Pflugers Arch - Eur J Physiol (2007) 454:721–734
DOI 10.1007/s00424-006-0196-9

INVITED REVIEW

Generation and modulation of chemosensory behaviors in *C. elegans*

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Received: 7 November 2006 / Revised: 21 November 2006 / Accepted: 27 November 2006 / Published online: 6 January 2007

Abstract *C. elegans* recognizes and discriminates among hundreds of chemical cues using a relatively compact chemosensory nervous system. Chemosensory behaviors are also modulated by prior experience and contextual cues. Because of the facile genetics and genomics possible in this organism, *C. elegans* provides an excellent system in which to explore the generation of chemosensory behaviors from the level of a single gene to the motor output. This review summarizes the current knowledge on the molecular and neuronal substrates of chemosensory behaviors and chemosensory behavioral plasticity in *C. elegans*.

Keywords *C. elegans* · Chemosensation · Plasticity · Circuit

Introduction

Of the classic five senses defined by Aristotle (*De Anima*, Book II), the microscopic free-living nematode *C. elegans* has just three. Worms can smell, taste, and respond to touch, but they cannot hear, and whether or not they respond to light is a matter of debate. As *C. elegans* lives in the soil and feeds on dead and decaying organic matter and bacteria, these animals must, therefore, rely on their chemosensory abilities to locate and navigate their way to food sources and mates, and to avoid toxic substances and predators. Thus, not surprisingly, *C. elegans* turns out to have a highly developed chemosensory system capable of not only recognizing, but also discriminating, among hundreds of chemical cues. Moreover, like sensory behaviors in all organisms, chemosensory responses in *C. elegans* retain the ability to be modulated by both contextual cues and past experience. In this review, I discuss recent findings on the molecular and neuronal bases of *C. elegans* chemosensation. For the sake of brevity, I will concentrate solely on these behaviors in the hermaphrodite. I refer the reader to several excellent publications and references therein addressing *C. elegans* male chemosensory behaviors [1–6].

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The importance of chemosensation

The ability to perceive chemical cues plays a critical role in shaping *C. elegans* behavior and development throughout its lifecycle. Although the chemosensory nervous system develops embryonically [7], it is unlikely that embryos are able to sense external chemicals because of the presence of the relatively impermeable eggshell. However, chemical signals sensed during the first larval stage are essential in directing the choice of the appropriate developmental program. *C. elegans* constitutively secretes a pheromone that serves as a measure of population density [8–10]. L1 larvae assess levels of this pheromone, as well as levels of food in their environment to make a critical developmental decision. Under overcrowded conditions and low food abundance, animals enter into the dauer developmental stage by downregulating the *daf-7* TGF-β and insulin signaling pathways, whereas under conditions more conducive to growth and reproduction, animals proceed in the reproductive cycle [11–17]. Dauer larvae can reenter the reproductive cycle when conditions improve. The ability to sense pheromone and food via the chemosensory neurons is

essential for correct regulation of this developmental choice [18]. Indeed, a hallmark of many mutants defective in chemosensory neuron structure or function is their compromised ability to regulate neuroendocrine signaling to correctly make this decision (e.g., [19–24]).

Chemosensory signals also regulate additional aspects of development and physiology including the regulation of body size and lipid homeostasis. Animals with compromised chemosensory neuron structure and/or function are small and accumulate fat [25–29]. A TGF-β signaling pathway has been implicated in regulating body size [30], and the DBL-1 TGF-β ligand is neuronally expressed [31]. However, it is not yet clear whether chemosensory inputs function via modulation of the *dbl-1* TGF-β pathway or via alternate pathways to regulate body size. A neuroendocrine signal from the chemosensory neurons released in response to external or internal nutrient signals has been proposed to regulate fat storage in the intestine [27–29], but pathway components have not yet been defined. As the requirement of chemosensation in regulating body size and fat storage may be independent of the ability of animals to locate food sources or the rate of food consumption and storage, internal metabolic state may be altered by chemosensory perception to regulate cell size and fat metabolism [26, 27].

The perception of environmental chemical cues also regulates *C. elegans* lifespan. A key signaling pathway regulating *C. elegans* lifespan and those of other organisms acts via insulin/IGF signaling [15, 32–37]. Mutations that prevent the worm from sensing the environment correctly, such as mutants with defective ciliary structures (see below), or compromised sensory signal transduction, exhibit lengthened lifespan via downregulation of insulin signaling [38, 39]. Moreover, ablation of specific subsets of chemosensory neurons results in increased or decreased longevity [40], suggesting that different chemosensory neurons promote or antagonize longevity. An attractive hypothesis is that chemosensory cues regulate levels of insulin, which in turn regulate longevity. Indeed, expression of the *daf-28* insulin peptide gene in sensory neurons appears to be regulated by sensory cues such as food [13].

In addition to regulating development via neuroendocrine signaling, chemosensory stimuli also play a role in regulating multiple motor programs. In particular, locomotory behaviors are modulated by food cues and prior experience of feeding or starvation. Upon encountering food in the form of a bacterial lawn, well-fed worms exhibit a slowed locomotory response, which is mediated by mechanosensory inputs [41]. However, if worms have been starved previously for a period of time and then placed on bacteria, their locomotion is further reduced (enhanced slowing response) [41]. This enhanced slowing response is mediated by chemosensory signals from food, and serotonin signaling [41]. Although it is unclear how bacterial chemosensory cues are integrated with a memory of prior starvation experience to regulate serotonin levels, it is likely that chemosensory neurons play an important role in transmitting food signals.

