Cell. Mol. Life Sci. 63 (2006) 1510–1522
1420-682X/06/131510-13
DOI 10.1007/s00018-006-6114-7

Cellular and Molecular Life Sciences

Chemical sensitivity in *Caenorhabditis elegans*

C. Bergamasco and P. Bazzicalupo*

Istituto di Genetica e Biofisica – A. Buzzati Traverso, CNR, Via P. Castellino 111, 80131 Napoli (Italy),
e-mail: bazzical@igb.cnr.it

Online First 29 May 2006

Abstract. The small nematode *Caenorhabditis elegans* lives in the soil, where mechanical, thermal and most of all chemical stimuli strongly influence its behavior. Here we briefly review how chemical sensitivity is organized at the cellular and molecular level in this organism. *C. elegans* has less than 40 chemosensory neurons. With few exceptions each neuron senses more than one substance and each substance is sensed by more than one neuron. At the molecular level, as in other organisms, also in *C. elegans*, seven transmembrane G-protein-coupled receptors (GPCRs), heterotrimeric G proteins, cyclic nucleotide-gated ion channels, TRP channels and Ca^{++} play crucial roles in chemical sensitivity. An unusual feature, possibly due to *C. elegans's* strong dependence on chemical cues, is the very large number of GPCR chemoreceptor genes (1300–1700) coded in its genome. Genetic approaches have also allowed the identification of new molecules involved in chemical sensitivity that would not have been discovered otherwise. In addition to the basic factors involved in primary signalling, the studies in *C. elegans* have revealed a network of regulatory pathways and molecules suggesting that fine modulation of the responsiveness of neurons is important, possibly to allow worms to negotiate a continuously changing environment. The experimental versatility of *C. elegans* has made it possible, in many cases, to determine precisely in which neuron a given molecule or pathway is required and for which biological response. This type of information can contribute to the general field of sensory signalling because it provides correlations between the biochemical properties of molecules and their cellular functions and between these and the *in vivo* behavioral responses of the animal.

Keywords. Taste, smell, chemoreceptor genes, G protein signalling, RGS proteins, chemical coding, chemical discrimination.

Introduction

The nematode worm *Caenorhabditis elegans* is proving to be a valuable experimental model to study the role of genes in the organization and functioning of a nervous system and in shaping behavior. *C. elegans* hermaphrodites have 302 neurons and males 381 neurons; shape, position and connectivity of each neuron have been described [1] (http://www.wormatlas.org), and this description represents a unique basis to approach the way the nervous system is organized and functions. A variety of powerful experimental approaches have been devised to study the function of cells and the role of genes in this organism. The study of the nervous system has also been facilitated by the fact that, under laboratory conditions, most of the neurons and many of the genes affecting behavior are in fact not required for viability and reproduction, and thus it has been easier to manipulate them experimentally. *C. elegans* enables study of chemoreception by testing behaviors that depend on or are modulated by chemical cues present in the environment. Most of the neurons and many genes affecting these behaviors have been identified [2–5]. Recently, the development of techniques for imaging Ca^{++} fluxes in selected sensory neurons in live, behaving animals has opened the possibility of monitoring the cellular responses of sensory neurons independently of the activity of the other cells (neuronal and non) that are involved in behavioral or developmental responses [6, 7]. In the soil where it lives, *C. elegans* moves to find food, mating partners, places with favorable conditions for laying its progeny. Mechanical, thermal and most of all chemical stimuli strongly influence direction and rate of movement. The worm chemotaxes toward food or indicators of the presence of food, follow-

* Corresponding author.

ing both volatile and water-soluble cues. In addition, the worms need to detect and avoid noxious substances and signals of the potential presence of toxics or of dangerous situations. The responses to these aversive cues include negative chemotaxis (moving in a gradient of repellent away from its source) and the avoidance reflex, in which the worm inverts the direction of its movement when it abruptly encounters a noxious or repellent stimulus. Signals from the environment influence not only movement but also feeding, mating, egg laying, defecation, social behavior, development and aging [2, 4, 5]. The different neuronal circuits responsible for these behavioral and developmental responses have been and are actively investigated and have recently been reviewed [5]. However, the information reviewed here derives mainly from studies of the way chemical stimuli influence movement (positive and negative chemotaxis as well as avoidance).

Here we review the *C. elegans* sensory neurons involved in detecting chemical cues, their sensory specificities and what we know about the molecules at work in them: those necessary for primary sensory transduction (if a neuron responds or not to a particular ligand and how) and those that regulate the responsiveness of sensory neurons (adaptation/modulation/plasticity). We then briefly discuss some of the implications and questions raised by the cellular and molecular organization of chemical sensitivity in the worm.

Cellular organization of chemical sensitivity

Chemosensory sensilla

The amphids, the phasmids and the inner labial sensilla (IL) are the three types of chemosensory sensilla of *C. elegans* present in both sexes. The specialized endings of their associated sensory neurons are exposed to the external environment (Fig. 1). The two bilaterally symmetric amphids and the six ILs are placed anteriorly at the sides and around the mouth respectively. The bilaterally symmetric phasmids are placed instead posteriorly in the tail. The ILs and the phasmids are simpler than the amphids and are each associated with the endings of two sensory neurons. In the phasmids, both neurons, PHA and PHB, are exposed to the outside, while, of the two neurons of each IL, only IL2 reaches the outside. The main and most complex chemoreceptor organs are the amphids. Each contains the endings of 12 different types of sensory neurons. Like most of the neurons of *C. elegans,* the bodies of the amphid neurons are located in the head between the two bulbs of the pharynx. Their axons connect with

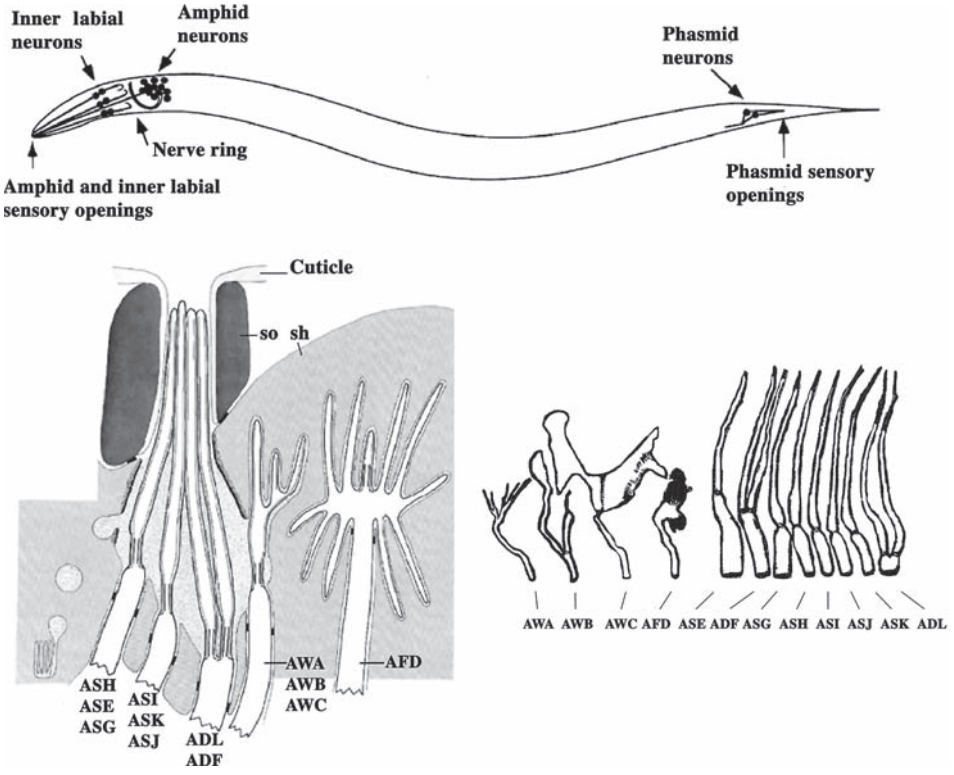

Figure 1. *C. elegans* chemosensory organs. Adapted from [2]. (*a*) Scheme of the position of main sensilla in the animal. (*b*) Details of amphid structure. The cilia of the AWA, AWB, AWC and AFD neurons are embedded in the sheath cell (sh). The cilia of the remaining eight chemosensory neurons reach the outside environment through the amphid channel constituted by the socket cell (so). (*c*) Structure of the sensory endings of the amphid neurons. Adapted from [60]. The cilia of the olfactory neurons AWA, AWB and AWC have flattened, fan-like endings. The cilium of the thermosensory neuron AFD branches in finger-like endings. The remaining neurons have long, thin, unbranched endings.

