al., 2003. The genome sequence of *Caenorhabditis briggsae*: a platform for comparative genomics. *PLoS Biology,* 1 (2), 166-192.

Strathmann, M.P. and Simon, M.I., 1991. G[alpha]12 and G[alpha]13 subunits define a fourth class of G protein alpha subunits. *Proceedings of the National Academy of Sciences of the United States of America,* 88 (13), 5582-5586.

Tesmer, J.J., Sunahara, R.K., Gilman, A.G., et al., 1997. Crystal structure of the catalytic domains of adenylyl cyclase in a complex with $G_{salpha}.GTP_{gamma}S$. *Science,* 278 (5345), 1907-1916.

Thomas, J.H., Birnby, D.A. and Vowels, J.J., 1993. Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. *Genetics,* 134 (4), 1105-1117.

Thomas, J.H., Kelley, J.L., Robertson, H.M., et al., 2005. Adaptive evolution in the SRZ chemoreceptor families of *Caenorhabditis elegans* and *Caenorhabditis briggsae*. *Proceedings of the National Academy of Sciences of the United States of America,* 102 (12), 4476-4481.

Treinin, M. and Chalfie, M., 1995. A mutated acetylcholine receptor subunit causes neuronal degeneration in *C. elegans*. *Neuron,* 14 (4), 871-877.

Troemel, E.R., Chou, J.H., Dwyer, N.D., et al., 1995. Divergent seven transmembrane receptors are candidate chemosensory receptors in *C. elegans*. *Cell,* 83 (2), 207-218.

Urwin, P.E., Lilley, C.J. and Atkinson, H.J., 2002. Ingestion of double-stranded RNA by preparasitic juvenile cyst nematodes leads to RNA interference. *Molecular Plant Microbe Interactions,* 15 (8), 747-752.

Van der Voorn, L., Gebbink, M., Plasterk, R.H.A., et al., 1990. Characterization of a G-protein [beta]-subunit gene from the nematode *Caenorhabditis elegans*. *Journal of Molecular Biology,* 213 (1), 17-26.

Ward, S., 1973. Chemotaxis by the nematode *Caenorhabditis elegans*: identification of attractants and analysis of the response by use of mutants. *Proceedings of the National Academy of Sciences of the United States of America,* 70 (3), 817-821.

Watson, N., Linder, M.E., Druey, K.M., et al., 1996. RGS family members: GTPase-activating proteins for heterotrimeric G-protein alpha-subunits. *Nature,* 383 (6596), 172-175.

White, J.G., Albertson, D.G. and Anness, M.A.R., 1978. Connectivity changes in a class of motoneurone during the development of a nematode. *Nature,* 271, 764-766.

White, J.G., Southgate, E. and Thompson, J.N., 1991. On the nature of undead cells in the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London,* 331, 263-271.

White, J.G., Southgate, E., Thomson, J.N., et al., 1986. The structure of the nervous system of the nematode *Caenorhabditis elegans*. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences,* 314 (1165), 1-340.

Zechman, J.M. and Labows, J.N., 1985. Volatiles of *Pseudomonas aeruginosa* and related species by automated headspace concentration-gas chromatography. *Canadian Journal of Microbiology,* 31 (3), 232-237.

Zhang, Y., Chou, J.H., Bradley, J., et al., 1997. The *Caenorhabditis elegans* seven-transmembrane protein ODR-10 functions as an odorant receptor in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America,* 94 (22), 12162-12167.