Prior experience of food signals also regulates the locomotory behavior of animals in the *absence* of food. Shortly upon removal of food, animals exhibit an "area-restricted search behavior" characterized by high frequency of reversals, whereas on prolonged removal of food the frequency of reversals is decreased with coordinated increase in the duration of forward movement [42–45]. These behaviors are regulated by inputs from distinct sets of chemosensory neurons [42, 43].

Chemosensory signals also regulate locomotory behaviors of continuously well-fed animals on bacterial lawns. When placed on a lawn, individual worms exhibit periods of either "dwelling" or "roaming" behavior characterized by low speeds/high turning rate and high speeds/low turning rates, respectively [26]. Mutants with altered chemosensory neuron function spend longer periods in the dwelling vs the roaming state, indicating that the periods spent in each of these states are modulated via chemosensory inputs [26]. Additional motor behaviors regulated by chemosensory inputs from food include egg-laying and pharyngeal pumping [46–50]. Taken together, these observations indicate that environmental chemosensory cues regulate multiple aspects of *C. elegans* behavior, development, and physiology.

Wiring the chemosensory circuit

The *C. elegans* adult hermaphrodite nervous system contains a total of 302 neurons, a full 10% of which are predicted to mediate responses to environmental chemicals [51–53]. Chemosensory neurons are localized at the head and tail, and grouped into sense organs. In the head, six chemosensory neurons are present in the inner labial sensilla, and 11 pairs in the bilateral amphid organs [51, 52]. The bilateral phasmid organs in the tail contain an additional two pairs of chemosensory neurons each [54]. Each chemosensory neuron type exhibits the typical invertebrate sensory neuron bipolar structure, with a single axon, and a single dendrite that terminates in cilia housing primary chemosensory signal transduction components [51, 52, 55–58]. Cilia, in turn are exposed either directly or indirectly to the external environment via openings in the cuticle lined by the processes of support cells [51, 52]. Some of these cilia have relatively simple structures, whereas other chemosensory neurons possess cilia of highly elaborate and complex structures, and are presumably specialized for their sensory functions [55].

Reconstruction of the neuronal connectivity map from serial section electron micrographs has shown that chemosensory neurons can be both presynaptic and postsynaptic to other chemosensory neurons as well as interneurons [53] (Fig. 1a,b), suggesting the possibility of cross-talk and feedback regulation of chemosensory function (discussed further below). Both electrical and chemical synapses are present, and individual chemosensory neurons also contain dense core vesicles and express neuropeptide genes [53, 59–62]. The major postsynaptic outputs of the chemosensory neurons are a few interneuron types, with each interneuron type receiving inputs from partly overlapping groups of chemosensory neurons [53] (Fig. 1a,b).

Chemosensory neurons that sense toxic chemicals exhibit largely distinct connectivity patterns from those sensing attractive chemicals. Thus, the ASH, ADL, and AWB sensory neuron types, which are the primary sensors of toxic chemicals or nociceptive stimuli, synapse directly onto backward command interneurons that direct backward locomotion via activation or inhibition of motor neurons [53] (Fig. 1a), enabling the worm to execute a rapid and robust escape response when these neurons are activated [63–65]. On the other hand, neurons that sense attractive chemicals synapse onto intervening layers of interneurons. The AIY interneurons receive inputs from the ASE, AWC, and AWA amphid chemosensory neurons, whereas the AIA interneurons receive inputs from the ASK, ASG, ASH, ADL, ASE, ASI, and AWC chemosensory neurons [53] (Fig. 1b). These first layer interneurons likely serve as important sites of coincidence detection, signal integration, and processing. It is not yet known whether the synaptic connections between these sensory neurons and interneurons are excitatory or inhibitory. Indeed, the AWC chemosensory neurons are predicted to inhibit the AIY, and activate the AIB interneurons under specific environmental conditions and in response to prior experience, although this remains to be shown physiologically [42–44]. Primary interneurons synapse onto a layer of secondary interneurons or motor neurons, which in turn are presynaptic to the command interneurons directing backward or forward locomotory movement [53, 66] (Fig. 1b). A network of additional interneurons and motor neurons direct more subtle but crucial aspects of chemosensory behaviors including the regulation of head and neck movement to allow efficient navigation of chemical gradients [43]. Integration of sensory inputs at these different layers ultimately dictates the duration of time spent by the animal in the forward, as opposed to the backward, moving state, thus regulating movement up or down a chemical gradient (see below)[43, 44, 64, 66–68]. These anatomical and functional mapping studies indicate that in contrast to chemosensory circuits in other model organisms such as *Drosophila* or the mouse, the chemosensory circuit in *C. elegans* is relatively shallow. In other words, only a few