Table 1. Molecules at work in some amphid chemosensory neurons.

Sensory neuron	Primary signalling				Regulation of signalling
	G protein Signaling	Effectors	Channels	Unknown	
ASE	GPA-3	?	TAX-2/TAX-4		GRK-2, TAX-6, OSM-9
AWC	ODR-3, GPA-3, GPA-2	ODR-1, DAF-11	TAX-2/TAX-4		ARR-1, GRK-2, TAX-6, OSM-9, TTX-4, TPA-1, EGL-4
AWB	ODR-3	ODR-1	TAX-2/TAX-4		ARR-1, GRK-2
ASH	ODR-3, GPA-3, GPC-1	PUFAs	OSM-9/OCR-2	OSM-10, QUI-1	ARR-1, GRK-2, GPC-1, TAX-6, TTX-4, GPA-11
AWA	ODR-10, ODR-3, GPA-3	PUFAs	OSM-9/OCR-2		GPA-5, ARR-1, GRK-2, TAX-6, TTX-4, TPA-1

each other and with other neurons in the head. Their dendritic processes bundle and extend anteriorly to reach the outside through the amphid channel and terminate with specialized sensory cilia (Fig. 1) [1, 2].

Chemosensory neurons

Cell-killing experiments using a laser microbeam [2], and cell-specific genetic rescue experiments [8, 9], have been used to assign a function to sensory neurons by testing the behavioral responses of operated or rescued animals. Figure 2 summarizes the results obtained by different groups on the 12 amphid and 2 phasmid sensory neurons.

Some considerations are suggested by these results. First, different sensory modalities appear to largely define three groups of amphid neurons. Eight pairs of neurons detect water-soluble chemicals, three pairs detect volatile odorants and one neuron is thermosensory. The structure of the endings of the sensory cilia is different in the three groups of neurons (Fig. 1c). The function of the two phasmid neurons, PHA and PHB, and the structure of their cilia make them more similar to the group of neurons that sense water-soluble chemicals. The correlation between morphology of the cilia and sensory modalities is, however, not absolute. For instance, the ASH and ADL neurons, which have long, thin cilia, detect both water-solu-

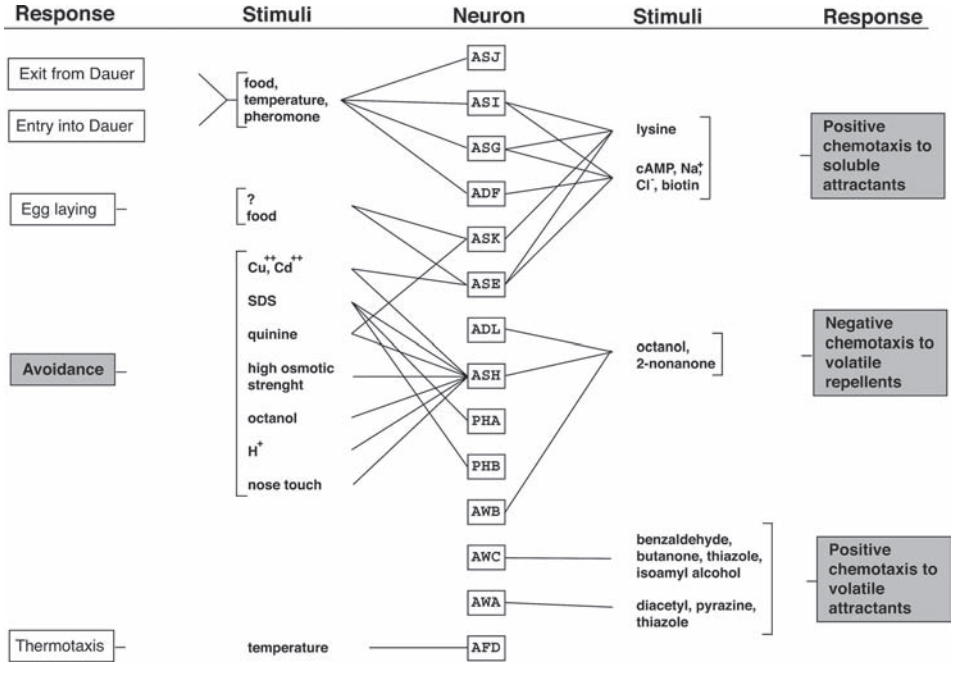

Figure 2. Schematic representation of the stimuli and of the main behavioral output of the 12 amphid and 2 phasmid sensory neurons. Most of the information for the assignement of a function to these neurons has been reviewed in [2], where references to the original works can be found. For the phasmid neurons see [9] and for some of the repellent stimuli see [13, 12].

ble and volatile repellents. In addition, ASH also senses osmolarity and a mechanical stimulus, nose touch. This broad spectrum of sensory modalities all linked to noxious stimuli suggests that ASH is a polymodal sensory neurons analogous to the nociceptive neuron of mammalians [10].

Figure 2 also indicates that the organization of the *C. elegans* chemosensory system is such that each substance is usually sensed by more than one neuron and each neuron senses more than one substance. Within this organization, however, sensitivity to repellents appears to be largely segregated to a group of neurons (ASH, ADL, AWB, PHA and PHB) different from those that detect attractants and/or signals influencing egg laying and dauer development (AWA, AWC, ADF, ASG, ASI, ASJ, ASE and ASK). This situation is somewhat analogous to the organization of taste in mammalians, in which individual taste cells express either receptors for bitter substances or receptors for sugars/amino acids [11]. Although segregation of the capacity to sense repellents and attractants is largely the rule, there are at least two documented exceptions: (i) ASK has been described as sensing the attractant lysine [2] but also the repellent quinine [12]; (ii) ASE, which appears to be the main neuron for several attractants (Na^{++}, Cl^{-}, cAMP) [2], also senses, as repellent, toxic concentrations of the divalent cations Cu^{++} and Cd^{++} [13]. How a single cell can connect to two different/opposite circuits is still not understood.

Another aspect of the cellular organization of chemical sensitivity regards the location of sensors in the head and in the tail and the role of amphid versus phasmid sensory neurons. Ablation of the PHA and PHB phasmid neurons does not result in chemotaxis or dauer formation defects [2]. However, PHA and PHB were shown to function as chemosensory neurons that sense repellents (e.g. the detergent SDS) and negatively modulate the avoidance response mediated by sensory neurons of the amphids [9]. In sensing repellents, antagonistic chemosensory inputs from head and tail neurons are integrated to generate appropriate behavioral responses. The integration most likely occurs at the level of the command interneurons (AVA, AVB, AVD and PVC) that receive inputs from sensory neurons to control the choice between forward and backward movement. The findings on the phasmid neurons indicate that for mapping chemicals in the environment, a key factor for its fitness, *C. elegans* uses at least two different strategies. For avoidance of noxious chemicals, for which fast responses may have a strong adaptive value, *C. elegans* has sense organs in the head and in the tail (amphids and phasmids) such that activation of sensory neurons immediately and directly reflects the spatial distribution of the chemical stimuli. For chemotaxis (e.g. toward food sources), chemosensory organs are clustered instead together in the head (amphids, IL?), and no input from the phasmids (tail) is necessary. The worms discern the location of chemicals by moving through the environment and integrating temporally separated signals with proprioceptive inputs. The temporal integration strategy, used for chemotaxis, is time consuming by definition but allows animals to accurately find the point source of an attractant in any direction. The simple head-to-tail sensory map, used instead for the avoidance reflex, allows for a very rapid response, but it is obviously limited in spatial resolution [9].

Molecules required for chemosensation

A powerful approach used in *C. elegans* to identify molecules involved in chemoreception has been the isolation of mutants with defects in behaviors dependent on chemical cues present in the environment. Additional molecules have been identified via reverse genetics by isolating deletions or overexpression mutants in candidate genes [3, 5, 14]. The molecular and genetic analysis of these mutants has led to the identification of molecules necessary for development, differentiation, morphogenesis and functioning of chemosensory neurons [15–18]. As for how chemosensory neurons function, which is the focus of this review, the results clearly indicate that, as in other organisms, also in *C. elegans*, seven transmembrane G-protein-coupled receptors (GPCRs), heterotrimeric G proteins, cyclic nucleotide-gated ion channels, TRP channels and Ca^{++} play crucial roles in chemical sensitivity [2–5].