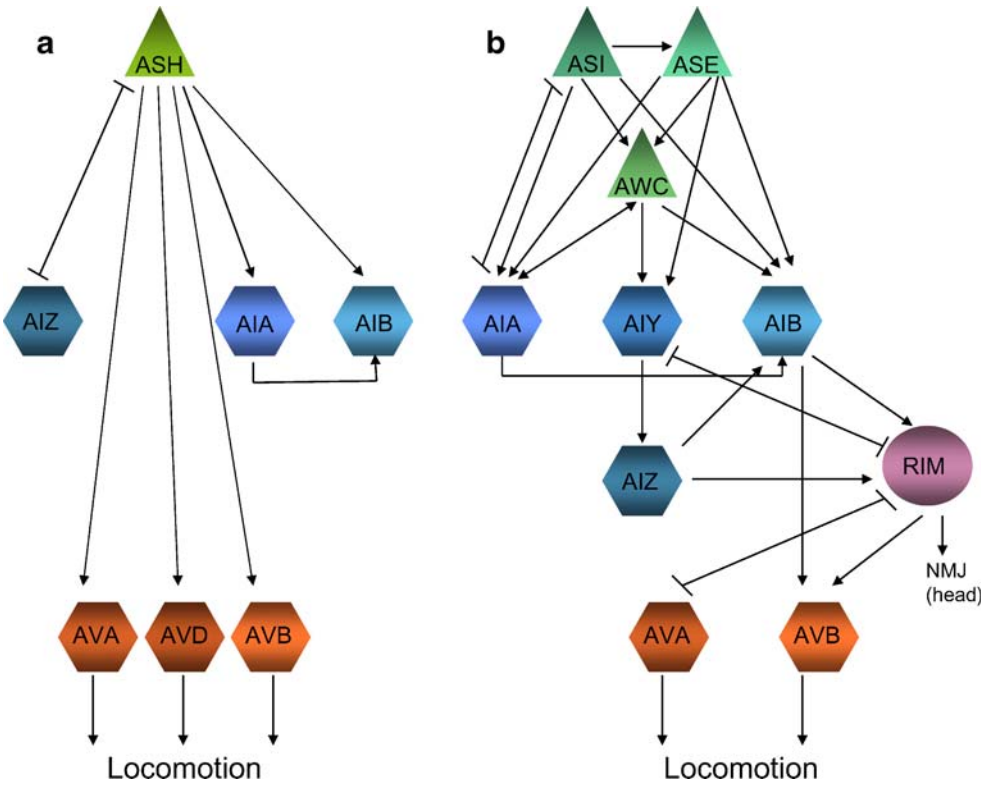

Fig. 1 Neural circuits for chemosensory navigation behaviors. Chemosensory neurons are indicated by *triangles*, interneurons by *hexagons*, and motorneurons by *ovals*. Only a subset of the pre- and postsynaptic partners of each neuron type is shown. *T-bars* represent gap junctions. Command interneurons driving backward or forward locomotory behaviors are indicated in shades of *brown*. Note that chemosensory neurons such as ASH that mediate avoidance, are directly connected to the command interneurons (**a**). In contrast, chemosensory neurons such as AWC that mediate attraction behaviors are indirectly connected to the command interneurons via layers of additional inter- and motorneurons (**b**). Adapted from [53]

synapses separate the chemosensory neurons from the motor neurons that direct behavioral output, suggesting that these circuits may use distinct mechanisms to process and integrate sensory stimuli.

Navigating towards or away from a chemical

A critical function of any chemosensory system is to enable the animal to detect and navigate towards the source of an attractive chemical. Chemotactic ability allows *C. elegans* to locate food sources or mates, and to avoid predators. In the laboratory, chemotaxis behaviors can be assayed simply by placing a single animal or populations of animals on an agar plate containing a point source of a chemical, and quantifying the number of animals at the source after a period of time [69–72]. Under these conditions, *C. elegans* exhibits robust chemotaxis behavior towards a wide range of chemicals at a range of concentrations. Thus, worms must be able to detect small changes in concentration and translate this information into the appropriate pattern of motor behavior so as to move up the chemical gradient.

Although in theory *C. elegans* could detect changes in chemical concentration by comparing concentrations at the head and tail, or between the left and right members of a sensory neuron pair, it has been shown previously that spatial comparisons likely do not play a role in driving nematode chemotaxis [69, 70]. Instead, worms navigate a chemical gradient using temporal comparisons of encountered concentrations [73, 74], similar to the mechanisms used in bacterial chemotaxis [75]. Worm movement on agar plates consists of periods of forward movement, punctuated by sudden turns or reversals that lead to changes in direction [76]. Quantitative analyses of worm movement on shallow gradients, or in response to abrupt changes in chemical concentration have shown that the probability of turns/reversals (collectively referred to as "pirouettes") is directly correlated with changes in concentration [73, 77, 78]. When moving up the gradient toward an attractive chemical ($dC/dt > 0$), worms suppress pirouettes while increasing the duration of forward movement, whereas when moving down the gradient ($dC/dt < 0$), worms increase the probability of pirouettes and decrease the duration of forward movement. Pirouettes not only serve to terminate movement in the direction of falling concentrations, but also reorient the worm up the gradient, with the degree of reorientation proportional to the degree that the animal was off-course [73]. The net result of this strategy is to allow the animal to move towards and accumulate at the point source. Similar mechanisms are also employed by worms to navigate thermal gradients above their preferred temperature [79, 80].