Receptors

The genome of *C. elegans* codes for a very large number of candidate chemoreceptor genes of the seven-transmembrane GPCR class (1300 apparently functional genes plus an additional 400 pseudogenes). *C. elegans* strong dependence on chemical cues is probably the reason for this unusually large number (about 7% of all its genes). Absolute number and percentage are higher than in any other species of vertebrate or invertebrate for which these figures are known. On the basis of sequence similarity and of the number and position of introns, *C. elegans* chemoreceptor genes have been grouped in four large superfamilies each made of several sub-families [19, 20]. The sequences of the *C. elegans* genes are highly divergent, and there is very low sequence similarity between the *C. elegans* genes and any of the mammalian chemoreceptor gene families. As in mammals, these genes are often found in clusters in the genome a condition that may favor frequent duplications and gene losses and which may underlie their rapid evolution both in terms of sequence diversification and gene number, as suggested by initial comparison with the genome of the closely related species *Caenorhabditis briggsiae* [20].

The first odorant receptor for which a ligand was identified in any organism is the *C. elegans* high-affinity receptor for diacetyl, ODR-10, which is expressed in the olfactory neuron AWA, and loss-of-function mutants fail to sense diacetyl [21]. Worms expressing ODR-10 in AWB instead of in AWA, as normally occurs, avoid diacetyl instead of being attracted to it. Thus, in *C. elegans*, the attractive or repellent quality of a stimulus is not encoded in the receptor itself but in the connectivity of the sensory neuron in which the receptor molecule is expressed [22]. An experiment arriving at similar conclusions has now been realized with taste receptors in mice [23].

Albeit these successes on *odr-10*, genetic screens for chemosensory mutants have been remarkably incapable of identifying genes for other receptors, although they have led to the identification of numerous other components of the signalling necessary for chemosensation. This resistance to genetic analysis is possibly due to extensive redundancy of the chemoreceptor genes necessary to sense a particular ligand and to the still-limited capacity of researchers to detect the subtle behavioral differences resulting from the loss of a single chemoreceptor gene.

That these genes code for chemoreceptors used to detect chemicals in the environment is also supported by studies of their expression patterns using fusions of their regulatory regions to the green fluorescent protein (GFP) as a reporter. Of the genes tested, 80% were expressed in sensory neurons (70% in amphid neurons) and only about 25% were also expressed in nonneuronal cells [19, 20]. In addition, translational fusions with GFP have shown that the corresponding proteins are localized to sensory cilia, the cellular compartment where detection of chemicals in the environment occurs.

One striking observation is that *C. elegans* and mammals have a roughly similar number of chemoreceptor genes despite the fact that mouse chemosensory neurons number in the order of 10^7, whereas *C. elegans* has fewer than 30 chemosensory neuron types. As a consequence, multiple receptors must be expressed in the same neuron. The unique combinations of neurons in which receptors for related chemicals are expressed may underlie, at least in part, the code with which natural mixtures encountered by *C. elegans* are sensed, recognized and discriminated.

Heterotrimeric G proteins

The Gα subunits

The genome of *C. elegans* encodes 21 Gα, two Gβ and two Gγ subunits. There is one clear ortholog for each of the four mammalian families of Gαs, with *C. elegans* GOA-1, GSA-1, EGL-30 and GPA-12 corresponding to mammalian G$\alpha_{o/i}$, Gα_s, Gα_q and Gα_{12}, respectively. The remaining 17 Gα subunits (ODR-3, GPA-1 to GPA-11 and GPA-13 to GPA-17), although perhaps more similar to the G$\alpha_{o/i}$ family, are referred to in this context as the *C. elegans* specific Gα subunits. While the four canonical α subunits are generally broadly expressed and are involved in a variety of developmental and behavioral functions, 14 of the *C. elegans* specific Gα subunits seem primarily to have a function in chemoreception since their expression is largely confined to subsets of chemosensory neurons [14, 24, 25]. Loss-of-function mutations *(lf)* in all the Gα-subunit genes have been isolated either in forward genetic screens (e.g. *odr-3*) or by reverse genetics. In addition, overexpression as transgenes either of the wild-type *(ox)* or of *in vitro* produced, constitutively activated gain-of-function alleles *(gf)* have been obtained [14, 24, 26]. The Gα subunits ODR-3 and GPA-3 have an important role in primary signalling because loss-of-function mutants have strong chemosensory phenotypes. ODR-3 is expressed in AWA, AWB, AWC, ASH, ADF, PHA and PHB, and it has been shown to be required for the responses of AWA and AWC to attractants and of AWB and ASH to repellents [24]. GPA-3 is expressed in all the chemosensory neurons of the amphid except for AWB. Loss-of-function mutants are defective for chemotaxis to some water-soluble and volatile attractants, for the response of ASH to some repellents and are also partially defective for dauer formation. For the responses mediated by the three neurons AWA, AWC and ASH, in which they both function, ODR-3 appears to be more important for some stimuli, while GPA-3 is more important for others, e.g. quinine avoidance mediated by ASH [12, 14, 24, 26, 27] (Figs. 3, 4). Imaging calcium fluxes in ASH has shown that the transient increase in intracellular calcium (Ca^{++} transients), observed in wild-type animals challenged with all classes of repellent stimuli, are reduced in *odr-3(lf)* mutants although they are not abolished. Instead, in *gpa-3(lf)* mutants, Ca^{++} transients are significantly reduced in response to quinine but are normal for the other classes of repellent stimuli. When both ODR-3 and GPA-3 are missing, no avoidance responses are obtained and no ASH activation occurs, indicating that these two Gα subunits act in redundant pathways that are essential to activate signalling in ASH [7]. ODR-3 and GPA-3 proteins localize to the cilia of the sensory neurons in which they are expressed, suggesting that they receive signals from chemosensory receptors and are directly involved in the transduction of chemical stimuli [24, 27].

GPA-1, GPA-2, GPA-5 and GPA-11 have also been shown to be involved in chemical signalling but possibly with a regulatory role. GPA-1, which is expressed in several chemosensory neurons, appears to have a role in the plasticity of the response to NaCl. Differently from wild-type, *gpa-1(lf)* mutants do not chemotax away from this salt after preexposure to it. The neuron(s) in which GPA-1 acts for plasticity remain to be established [28]. GPA-2 is expressed in the AWC neuron and has a role in sensing volatile attractants. However, results were somewhat con-