Presumably, sensory neurons detect concentration changes and transmit this information to the downstream circuitry to modulate forward movement and pirouettes. Although sensory neurons responding to specific chemicals have been identified, the exact contribution of these neurons to specific aspects of chemotaxis navigation behaviors have not been explored in detail. An exception is the ASE chemosensory neuron. Ablation of the ASE neurons reduces but does not completely abolish the ability of worms to chemotax towards a point source of aqueous attractants including NaCl, while perturbation of ASE function, together with ADF, ASG, and ASI results in a more complete defect [72] (Table 1). Intriguingly, it has been shown that in ASE-ablated animals, the ability to respond to *decreases* in salt concentration is abolished,

Table 1 Chemical responses mediated by individual chemosensory neurons

Neuron	Attraction to:	Avoidance of:	References
AWA	Diacetyl, pyrazine, trimethylthiazole, 2,3-pentanedione [high]		[71, 83]
AWB		2-nonanone, 1-octanol (off food)	[65, 91]
AWCL	Benzaldehyde, Isoamyl alcohol, Trimethylthiazole, Butanone or 2,3-pentanedione, Diacetyl [high]		[71, 83, 99]
AWCR	Benzaldehyde, Isoamyl alcohol, Trimethylthiazole, Butanone or 2,3-pentanedione, Diacetyl [high]		[71, 83, 99]
ASE	Na^+ (ASEL), Cl^- (ASER), K^+ (ASER), Biotin, cAMP	Cd^{2+}, Cu^{2+}	[72, 98, 178]
ADF	Na^+, Cl^-, Biotin , cAMP (minor)		[72]
ASG	Lysine, Na^+, Cl^-, Biotin , cAMP (minor)		[72]
ASH		1-octanol, Cd^{2+}, Cu^{2+}, SDS, quinine	[58, 104, 179] [65, 178]
ASI	Lysine, Na^+, Cl^-, Biotin , cAMP (minor)		[72]
ASJ			
ASK	Lysine	Quinine, SDS (minor)	[72, 104, 179]
ADL		Cu^{2+}, Cd^{2+}, 1-octanol (off food)	[91, 178] [58, 65]
PHA/PHB		SDS (antagonistic)	[104]

whereas animals continue to respond to concentration increases, perhaps via the ADF, ASG, and ASI neurons [77]. It will be interesting to determine whether the ability to respond to positive or negative changes in concentration is segregated to different chemosensory neurons, to the left/right members of a neuron pair, or is a property of downstream components of the chemosensory circuit.

Mapping chemicals to chemosensory neurons

Each of the 11 amphid chemosensory neuron pairs mediates responses to distinct subsets of aqueous or volatile chemicals (Table 1). (As the distinction between smell and taste is somewhat arbitrary for the worm, I will henceforth refer to both sensory modalities together as chemosensation). Individual sensory neurons have been associated with their ability to respond to specific chemicals by both genetic and physical perturbation methods. In the laboratory, the majority of the nervous system appears to be dispensable for worm survival [81]. Thus, single neuron types can be selectively killed using a tightly focused laser beam and the resulting behavior of the operated animals assessed to determine the contribution of that neuron to the behavior [82]. These experiments have revealed a number of shared and unique features of chemosensory coding in *C. elegans*.

First, similar to other olfactory systems, low concentrations of chemicals are sensed by smaller numbers of neurons than higher concentrations [83] (Table 1). Second, the same chemical may act as a repellent at one concentration, and as an attractant at a different concentration [70, 71, 84–86]. Third, even at low concentrations, the ability to sense a particular chemical may be distributed among several neurons. For instance, low concentrations of NaCl (0.4 M) and biotin (0.2 M) are sensed primarily by the ASE neurons, with contributions from the ADF, ASG, and ASI chemosensory neurons [72] (Table 1). As mentioned above, it is possible that a subset of neurons respond to a negative change in chemical concentration, whereas others respond to a positive change [77]. Fourth, as *C. elegans* responds to a large repertoire of chemicals [69–72, 87, 88] using a small number of chemosensory neurons, each neuron type recognizes multiple chemicals of unrelated structures, pointing to the presence of multiple chemoreceptors in each neuron type. Fifth, to further emphasize the importance of avoiding noxious chemicals, the ability to sense noxious chemicals is largely segregated from the ability to respond to attractive chemicals at the level of the sensory neurons themselves (Table 1). Thus, the ASH, ADL, and AWB neurons mediate aversive behaviors more or less exclusively, whereas attraction is mediated by the remaining eight pairs of amphid chemosensory neurons [58, 65, 71, 72, 89–91]. This organization is similar to observations in the mammalian and *Drosophila* gustatory system, where sensory cells responding to sugars and other palatable compounds are segregated from those sensing bitter and hence, toxic compounds [92–97]. This strategy of separating attractive and aversive responsiveness at the sensory neuron level is also efficient in terms of circuit wiring in *C. elegans* as repellent-sensing neurons are directly connected to the backward command interneurons to effect rapid reversal responses (Fig. 1a). An obvious prediction from these observations is that expression of a receptor for an attractive odorant in a neuron that mediates avoidance should trigger avoidance of that chemical. This has been shown to be the case [91], underscoring that the sensory neuron, and not the molecules it expresses, is the arbiter of the behavioral outcome.

Although the left/right pairs of a neuron type were initially thought to be equivalent in terms of chemosensory responses, it is now increasingly evident that *C. elegans* generates further functional diversity by assigning distinct chemosensory response profiles to each of a left/right sensory neuron pair. Thus, the left ASE neuron is the primary sensor of Na^+ ions, whereas the right ASE neuron responds to Cl^- and K^+ [98] (Table 1). On the other hand, while both the left and right AWC neurons sense benzaldehyde, either the left or the right AWC neuron responds to the volatile odorant butanone, and the other neuron responds to 2,3-pentanedione [71, 99] (Table 1). The advantages of assigning the sensory functions stochastically as in the AWC neurons [100], or via a developmentally hardwired mechanism as for the ASE neurons [101–103] is not immediately obvious. A suggestion from these findings is that other chemosensory neuron types are likely to also exhibit left/right functional diversification, essentially doubling the sensory neuron repertoire.