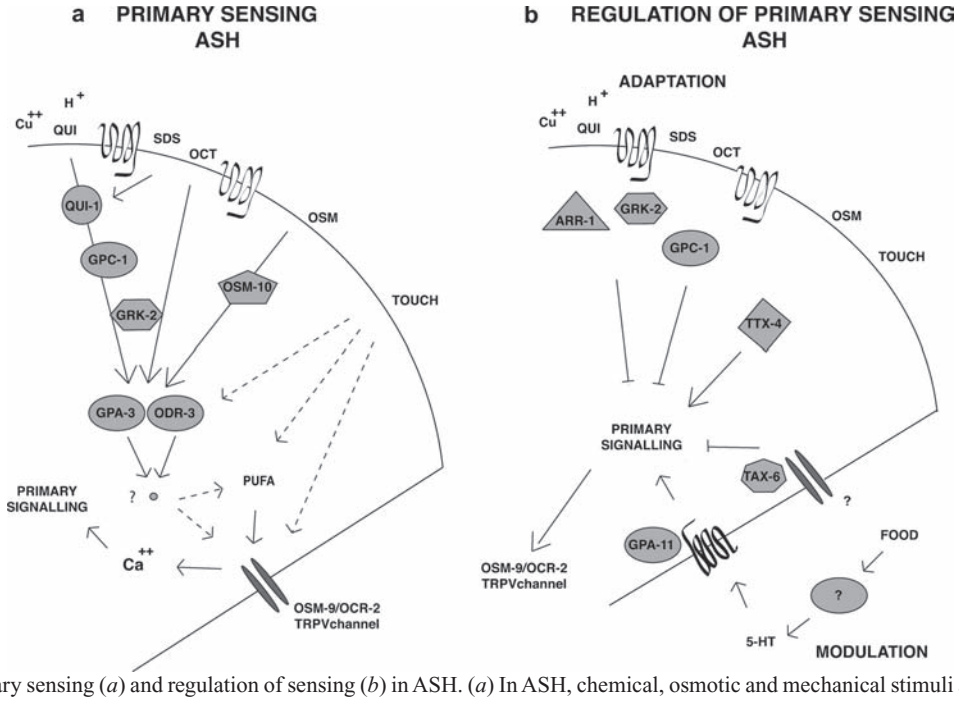

Figure 3. Primary sensing (*a*) and regulation of sensing (*b*) in ASH. (*a*) In ASH, chemical, osmotic and mechanical stimuli activate initially separated signalling cascades. Osmotic stimuli require OSM-10 and the Gα subunits ODR-3. Chemical signals require QUI-1, GRK-2 and, for quinine, also GPC-1. The Gα subunits ODR-3 and GPA-3 mediate chemical signals and have partially redundant functions. All stimuli, including the mechanical stimulus nose touch, converge, either directly or through PUFA, on the OSM-9/OCR-2 TRPV channel that is required for all primary signalling in ASH. In (*b*) primary signalling is regulated by multiple mechanisms/pathways. GRK-2 and ARR-1 may be involved in receptor desensitization. The nPKC TTX-4 increases signalling by inhibiting adaptation, while the Gγ subunit, GPC-1 and the calcineurin TAX-6 stimulate adaptation. Signalling is also positively modulated by inputs from other neurons responding to the presence of food. Serotonin (5HT) and the Gα subunit GPA-11 mediate this modulation. The stimuli are Cu^{++}, copper ions; H^{+}, high pH; SDS, the detergent sodium dodecyl sulphate; QUI, quinine; OCT, octanol; OSM, high osmotic concentration; touch, nose touch.

tradictory on whether that role is stimulatory or inhibitory [24, 27]. GPA-5 appears to play an inhibitory role in the responses of AWA to some odorants, because the loss of GPA-5 function results in increased sensitivity to some attractants and partially rescues the chemotaxis defects of *odr-3(lf)* mutations [27, 29]. GPA-11 is necessary in ASH to mediate the positive modulatory effect of serotonin, an internal indicator of food abundance, on the responsiveness of ASH to octanol [30] (Fig. 3).

Loss-of-function mutations in the other *C. elegans* specific Gα subunits showed modest or no chemosensory defects. For several of these genes, overexpression *(ox)* or gain-of-function *(gf)* alleles show some chemosensory defect, but it is still not clear whether these are due to a specific function in signalling or to a more general disruption of neuron physiology when they are overexpressed [14, 27]. The limited effects of mutations in most of the Gα subunits expressed in chemosensory neurons indicates that they are probably not required for primary signalling but play modulatory and partially redundant roles. A network of Gα subunits may be necessary to finely modulate the responsiveness of chemosensory neurons in a continously changing environment. The large number of Gα-subunit genes and the fact that they are expressed in unique combinations in the limited number of *C. elegans* chemosensory neurons [14] is another element (in addition to the large number of GPCR genes) that probably contributes to coding chemical signals in *C. elegans*.

The Gγ subunit GPC-1

Of the two Gβ and two Gγ subunits coded in the *C. elegans* genome, the only one for which a role in chemosensation has been demonstrated is the Gγ subunit, GPC-1, which is expressed in most sensory neurons, including ASH. Behavioral analysis has shown that primary sensory responses of *gpc-1(lf)* mutants are normal [31] with the exception of quinine avoidance, which is instead reduced in these mutants. Ca^{++} imaging of ASH showed that its cellular response to quinine, but not to other repellents, was reduced in *gpc-1* mutants [7]. The main role of GPC-1 in chemosensation, however, appears to be in adaptation: the reduction in response to a stimulus after persistent or repeated exposure to it. Behavioral studies have shown that *gpc-1* loss-of-function mutants adapt poorly to some water-soluble attractants [31] and to noxious stimuli that trigger an avoidance response, although the mutants are still capable of adapting to volatile attractants. Ca^{++} imaging showed a reduced capacity of *gpc-1* mutants to modify the cellular response of ASH, after either repeated or prolonged stimulation with a repellent

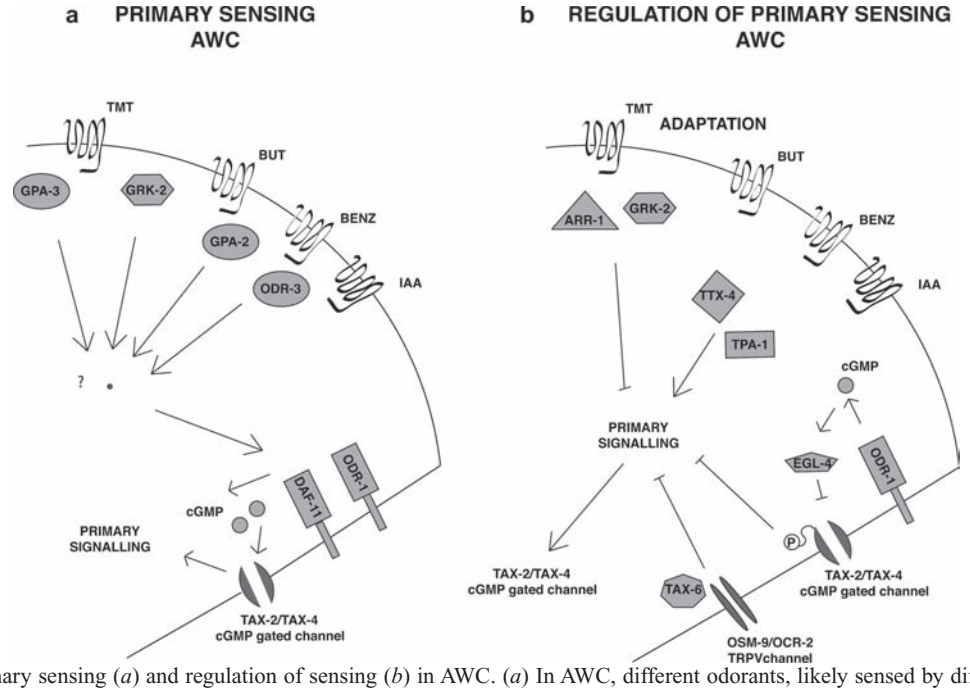

Figure 4. Primary sensing (*a*) and regulation of sensing (*b*) in AWC. (*a*) In AWC, different odorants, likely sensed by different GPCRs, activate separate signalling cascades that use one or more Gα subunits, with ODR-3 playing the main role. Activation of the Gα subunits leads, by unknown mechanisms, to activation of the guanylate cyclases ODR-1 and DAF-11, which control the level of cGMP. The nucleotide in turn controls the activity of the cGMP-gated channel TAX-2/TAX-4 that is required for all primary signalling in AWC. In (*b*) primary signalling is regulated positively and negatively via different pathways. Receptor desensitization requires GRK-2 and ARR-1. Pathways that stimulate adaptation and thus downregulate signalling: the OSM-9 dependent TAX-6 adaptation pathway and the cGMP-dependent EGL-4 kinase pathway, which may involve phosphorylation of the TAX-2 subunit of the TAX-2/TAX-4 channel. Pathways that inhibit adaptation and increase primary signalling use the nPKCs TTX-4 and TPA-1. Odorants areTMT, trimethyl thiazole; BUT, butanone; BENZ, benzaldehide; IAA, indol acetic acid.

[7]. GPC-1 has been shown recently to have a role also in the plasticity of the response of worms to NaCl. After prolonged exposure to NaCl, wild-type worms chemotax away instead of toward an otherwise attractive concentration of this salt. In contrast, *gpc-1(lf)* mutants do not [28]. It is not clear whether the function of GPC-1 in adaptation underlies this effect on plasticity.

Downstream pathways

Molecular, cellular and genetic studies indicate that in, *C. elegans,* two major ion channels operate to couple receptor activation to the electrical activity of chemosensory neurons. In one group of neurons, signals from diverse receptors converge toward the cGMP-gated channel formed by the two subunits TAX-2 and TAX-4 [32, 33]. In other cells, signals converge toward the transient receptor potential vanilloid-related channel (TRPV) formed by the two subunits OSM-9 and OCR-2 [10, 34].