What are the functions of the inner labial and the phasmid sensilla? To date, no specific chemosensory functions have been assigned to the inner labial chemosensory neurons. However, the PHA and PHB phasmid chemosensory neurons may sense chemical repellents and antagonize ASH-mediated avoidance behaviors [104] (Table 1).

The neurons required for responses to complex biologically relevant cues are not fully defined. Worms are attracted to, or avoid, different bacteria [68, 105, 106], and it is not yet known whether these behaviors are mediated primarily via a single bacterially produced chemical, or whether a set of chemicals must be recognized as an ensemble to provide a chemical signature for a specific bacterial strain. Similarly, although a major regulator of worm behavior and development is levels of the constitutively produced dauer pheromone [8–10, 107–109], the neurons that respond to pheromone are also not

yet defined. Presumably, males and hermaphrodites also produce signals to attract or repel each other, but these cues and the relevant sensory neurons are unknown [3, 4]. As "...behavior is messy" (with apologies to [110]), the development of technologies for imaging neuronal activity in single neuron types in vivo in response to an applied stimulus [111–115] may allow for more precise functional mapping of chemicals to sensory neuron types.

The molecules for taste and smell

As in *Drosophila* and vertebrates, many chemicals are sensed by seven transmembrane domain G protein-coupled receptors (GPCRs) in *C. elegans*. Given the critical importance of chemosensation for the worm's survival, it is perhaps not surprising that nearly 10% of the *C. elegans* genome is devoted to encoding predicted chemosensory receptors (CRs), a current total of ~1,500 molecules [58, 116–118]. In comparison, the *Drosophila* genome is predicted to encode ~62 olfactory and ~68 gustatory receptors [119–123], whereas the mouse genome encodes ~1,200 olfactory and 38 gustatory GPCRs [93, 94, 124–128]. Although the expression patterns of only a handful of CR genes have been examined [58, 129], it is clear that in stark contrast to the vertebrate or *Drosophila* olfactory systems, each chemosensory neuron in *C. elegans* expresses multiple CR genes, perhaps as many as 20 per neuron type (Fig. 2). In this respect, the worm chemosensory system is similar to the bitter-sensing taste cells in vertebrates, which express multiple bitter receptors per cell, with each receptor being selectively tuned to a small subset of bitter compounds [93, 127, 128, 130].

What is the molecular receptive range (MRR) of each *C. elegans* CR? Does each CR in *C. elegans* respond selectively to one chemical, a small set of chemicals of related structure, or to an "odotope" present on multiple chemicals of overall unrelated structures? Unfortunately, the answer to this issue is still not resolved. Only one chemical has been linked to its cognate receptor [56, 131]; the ligands for other receptors are as yet unknown. Remarkable strides have been made in defining receptor–ligand interactions in other systems (e.g., [132–135]), so it is surprising that so little is known about this issue in the *C. elegans* chemosensory system. Part of this lack of knowledge may be attributed to the large size of the *C. elegans* CR gene family, which makes it difficult to employ the type of analysis that has been used so elegantly and effectively to "de-orphanize" *Drosphila* olfactory receptor genes [132, 133]. Genetic screens have also failed to yield additional CR gene mutants, perhaps pointing to some degree of redundancy in this system. One critical issue, however, has been a paucity of knowledge regarding the identities of ecologically relevant chemical cues for *C. elegans*. As *C. elegans* are free-living, they are likely to be generalists responding to many chemicals produced by different strains of bacteria, most of which are uncharacterized. Thus, it has been difficult to make educated guesses regarding the set of chemicals that could be used to systematically examine CR ligand selectivity. Complicating matters, chemosensory neurons in *C. elegans* also express multiple members of other protein families such as transmembrane guanylyl cyclases, which may also act as chemoreceptors [136, 137], further increasing the complexity of the chemosensory receptor repertoire.

As in other chemosensory systems, interaction of an odorant with its cognate ligand is predicted to either activate or inhibit synaptic output of a chemosensory neuron [42, 43] (C. Bargmann, personal communication). Although the physiological mechanisms by which activation or inhibition is mediated have not yet been described, many of the molecules required for chemosensory signal transduction have been identified using forward or reverse