The cGMP-gated channel TAX-2/TAX-4

C. elegans TAX-4 and TAX-2 are the α and β subunits of a cGMP-gated channel expressed in some amphidial neurons. The activity of this channel is required for the response of these neurons to the stimuli they each sense. Accordingly, loss-of-function mutants of *tax-2* and *tax-4* show defects in chemotaxis toward NaCl (ASE) and toward some volatile attractants (AWC) and repellents (AWB). They also show thermotaxis defects (AFD) and defects in dauer formation (ASI) [32, 33]. Expression of these two *C. elegans* channel subunits in HEK293 cells has shown that the channel is gated by cGMP and that it is permeable to Na^{+} and Ca^{++} [35]. The TAX-2/TAX-4 channel is also the target of an adaptation pathway, and a phosphorylation site on TAX-2 appears to be required for the adaptation that occurs after short exposure to odors. The cGMP-dependent protein kinase EGL-4 [36] is one of the potential candidate kinases for the phosphorylation of TAX-2 (Fig. 4). Thus in AWC, but possibly also in other neurons, downmodulation via phosphorylation of the activity of the channel involved in primary signalling appears to be an important early step in adaptation. [36]. It is worth pointing out that the TAX-2/TAX-4 channel is used both by neurons sensing water-soluble cues (ASE) and by neurons sensing odorants (AWB and AWC). Also, the channel is used independent of whether the neuron responds to attractants (AWC and ASE) or to repellents (AWB). This crucial role of the TAX-2/TAX-4 channel indicates that in *C. elegans* cGMP is an important intracellular messenger for transduction of various sensory modalities.

Guanylate cyclases

The transmembrane guanylate cyclase ODR-1 regulates the level of cGMP in the olfactory neurons AWC and AWB and thus controls the activity of the cGMP-gated channel TAX-2/TAX-4. *odr-1(lf)* mutants fail to chemotax toward all the volatile attractants sensed by AWC and away from the volatile repellents sensed by AWB. Anti-ODR-1 antibodies showed that the protein is specifically localized in the sensory cilia of AWC and could not be detected in cell bodies and axons, suggesting a direct role in signalling. While the cytoplasmic, cyclase domain of ODR-1 is required for the response of AWC to attractants, its extracellular domain is not, suggesting that for this function it is not acting as a receptor for the stimuli but as a component of the transduction pathway downstream of the receptors and of the G proteins (Fig. 4). Overexpression of ODR-1 does not affect primary sensing of odorants, but causes odorant-specific defects in adaptation to and discrimination between AWC-sensed odorants [37].

A second, ODR-1-related transmembrane guanylate cyclase, DAF-11, is also expressed in AWC and required for AWC olfaction. DAF-11 is also required for chemotaxis to water-soluble attractants and for dauer formation, but the neurons in which it acts for these latter functions have not been unambiguously identified [38, 39].

In addition to ODR-1 and DAF-11, *C. elegans* codes for many additional guanylate cyclases. As many as 30 of these are transmembrane cyclases and may also function as receptors. Some are expressed in chemosensory neurons, but their function has not been analysed [40]. Two soluble guanylate cyclases, *gcy-35* and *gcy-36,* have been implicated in social behavior and aerotaxis by acting in three body cavity neurons. They appear to directly sense oxygen concentration and control the TAX-2/TAX-4 channel [41–43]. The *C. elegans* genome codes for four adenylate cyclases, but mutants in two of these genes have not shown chemosensory defects [44]. The fact that no chemosensory mutant has been mapped to these genes suggests that cAMP is rarely if at all used as a second messenger in *C. elegans* chemical sensitivity.

The TRPV channel OSM-9/OCR-2

The *C. elegans* genome codes for five TRPV-related channel proteins. The first TRPV gene, *osm-9*, was identified in forward genetic screens for mutants failing to avoid a high osmotic strength stimulus [34]. The other four genes were identified on the basis of sequence similarity to *osm-9* and were named *ocr-1*, *-2*, *-3* and *-4* for osm-9/capsaicin related [10]. The gene *osm-9* is expressed in all the amphid and phasmid neurons, except for AFD and AWB, and in a few other neurons. The four *ocr* genes are each expressed in a subset of the *osm-9* expressing neurons, suggesting that they may act in combination with OSM-9, although several amphidial neurons express only OSM-9 [10].

Loss-of-function of OSM-9 causes defects in all the functions of the chemosensory neurons AWA and ASH [34]. Ca^{++} imaging of the ASH neuron also showed that, in *osm-9(lf)* mutants, Ca^{++} transients in response to all classes of repellents were abolished [7], confirming that, in this cell, signals triggered by different stimuli all converge toward the OSM-9 ion channel, which is thus necessary for all ASH primary sensory responses (Fig. 3). Of the four *ocr* genes, only *ocr-2* has an essential role in primary sensation. Indeed, both OSM-9 and OCR-2 are required for AWA and ASH primary signalling. In cells in which they are both expressed, OSM-9 and OCR-2 are localized to the sensory cilia, and it was shown that the two proteins are mutually dependent on each other for proper localization [10]. In contrast to AWA and ASH, in the olfactory neuron AWC, primary sensing is mediated by the TAX-2/TAX-4 channel, and OSM-9 is not required for primary sensing but for adaptation [34] (Fig. 4). AWC is one of the neurons in which OSM-9 is expressed by itself and not with OCR-2 or with any other OCR protein. OSM-9 in AWC is not localized to the sensory cilia but in the cell body [10]. This different cellular localization may underlie its different function in this cell. In addition to their role in primary sensing and in adaptation, *osm-9*, *ocr-1* and *ocr-2* have also been implicated in the plasticity of the response to NaCl [28].

ASH senses chemical, osmotic and mechanic stimuli. Most of them are mediated by one or both of the Gα subunits ODR-3 and GPA-3 [7, 12], and all converge on the OSM-9/OCR-2 ion channel. How is the activity of this channel regulated? Studies in mammalian cell cultures and in *Drosophila melanogaster* have provided evidence for the involvement of various molecules in the activation of TRP ion channels in different cell types. These include phospholipase Cβ (PLCβ), diacyl glycerol (DAG), inositol trisphosphate (IP3) and its receptor (IP3R), phosphokinase C (PKC), polyunsaturated fatty acids (PUFA) etc., reviewed in [45]. However, the transduction cascades by which the different signals are conveyed to the OSM-9/OCR-2 TRPV channel in AWA and ASH are not understood, although an important role has been shown for PUFA [46]. *C. elegans* strains defective for PUFA synthesis display sensory deficits in TRPV-dependent neurons (e.g. ASH). Addition of specific PUFA to the diet bypasses the normal requirement for PUFA synthesis and rescues these deficits. In addition, delivery of a specific PUFA, eicosapentaenoic acid (EPA), appears to largely bypass the stimulus transduction cascade and directly elicits an immediate and strong avoidance response of the worms, which is characteristic of ASH activation. Accordingly, Ca^{++} imaging showed a rapid increase of intracellular calcium in the ASH neurons of animals challenged with exogenous EPA. The effects of PUFA and of EPA did not occur in *osm-9(lf)* mutants, indicating that their action requires the presence of a functional OSM-9 channel [46] (Fig. 3).

The general relevance of studying the function of the TRPV channel in ASH is supported by the effects of expressing mammalian TRPV channels in *C. elegans*. Worms expressing the mammalian TRPV1, capsaicin receptor, in ASH avoid capsaicin, while wild-type worms do not respond to it [10]. This indicates that, within ASH, the mammalian protein can interact with the downstream pathway leading to avoidance. Expression of the mammalian TRPV4 can rescue some of the avoidance defects of *osm-9(lf)* mutants, namely the response to touch and to high osmotic stress stimulus. The rescue of the Osm phenotype is dependent on the presence of a functional OSM-10 protein (see below). This indicates that TRPV4 can also interact correctly with the upstream factors normally acting in *C. elegans* ASH in response to the osmotic stimulus [47].

Regulators of signalling

The GPCR kinase GRK-2

The *C. elegans* genome codes for two GPCR kinases, GRK-1 and GRK-2. GRK-2 is expressed in many neurons, including chemosensory ones, and *grk-2 (lf)* mutants are severely impaired in chemotaxis to some odorants, both attractive and repellent [48], and in chemotaxis to NaCl [28]. These mutants also fail to avoid quinine, octanol and a high osmotic strength stimulus (1 M glycerol). Genetic and Ca^{++} imaging experiments showed that, the function of GRK-2 is required in ASH, for avoidance of repellents [48] and also for chemotaxis toward 1–100 mM NaCl [28]. In mammals, G-coupled receptor kinases (GRKs) are involved in desensitization and interruption of receptor signalling, and loss or reduction of function results in hypersensitivity [49]. The defect in chemotaxis toward attractive concentrations of NaCl has been interpreted on the basis of this mechanism of action. NaCl is sensed both by neurons mediating repulsion (ASH and perhaps ADL) and by neurons mediating attraction (ASE). Chemotaxis toward or away from NaCl appears to result from a balance between these opposing inputs from different neurons. Loss of GRK-2 function would result in hypersensitivity of ASH to NaCl and thus in excessive repulsion signals that overcome the attractive signals from ASE and prevent positive taxis. Expression of GRK-2 in ASH would restore a more balanced situation and rescue the chemotaxis defect [28]. In contrast, on the basis of the role of GRK-2 in desensitization and signal interruption, it is difficult to explain the fact that loss of GRK-2 function reduces or even abolishes, instead of enhancing, the avoidance response and concomitant Ca^{++} increases in ASH [48]. It is possible that, in the nematode, GRK-2 also acts via different mechanisms that remain to be elucidated.

The arrestin ARR-1

Arrestins are multifunctional adaptor proteins that interact with signalling molecules (e.g. phosphorylated GPCRs) to downregulate receptor signalling (desensitization), promote receptor endocytosis and activate downstream pathways [50]. In the *C. elegans* genome, Arrestin-1 (*arr-1)*, which appears to be a β-arrestin, is the only gene that has significant homology to arrestins in other organisms. ARR-1 is expressed in many neurons, including chemosensory ones. Loss-of-function mutants have normal primary chemosensory responses [28, 48, 51] but exhibit significant defects in adaptation to volatile odorants and in recovery from adaptation [51], and also in the plasticity of the response to NaCl [28]. In agreement with what is known in other systems, the failure of *arr-1(lf)* mutants to adapt has been interpreted as a failure to desensitize the relevant GPCR receptors. Consistent with this finding, for both adaptation and recovery from adaptation, ARR-1 function is required in the relevant olfactory neurons (e.g. AWC, for isoamyl alcohol) (Fig. 4). Molecular manipulation of the gene combined with *in vivo* and with biochemical studies has in addition shown that the regions of ARR-1 required for adaptation and for recovery can be separated. The N-terminal region is required for arrestin to prevent binding of phosphorylated GPCR to the G proteins and thus for desensitization and adaptation. The C-terminal region, which contains binding sites for components of the endocytic machinery (clathrin and β_2-adaptin), is not required for adaptation itself but for recovery from adaptation. It does so by possibly contributing to GPCR endocytosis, which may be necessary for the receptor to recover its responsiveness to stimuli and to recycle to the cell membrane (resensitization/recovery) [51]. The analysis of ARR-1 in *C. elegans* has provided new insight into the correlation between the molecular interactions of arrestin, the cellular processes of desensitization and resensitization, and the behavioral phenomena of adaptation and recovery [51].

The calcineurin TAX-6

C. elegans TAX-6 is the A subunit of calcineurin, a calcium/calmodulin-dependent protein phosphatase. It is expressed in muscle cells and several neurons, including chemosensory neurons of amphids and phasmids, and has been shown to have a role in sensory signalling and in adaptation. TAX-6 appears to act as a negative regulator of the responsiveness (gain) of Ca^{++} signalling pathways. Loss of TAX-6 function causes a thermophilic phenotype resulting from hyperactivation of the amphidial thermosensory neuron AFD. In contrast, constitutively activated TAX-6 results in downregulation of AFD signalling and in a cryophilic or athermotactic phenotype [52]. In AFD, TAX-6 may regulate, directly or indirectly, the TAX-2/TAX-4 channel, which is necessary for primary signalling in this cell. *tax-6(lf)* mutants are also partially defec-

tive for chemotaxis toward odorants sensed by AWC, and the defect was shown to result from hyperadaptation. In AWC, adaptation but not primary signalling is mediated by the OSM-9 channel, and indeed the *tax-6(lf)* defect is dependent on the presence of a functional OSM-9, suggesting that TAX-6 can also negatively regulate the OSM-9-dependent adaptation pathway [52].

The cGMP-dependent kinase EGL-4

The cGMP-dependent protein kinase EGL-4 has been shown to be necessary for adaptation to odorants sensed by the olfactory neuron AWC. *egl-4* null mutants disrupt both early and late adaptation. After short odor exposure, for early adaptation, EGL-4 may downregulate signalling by phosphorylating some component, possibly the β subunit of the cGMP-gated channel TAX-2/TAX-4 (Fig. 4). For later phases of adaptation, after long exposure to odors, a different mechanism might be involved: a nuclear localization signal present in EGL-4 is required only for this late phase of adaptation. This suggests that long-term adaptation may involve nuclear translocation of EGL-4 and possibly novel gene expression and protein synthesis [36].

The nPKC TTX-4

The gene *ttx-4* was identified in a screen for mutants defective in thermotaxis and codes for a protein kinase C of the *novel* subgroup (nPKC) [53]. TTX-4 mutants show defects in thermotaxis (AFD), in chemotaxis to odorants (AWA and AWC) and to NaCl (ASE), and in avoidance (ASH). TTX-4 is expressed and required in these amphid neurons to regulate signal transduction. Genetic analysis using mutations in other genes and both loss-of-function and gain-of-function alleles of *ttx-4*, indicate that its normal function is to downregulate signalling in AFD, and stimulate it in ASH, AWA and AWC. The results also indicate that it acts upstream of TAX-4 in AFD and AWC, and its role is partially redundant with that of another nPKC, TPA-1, in AWA and AWC. TTX-4 appears to be a regulator rather than an essential component of the signal transduction pathway. Like mammalian nPKCs, it appears to be activated by DAG. Whether it acts directly or indirectly on the TAX-2/TAX-4 channel in AWC and AFD and on the OSM-9 channel in ASH and AWA is not known [53].

Stimulus-specific molecules

The possibility offered by *C. elegans* of performing powerful genetic screens for chemoreception-defective mutants has led to the discovery of new molecules involved in chemical signalling that could not have been identified otherwise. Two of them, OSM-10 and QUI-1, appear to have stimulus-specific roles within the polymodal avoidance neuron ASH. OSM-10 and QUI-1 are not required for ASH survival nor for its general functioning. In *osm-10* and *qui-1* mutants, the morphology of ASH and of its sensory cilia are apparently normal [12, 54]. OSM-10 is a novel cytoplasmic protein sharing no significant homology with known proteins and which is expressed in ASH, ASI, PHA and PHB. Mutations in *osm-10* eliminate the response to the high osmotic strength stimulus but have no effect on responses to other stimuli [54] also detected by ASH: chemical repellents (e.g. quinine, octanol) and the mechanical stimulus nose touch. *qui-1(lf)* mutants fail to avoid quinine and other aversive chemical stimuli but are still capable of responding to the nose touch and to a high osmotic strength stimulus [12]. The gene *qui-1* is expressed in a small group of neurons, including ASH, in which expression is strongest. QUI-1 is a novel protein containing WD40 repeats in the C-terminal region. It is 1592 amino acids long and contains no special localization signals, and no secretion peptide or transmembrane domains [12]. More accurate informatics analysis of its sequence has revealed that, in addition to the WD40 repeats, QUI-1 also contains a NACHT domain, predicted to have NTPase activity [55], and, most significantly, a regulator of G protein signalling domain (RGS) [C. Bergamasco and P. Bazzicalupo, in preparation]. The *C. elegans* genome contains a paralog of QUI-1, and both genes have been conserved in evolution: orthologs of both are encoded in the genome of insects and mammals. QUI-1 and its homologs define a new class of RGS proteins since their domain composition is different from that of the 10 classes of RGS domain-containing proteins thus far identified [56]. While the data indicate that QUI-1 and OSM-10 are involved in stimulus-specific ASH signalling, mechanistic models for their action remain to be discovered.

Two more molecules, ODR-4 and ODR-8, which have been discovered in behavioral screens, are also novel and also appear to affect the sensitivity of olfactory neurons to some chemicals but not to others. They appear to be necessary to localize some but not all GPCRs to sensory cilia [57].

Concluding remarks

Sensitivity range and the problem of discrimination

The very large number of GPCR genes indicates that *C. elegans* can detect a vast variety of chemicals, comparable or superior to that of much more complex animals. It does so even with a limited number of chemosensory neuron types (between 20 and 30). With more than a thousand different chemoreceptor genes, each neuron must express many receptors and is thus very broadly tuned. Broadly tuned sensory neurons are reminiscent of mammalian taste cells.