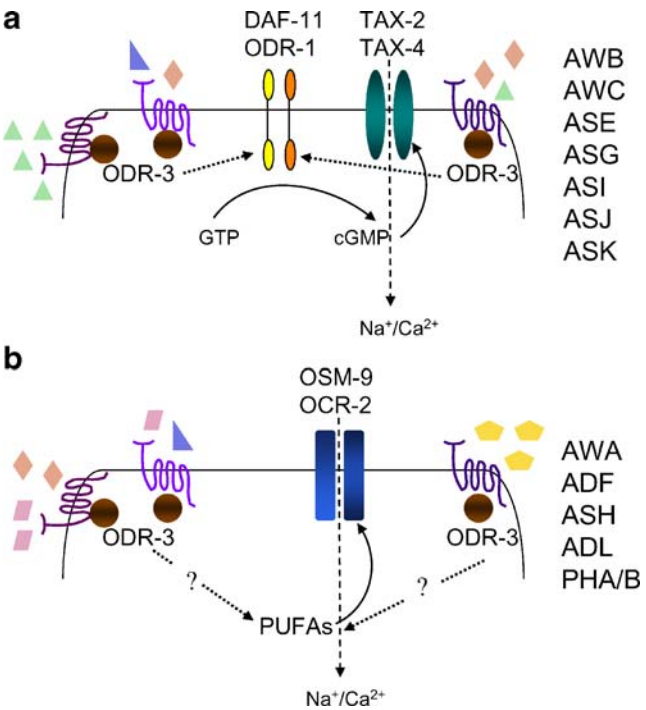

Fig. 2 Chemosensory signaling pathways. Chemosensory signal transduction is mediated either via cGMP **(a)** or polyunsaturated fatty acid (PUFA) **(b)** mediated signaling. Sensory neurons in which the proposed pathways are believed to function are indicated at *right*. Each chemosensory neuron expresses multiple CRs. The major Gα subunit (ODR-3), receptor guanylyl cyclases (DAF-11, ODR-1), cGMP-gated channels (TAX-2, TAX-4), and TRPV channels (OSM-9, OCR-2) implicated in chemosensation are shown. Additional members of these families are also expressed in, and required for the functions of these neurons. See text for references

genetic approaches. One of the conclusions from these results is that multiple CRs expressed in a given cell type converge onto a common set of downstream signal transduction molecules. For example, a number of sensory neuron-specific Gα proteins appear to act in a complex manner to activate or inhibit signaling upon interaction of any ligand with its cognate CR in a given cell type [19, 57, 138–140]. These G proteins act via different signaling pathways in different chemosensory neuron types.

In the AWC olfactory neurons and in several other neuron types, G proteins may activate guanylyl cyclases, resulting in gating of the TAX-2/4 cGMP-gated channels [20, 22, 141–143] (Fig. 2a). Thus, in the absence of TAX-2/4 function, these neurons fail to respond to any odorants. However, in a subset of additional neuron types, primary chemosensory signal transduction is likely mediated via the OSM-9 and OCR-2 TRPV channels, which may be gated by polyunsaturated fatty acids (PUFAs) or their derivatives [63, 113, 144] (Fig. 2b). Neurons use either the cyclic nucleotide or the PUFA-mediated signaling pathway, although CRs are capable of coupling to the alternate pathway upon misexpression [91].

As signaling mediated by chemoreceptors converges onto a common downstream set of signal transduction molecules, can the animal discriminate among odorants sensed by the same neuron type? In the background of a high constant concentration of one chemical, animals fail to respond to a point source of that chemical, but continue to respond to other chemicals sensed by that neuron type [71]. Similarly, prolonged exposure to one odorant decreases the response to that odorant while sparing responses to other chemicals sensed by that neuron [142, 145–147]. These observations indicate that the chemosensory system is able to discriminate among chemicals sensed by one neuron type. In the case of the L/R asymmetric ASE and AWC neurons, this feat is achieved simply by segregating responsiveness to different chemicals to the left or the right neuron [98, 99]. However, for cues sensed by both left and right neurons, one mechanism by which the signal transduction pathways could be insulated is via segregation of individual signaling pathways into signaling microdomains [142, 148–151]. It is also possible that while the core components of the signal transduction pathways are shared, additional molecules act in an odorant pathway-specific manner [140], allowing the neuron to discriminate between multiple chemical cues. Finally, discrimination may also be effectively achieved by the activation or inhibition of different CRs expressed in a given neuron by different chemicals, or via differential temporal dynamics of CR function [132, 133, 152]. Identification of additional signaling molecules, and characterization of protein function, including CRs, will be necessary to fully address this issue.

Modulation of chemosensory behaviors

Animals must not only be able to respond to chemical cues, but must also be able to modulate their response based on the context of presentation and their past experience. This behavioral plasticity may arise at the level of changes in intracellular signaling pathways, in intercellular communication, or both. Examples of both intracellular and intercellular plasticity mechanisms have now been described in the *C. elegans* chemosensory system, revealing a high degree of functional complexity in these relatively simple neural circuits.

Intracellular mechanisms of behavioral plasticity A common feature of all sensory systems is the ability to adapt to the ambient stimulus level, so as to maintain responsiveness. As mentioned above, prolonged exposure to high concentrations of a chemical results in worms failing to respond to a point source of the chemical. Adaptation appears to be biphasic with an early, rapid stage, and a later prolonged stage [145, 146]. Chemosensory responses are restored upon removal from the adapting chemical, with the time period of exposure correlating with the time required for recovery [146]. Early steps in adaptation may be mediated via modulation of activity of signaling components such as receptors, channels, and other signaling molecules via posttranslational mechanisms, resulting in cue-specific changes in sensory neuron responses [115, 145, 153, 154] (Fig. 3a). Thus, in the AWC olfactory neurons, phosphorylation of the TAX-2 channel appears to play an important role in the early adaptation stage [145], and this phosphorylation may be antagonized by the TAX-6 calcineurin phosphatase [155] (Fig. 3a). Cytoplasmic activity of the TBX-2 transcription factor also affects early adaptation steps via as yet unknown mechanisms [156] (Fig. 3a). However, later steps may require changes in gene expression. In the AWC neurons, cGMP-dependent protein kinase EGL-4 affects both early and late steps in adaptation, but interestingly, must be nuclear-translocated to effect the later stages [145] (N. L'Etoile, personal communication; Fig. 3a).