The limited number of neurons poses clear limitations to the capacity of the nematode to discriminate between related chemicals. In *C. elegans*, saturation experiments, which test whether high uniform concentrations of one attractant prevent chemotaxis to a second attractant, have shown that *C. elegans* can discriminate between at least five classes of water-soluble and seven classes of volatile attractants, reviewed in [2]. Adaptation experiments have also shown discrimination between repellents [7]. The capacity of *C. elegans* for discrimination may in fact be much greater since the numbers above derive from the relatively few substances tested. How is discrimination achieved? The variety of sensory neurons is increased by generating, through differential gene expression, left-right asymmetry between the bilaterally symmetric left-right pairs that define a given neuron type. Differential expression has been documented for some GPCR-coding genes between AWC left and AWC right [58] and for some guanylate cyclases between ASE left and ASE right [40]. The generation of asymmetry between the two pairs of neurons has indeed been shown to be necessary for discrimination between different chemicals [59] and may regard the expression of many more genes. Some of the genes that control and realize neuronal asymmetry have been identified [15, 16]. Neuronal asymmetry cannot, however, account for all the odor discrimination observed in *C. elegans,* since for instance the two AWC neurons can discriminate at least five odors, and worms carrying a mutation that abolishes AWC asymmetry can still discriminate between some odors sensed by AWC [58]. For discrimination within a single neuron, signalling by different receptors should activate and/or be regulated by distinct downstream molecules. In addition, sensing complexes for different ligands should be spatially and functionally isolated within the cell, such that activation by one ligand does not influence signalling complexes that sense other ligands. Indeed, genes such as *osm-10* and *qui-1* affect the capacity of a single neuron, ASH, to sense some stimuli but not others [12, 54]. Also, the discovery of genes such as *odr-4* and *odr-8*, required to localize some but not all GPCRs to sensory cilia [57], suggests that different signalling complexes may in fact be assembled separately.

Chemical coding and regulation

The available data suggest that each chemosensory neuron of *C. elegans* expresses a unique combination of GPCR genes and of chemosensory-specific Gα subunits. This combinatorial makeup of sensory neurons and the fact that each substance is sensed by more than one neuron suggest a strategy for chemical coding in which chemicals present in the environment activate unique combinations of neurons. As the number of neurons activated in any given situation increases, the number of different combinations can rapidly become very large. This type of strategy may be particularly useful in natural conditions where the task is usually not that of distinguishing between single related molecules but between the complex natural mixtures of chemicals that are present in the environment.

The activity of each sensory neuron is also regulated by unique combinations of modulatory molecules acting in pathways that stimulate or inhibit the responsiveness of neurons and that are responsible for phenomena such as desensitization, adaptation, modulation and plasticity. Integration of these pathways probably underlies the flexible response required of worms to negotiate the continuous changes in the chemical composition of the environment. However, a full understanding of the way chemical cues are coded in *C. elegans* and how worms adjust their responses remains to be elucidated and is a challenging task for future research.

Future perspectives

The results reported in this review indicate that at the molecular level *C. elegans* chemosensory neurons largely use the same molecules that have been shown to be important in sensory systems in other organisms. The results also indicate two directions in which future work on *C. elegans* can provide original contributions to the general field of sensory signalling. First, it can identify new molecules whose function in sensory system can only be discovered by powerful genetic screens. Some such molecules have already been identified, and the list may become larger in the future as more sophisticated screens are devised. Second, the experimental versatility of *C. elegans* makes it possible to determine precisely in which neuron molecules and pathways are acting and which behavioral responses they are affecting *in vivo*. This possibility can provide detailed correlations, not only between the biochemical properties of molecules and their cellular function in relevant neurons, but also between these cellular functions and the *in vivo* behavioral responses of the animal.

Acknowledgements. We thank Giovanni Esposito and Massimo Hilliard who contributed many of the ideas summarized in this review, We also wish to acknowledge all the work of *C. elegans* researchers on sensory signaling that, because of space limitations, could only be cited through review articles. Finally, we wish to thank Umberto Di Porzio, Elia Di Schiavi and Franco Graziani for critically reading the manuscript. This work was supported by grants to P. B. from the Human Frontiers Science Program, Telethon Foundation (grant GGP030288) and FIRB-Neuroscienze (grant RBNE01WY7P).

1 White J. G., Southgate E., Thomson J. N., Brenner S. (1986) The structure of the nervous system of the nematode Caenorhabditis elegans. Phil. Trans. R. Soc. Lon. **314:** 1–340

2 Bargmann C. I., Mori I. (1997) Chemotaxis and thermotaxis. In: C. elegans II, pp. 717–737, Riddle D. L., Blumenthal T., Meyer B. J. and Priess J. R. (eds.), Cold Spring Harbor Press, New York