Another mechanism by which worms can rapidly alter their behavioral responses is via modulation of expression of individual chemoreceptor genes expressed in a single chemosensory neuron type. As each chemosensory neuron expresses multiple chemoreceptors, alteration of expression of individual receptors provides a mechanism by which worms can selectively alter their response to a single chemical sensed by that neuron type, without altering responses to other chemicals. Indeed, individual chemoreceptor genes have been shown to be regulated by a plethora of mechanisms including neuronal activity, internal metabolic state, levels of pheromone, and intercellular signaling

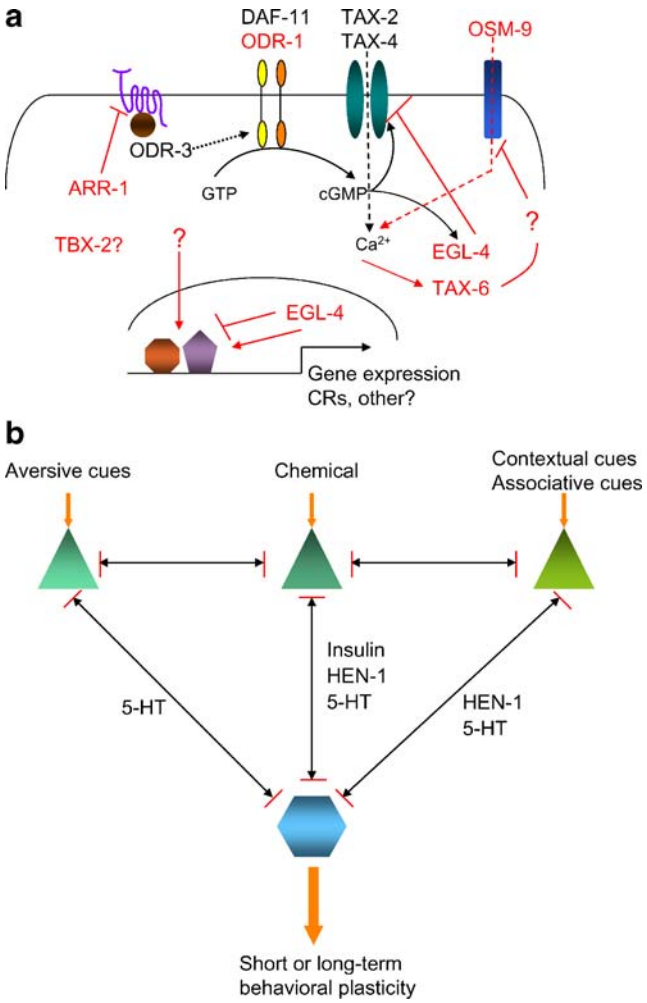

Fig. 3 Intra- and intercellular mechanisms of chemosensory neuronal plasticity. **a** Molecules acting in the AWC neurons to modulate AWC neuron plasticity are shown in *red*. ARR-1 encodes an arrestin possibly playing a role in receptor desensitization [154]; overexpression of the ODR-1 receptor guanylyl cyclase alters adaptation to a subset of AWC-sensed odorants [142]; the TBX-2 transcription factor acts cytoplasmically to regulate olfactory adaptation via as yet unknown mechanisms [156]; the EGL-4 cGMP-dependent protein kinase phosphorylates the TAX-2 channel, and also translocates to the nucleus to regulate early and late steps in olfactory adaptation, respectively [145]; Ca^{2+} entry through the OSM-9 TRPV channel is required for olfactory adaptation to a subset of AWC-sensed chemicals [146]; the TAX-6 Ca^{2+}-dependent calcineurin phosphatase negatively regulates adaptation [155]. **b** Simplified summary of intercellular mechanisms proposed to mediate chemosensory behavioral plasticity. *Triangles* represent chemosensory neurons; *hexagon* represents an interneuron. Both feedforward and feedback mechanisms may act in these circuits, and these mechanisms may result in facilitation or inhibition of neuronal output. A subset of molecules implicated in mediating neuronal circuit plasticity are shown. See text for references

[25, 63, 100, 107, 108, 157] (A. van der Linden, K. Kim and P.S., unpublished observations; Fig. 3a). Thus, dynamic regulation of chemoreceptor gene expression may drive a subset of the behavioral changes observed under different conditions. This mechanism may not necessarily be restricted to *C. elegans*; the altered host-seeking behavior of the mosquito *Anopheles gambiae* after a blood meal may result from downregulation of olfactory receptors that respond to components in human sweat [158–160].

Intercellular mechanisms of plasticity In addition to intracellular mechanisms, intercellular communication also plays an important role in regulating adaptation behaviors [147, 161] (Fig. 3b). Adaptation is regulated by the animal's experience such that the presence of food suppresses adaptation, whereas the absence of food promotes adaptation [84, 162]. Adaptation has also been shown to be state-dependent, such that if adaptation to chemical A is performed in a specific context of chemical B, subsequent adaptation to chemical A is observed only in the presence of chemical B [163, 164]. These experience-dependent modulation of adaptation behaviors likely requires integration and processing of information at downstream loci in chemosensory neural circuits [164, 165]. However, cell–cell communication between different chemosensory neurons may also play a role in the regulation of responses to a chemical after prolonged exposure [138] (Fig. 3b).

Chemosensory behaviors of *C. elegans* may also be modulated via associative conditioning. Upon pairing of an attractive chemical (conditioned stimulus or CS) with an aversive stimulus such as a noxious chemical or starvation (unconditioned stimulus or US), worms will avoid the CS upon subsequent encounters [166, 167]. Similarly, worms will preferentially migrate towards the chemical paired with an attractive stimulus such as food [168]. Two interesting pathways have been implicated in these associative behavioral paradigms. Both *hen-1* mutants and mutants in the insulin signaling pathway, including animals mutant for the *daf-2* insulin receptor, show a compromised ability to avoid an attractive salt stimulus when paired with the aversive stimulus of starvation (salt chemotaxis learning) [169, 170]. Mutations in these genes do not affect responses to salt in the absence of conditioning. *hen-1* encodes a secreted protein that acts cell non-autonomously, suggesting that HEN-1 may modulate circuit function by acting on presynaptic chemosensory neurons, or postsynaptically on interneurons [169] (Fig. 3b). The DAF-2 insulin receptor and its effector PI3 kinase have been shown to act only in the ASER neurons to mediate salt chemotaxis learning, possibly via regulation of ASER synaptic output [170] (Fig. 3b). The ligand for DAF-2 in this pathway is likely INS-1, which is produced by the AIA postsynaptic interneurons [170]. Thus, a provocative model for salt chemotaxis learning is that upon conditioning, insulin is produced by the AIA interneurons, which in turn feeds back onto the ASER chemosensory neurons to regulate ASER synaptic output,

and hence ASER functions [170]. These findings on the role of insulin in regulating neuronal functions provides the opportunity to define how internal metabolic state may modulate chemosensory neuron functions. As insulin signaling has been implicated in neuronal plasticity in other organisms [171, 172], it will be interesting to explore whether insulin acts via similar feedback mechanisms to regulate neuronal function.

Another example of associative conditioning has recently been described [106]. Upon infection by pathogenic bacteria, *C. elegans* will subsequently avoid odors associated with the pathogenic bacteria and increase its preference towards non-pathogenic bacteria. This plasticity requires serotonergic signaling from the ADF chemosensory neurons, which then acts via serotonin receptors in downstream interneurons to modulate aversive learning [106] (Fig. 3b). This aversive conditioning is highly relevant for the animal biologically, as it allows *C. elegans* to preferentially locate food sources that are non-toxic based on prior experience.

The context of a presented chemical cue is also an important modulator of behavior. The presence or absence of food can rapidly and reversibly alter the responses of animals to the volatile repellent 1-octanol, and this modulation may occur presynaptically via serotonergic signaling [65]. In another behavioral paradigm, animals are presented simultaneously with an attractive odorant such as diacetyl, and a repellent chemical such as Cu^{2+} ions. These attractive and repulsive cues are sensed, integrated, and balanced against each other, to result in attraction towards diacetyl, avoidance of Cu^{2+}, or no net directed movement depending on the relative concentrations of each chemical [169]. Animals mutant for the secreted peptide HEN-1 fail to correctly integrate these stimuli, although these mutants retain normal responses to each odorant when presented alone. As the receptor for HEN-1 has not yet been identified, the locus of action of the HEN-1 signaling pathway is unknown.

Although all the behavioral plasticity mechanisms described above act acutely to alter circuit output, it has recently been shown that chemosensory cues experienced during a specific period in development can alter chemosensory behaviors at a later stage [173]. Animals exposed to a chemical during the L1 developmental stage show a marked preference for that chemical as adults [173]. Chemicals sensed during the juvenile stage in animals such as salmon also play a critical role in allowing the adult animal to return to their natal area to spawn—a phenomenon referred to as olfactory imprinting [174, 175]. The coincident detection of food and the chemical is necessary for this imprinting to occur in *C. elegans*, and the AIY interneurons play an important role [173]. It will be very interesting to investigate how the memory of the chemosensory experience is stored during the L1 stage, and how it is retrieved upon encountering the chemical at the adult stage to modulate the response.

Future directions

C. elegans has proved to be an excellent model organism in which to explore the generation of chemosensory behavior from the level of a single gene to the behavioral output of the whole organism. The development of quantitative behavioral assays and measurements of neuronal activity [73, 77, 111, 113–115, 176, 177] will allow researchers to further investigate the mechanisms by which neuronal circuits encode specific behavioral responses. Several outstanding questions remain in this field. If we are to understand how worms sense their chemical environment, it will be important to identify the ligands for individual chemoreceptors. It will also be critical to understand how information about chemical cues is encoded in spatial and temporal patterns of neuronal and neuronal circuit activity to alter behavior. Further dissection of the molecular and neuronal mechanisms underlying behavioral plasticity is also likely to provide new information regarding both intracellular and intercellular contributions to short-term and long-term behavioral changes, and to determine whether similar mechanisms operate in other systems. Finally, it will be very interesting to explore mechanisms of polymodal sensory integration. All animals respond to multiple types of sensory stimuli simultaneously in their natural habitat, but how these stimuli are integrated to result in a coherent motor output has been a difficult issue to study experimentally. *C. elegans* may provide an ideal system in which to examine how a nervous system translates spatiotemporally complex sensory cues into the appropriate behavioral response.

Acknowledgments I thank Oliver Hobert, Alexander van der Linden, Kyuhyung Kim, and Cori Bargmann for comments and discussion. I acknowledge financial support from the NSF and NIH (NIGMS and NINDS).

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