3 Bargmann C. I., Kaplan J. M. (1998) Signal transduction in the Caenorhabditis elegans nervous system. Annu. Rev. Neurosci. **21:** 279–308
4 Mori I. (1999) Genetics of chemotaxis and thermotaxis in the nematode Caenorhabditis elegans. Annu. Rev. Genet. **33:** 399–422
5 de Bono M., Maricq A. V. (2005) Neuronal substrates of complex behaviors in C. elegans. Annu. Rev. Neurosci. **28:** 451–501
6 Suzuki H., Kerr R., Bianchi L., Frokjaer-Jensen C., Slone D., Xue J. et al. (2003) *In vivo* imaging of C. elegans mechanosensory neurons demonstrates a specific role for the MEC-4 channel in the process of gentle touch sensation. Neuron **39:** 1005–1017
7 Hilliard M. A., Apicella A. J., Kerr R., Suzuki H., Bazzicalupo P., Schafer W. R. (2005) *In vivo* imaging of C. elegans ASH neurons: cellular response and adaptation to chemical repellents. EMBO J. **24:** 63–72
8 Fujiwara M., Ishihara T., Katsura I. (1999) A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of C. elegans sensory cilia. Development **126:** 4839–4848
9 Hilliard M. A., Bargmann C. I., Bazzicalupo P. (2002) C. elegans responds to chemical repellents by integrating sensory inputs from the head and the tail. Curr. Biol. **12:** 730–734
10 Tobin D., Madsen D., Kahn-Kirby A., Peckol E., Moulder G., Barstead R. et al. (2002) Combinatorial expression of TRPV channel proteins defines their sensory functions and subcellular localization in C. elegans neurons. Neuron **35:** 307–318
11 Adler E., Hoon M. A., Mueller K. L., Chandrashekar J., Ryba N. J., Zuker C. S. (2000) A novel family of mammalian taste receptors. Cell **100:** 693–702
12 Hilliard M. A., Bergamasco C., Arbucci S., Plasterk R. H., Bazzicalupo P. (2004) Worms taste bitter: ASH neurons, QUI-1, GPA-3 and ODR-3 mediate quinine avoidance in Caenorhabditis elegans. EMBO J. **23:** 1101–1111
13 Sambongi Y., Nagae T., Liu Y., Yoshimizu T., Takeda K., Wada Y. et al. (1999) Sensing of cadmium and copper ions by externally exposed ADL, ASE, and ASH neurons elicits avoidance response in Caenorhabditis elegans. Neuroreport **10:** 753–757
14 Jansen G., Thijssen K. L., Werner P., van der Horst M., Hazendonk E., Plasterk R. H. (1999) The complete family of genes encoding G proteins of Caenorhabditis elegans. Nat. Genet. **21:** 414–419
15 Chang S., Johnston R. J. Jr., Frokjaer-Jensen C., Lockery, S. and Hobert O. (2004) MicroRNAs act sequentially and asymmetrically to control chemosensory laterality in the nematode. Nature **430:** 785–789
16 Melkman T., Sengupta P. (2004) The worm's sense of smell. Development of functional diversity in the chemosensory system of Caenorhabditis elegans. Dev. Biol. **265:** 302–319
17 Scholey J. M., Ou G., Snow J. J., Gunnarson A. (2004) Intraflagellar transport motors in Caenorhabditis elegans neurons. Biochem. Soc. Trans. **32:** 682–684
18 Ou G., Blacque O. E., Snow J. J., Leroux M. R., Scholey J. M. (2005) Functional coordination of intraflagellar transport motors. Nature **436:** 583–587
19 Troemel E. R. (1999) Chemosensory signaling in C. elegans. Bioessays **21:** 1011–1020
20 Robertson H. M. and Thomas J. H. (2005) The putative chemoreceptor families of *C. elegans* In: WormBook, The C. elegans Research Community (eds.), http://www.wormbook.org, doi/10.1895/wormbook.1.7.1
21 Sengupta P., Chou J. H., Bargmann C. I. (1996) odr-10 encodes a seven transmembrane domain olfactory receptor required for responses to the odorant diacetyl. Cell **84:** 899–909
22 Troemel E. R., Kimmel B. E., Bargmann C. I. (1997) Reprogramming chemotaxis responses: sensory neurons define olfactory preferences in C. elegans. Cell **91:** 161–169
23 Mueller K. L., Hoon M. A., Erlenbach I., Chandrashekar J., Zuker C. S., Ryba N. J. (2005) The receptors and coding logic for bitter taste. Nature **434:** 225–229
24 Roayaie K., Crump J. G., Sagasti A., Bargmann C. I. (1998) The G alpha protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron **20:** 55–67
25 Cuppen E., Van Der Linden A. M., Jansen G. R. and Plasterk H. A. (2003) Proteins interacting with Caenorhabditis elegans Gα subunits. Comp. Funct. Genomics **4:** 479–491
26 Zwaal R. R., Mendel J. E., Sternberg P. W., Plasterk R. H. (1997) Two neuronal G proteins are involved in chemosensation of the Caenorhabditis elegans Dauer-inducing pheromone. Genetics **145:** 715–727
27 Lans H., Rademakers S., Jansen G. (2004) A network of stimulatory and inhibitory Galpha-subunits regulates olfaction in Caenorhabditis elegans. Genetics **167:** 1677–1687
28 Hukema R. K., Rademakers S., Dekkers M. P., Burghoorn J., Jansen G. (2006) Antagonistic sensory cues generate gustatory plasticity in Caenorhabditis elegans. EMBO J. **25:** 312–322
29 Battu G., E. F. Hoier, and A. Hajnal (2003) The C. elegans G-protein-coupled receptor SRA-13 inhibits RAS/MAPK signalling during olfaction and vulval development. Development **130:** 2567–2577
30 Chao M. Y., Komatsu H., Fukuto H. S., Dionne H. M., Hart A. C. (2004) Feeding status and serotonin rapidly and reversibly modulate a Caenorhabditis elegans chemosensory circuit. Proc. Natl. Acad. Sci. USA **101:** 15512–15517
31 Jansen G., Weinkove D., Plasterk R. H. (2002) The G-protein gamma subunit gpc-1 of the nematode C.elegans is involved in taste adaptation. EMBO J. **21:** 986–994
32 Coburn C. M., Bargmann C. I. (1996) A putative cyclic nucleotide-gated channel is required for sensory development and function in C. elegans. Neuron **17:** 695–706
33 Komatsu H., Mori I., Rhee J. S., Akaike N., Ohshima Y. (1996) Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in C. elegans. Neuron **17:** 707–718
34 Colbert H. A., Smith T. L., 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. J. Neurosci. **17:** 8259–8269
35 Komatsu H., Jin Y. H., L'Etoile N., Mori I., Bargmann C. I. (1999) Functional reconstitution of a heteromeric cyclic nucleotide-gated channel of Caenorhabditis elegans in cultured cells. Brain Res. **821:** 160–168
36 L'Etoile N. D., Coburn C. M., Eastham J., Kistler A., Gallegos G., Bargmann C. I. (2002) The cyclic GMP-dependent protein kinase EGL-4 regulates olfactory adaptation in C. elegans. Neuron **36:** 1079–1089
37 L'Etoile N. D., Bargmann C. I. (2000) Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron **25:** 575–586
38 Vowels J. J., Thomas J. H. (1994) Multiple chemosensory defects in daf-11 and daf-21 mutants of Caenorhabditis elegans. Genetics **138:** 303–316
39 Birnby D. A., Link E. M., Vowels J. J., Tian H., Colacurcio P. L., Thomas J. H. (2000) A transmembrane guanylyl cyclase (DAF-11) and Hsp90 (DAF-21) regulate a common set of chemosensory behaviors in Caenorhabditis elegans. Genetics **155:** 85–104
40 Yu S., Avery L., Baude E., Garbers D. L. (1997) Guanylyl cyclase expression in specific sensory neurons: a new family of chemosensory receptors. Proc. Natl. Acad. Sci. USA **94:** 3384–3387
41 Cheung B. H., Arellano-Carbajal F., Rybicki I., de Bono M. (2004) Soluble guanylate cyclases act in neurons exposed to the body fluid to promote C. elegans aggregation behavior. Curr. Biol. **14:** 1105–1111

42 Gray J. M., Karow D. S., Lu H., Chang A. J., Chang J. S., Ellis R. E. et al. (2004) Oxygen sensation and social feeding mediated by a C. elegans guanylate cyclase homologue. Nature **430:** 317–322
43 Cheung B. H., Cohen M., Rogers C., Albayram O., de Bono M. (2005) Experience-dependent modulation of C. elegans behavior by ambient oxygen. Curr. Biol. **15:** 905–917
44 Korswagen H. C., van der Linden A. M., Plasterk R. H. (1998) G protein hyperactivation of the Caenorhabditis elegans adenylyl cyclase SGS-1 induces neuronal degeneration. EMBO J. **17:** 5059–5065
45 Montell C. (2005) The TRP superfamily of cation channels. Sci. STKE **2005:** re3
46 Kahn-Kirby A. H., Dantzker J. L., Apicella A. J., Schafer W. R., Browse J., Bargmann C. I. et al. (2004) Specific polyunsaturated fatty acids drive TRPV-dependent sensory signaling *in vivo*. Cell **119:** 889–900
47 Liedtke W., Tobin D. M., Bargmann C. I., Friedman J. M. (2003) Mammalian TRPV4 (VR-OAC) directs behavioral responses to osmotic and mechanical stimuli in Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA **100 Suppl 2:** 14531–14536
48 Fukuto H. S., Ferkey D. M., Apicella A. J., Lans H., Sharmeen T., Chen W. et al. (2004) G protein-coupled receptor kinase function is essential for chemosensation in C. elegans. Neuron **42:** 581–593
49 Gainetdinov R. R., Bohn L. M., Sotnikova T. D., Cyr M., Laakso A., Macrae A. D. et al. (2003) Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. Neuron **38:** 291–303
50 Lefkowitz R. J., Shenoy S. K. (2005) Transduction of receptor signals by beta-arrestins. Science **308:** 512–517
51 Palmitessa A., Hess H. A., Bany I. A., Kim Y. M., Koelle M. R., Benovic J. L. (2005) Caenorhabditus elegans arrestin regulates neural G protein signaling and olfactory adaptation and recovery. J. Biol. Chem. **280:** 24649–24662
52 Kuhara A., Inada H., Katsura I., Mori I. (2002) Negative regulation and gain control of sensory neurons by the C. elegans calcineurin TAX-6. Neuron **33:** 751–763
53 Okochi Y., Kimura K. D., Ohta A., Mori I. (2005) Diverse regulation of sensory signaling by C. elegans nPKC-epsilon/eta TTX-4. EMBO J. **24:** 2127–2137
54 Hart A. C., Kass J., Shapiro J. E., Kaplan J. M. (1999) Distinct signaling pathways mediate touch and osmosensory responses in a polymodal sensory neuron. J. Neurosci. **19:** 1952–1958
55 Koonin E. V., Aravind L. (2000) The NACHT family – a new group of predicted NTPases implicated in apoptosis and MHC transcription activation. Trends Biochem. Sci. **25:** 223–224
56 Siderovski D. P., Willard F. S. (2005) The GAPs, GEFs, and GDIs of heterotrimeric G-protein alpha subunits. Int. J. Biol. Sci. **1:** 51–66
57 Dwyer N. D., Troemel E. R., Sengupta P., Bargmann C. I. (1998) Odorant receptor localization to olfactory cilia is mediated by ODR-4, a novel membrane-associated protein. Cell **93:** 455–466
58 Wes P. D., Bargmann C. (2001) Caenorhabditis elegans senses at least five attractive odours with a single pair of olfactory neurons, AWC, but can distinguish among these odours in behavioural. Nature **410:** 698–701
59 Pierce-Shimomura J. T., Faumont S., Gaston M. R., Pearson B. J., Lockery S. R. (2001) The homeobox gene lim-6 is required for distinct chemosensory representations in C. elegans. Nature **410:** 694–698
60 Ward S., Thomson N., White J. G., Brenner S. (1975) Electron microscopical reconstruction of the anterior sensory anatomy of the nematode Caenorhabditis elegans. J. Comp. Neurol. **160:** 313–337