The DAF-7 TGF-β signaling pathway regulates chemosensory receptor gene expression in C. elegans

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Regulation of chemoreceptor gene expression in response to environmental or developmental cues provides a mechanism by which animals can alter their sensory responses. Here we demonstrate a role for the daf-7 TGF-β pathway in the regulation of expression of a subset of chemoreceptor genes in Caenorhabditis elegans. We describe a novel role of this pathway in maintaining receptor gene expression in the adult and show that the DAF-4 type II TGF-β receptor functions cell-autonomously to modulate chemoreceptor expression. We also find that the alteration of receptor gene expression in the ASI chemosensory neurons by environmental signals, such as levels of a constitutively produced pheromone, may be mediated via a DAF-7-independent pathway. Receptor gene expression in the ASI and ASH sensory neurons appears to be regulated via distinct mechanisms. Our results suggest that the expression of individual chemoreceptor genes in C. elegans is subject to multiple modes of regulation, thereby ensuring that animals exhibit the responses most appropriate for their developmental stage and environmental conditions.

Keywords: TGF-β; C. elegans; chemosensory receptor; dauer

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animals. Expression in the PVQ interneurons is unaffected (arrowhead). Lateral view; anterior is at

interneurons in the nerve ring. (Albert et al. 1981; Golden and Riddle 1982, 1984; Gottlieb and Ruvkun 1994; Ren et al. 1996; Schackwitz et al. 1996). The nervous system and other tissues undergo extensive remodeling in the dauer stage [Albert and Riddle 1983; Riddle and Albert 1997], and dauer animals exhibit distinctive stage-specific sensory behaviors [Hedgecock and Russell 1975; Albert and Riddle 1983; Riddle and Albert 1997]. As suggested previously [Peckol et al. 2001], a simple mechanism by which olfactory behaviors could be modified in C. elegans is via the altered expression of olfactory receptor genes. Similar to other organisms, nematodes respond to odorants using G protein-coupled receptors [Troemel et al. 1995; Sengupta et al. 1996]. However, in contrast to both Drosophila and rodents, C. elegans expresses multiple, partially overlapping sets of receptors in each chemosensory neuron type [Chess et al. 1994; Troemel et al. 1995; Clyne et al. 1999; Gao and Chess 1999; Malnic et al. 1999; Troemel 1999a; Vosshall et al. 1999; Scott et al. 2001]. Chemoreceptors expressed in a given neuron type share downstream signal transduction components [Coburn and Bargmann 1996; Komatsu et al. 1996; Colbert et al. 1997; Roayaie et al. 1998; L'Etoile and Bargmann 2000], suggesting that the ability of chemosensory neurons to respond to multiple, structurally unrelated odorants is determined primarily by the set of expressed chemoreceptors. Because each chemosensory neuron expresses multiple chemoreceptors, altering the synaptic efficacy of the circuit would alter the responses to multiple chemicals. However, altering expression of a single chemoreceptor gene would result in a specific change in the response to one or a small subset of chemicals. Consistent with this, misexpression of the olfactory receptor for the normally attractive chemical diacetyl in a neuron type that senses repellents has been shown to be sufficient to trigger avoidance of diacetyl [Troemel et al. 1997]. Moreover, it has been shown that exposure to pheromone and entry into the dauer stage results in dramatic alterations of olfactory receptor gene expression in the ASI chemosensory neurons. This modulation may underlie some aspects of the altered chemosensory responses exhibited under conditions of overcrowding or by dauer animals [Peckol et al. 2001]. Thus, given the critical role of chemoreceptors in directing the functions of chemosensory neurons in C. elegans, the correct spatial and temporal expression of these genes is likely to be regulated in a complex manner.

In this study, we demonstrate that the daf-7 TGF-β pathway regulates the expression of all chemoreceptors known to be expressed in the ASI chemosensory neurons, as well as a chemoreceptor expressed in the ASH sensory neurons. Our results demonstrate that TGF-β signaling is required at multiple developmental stages to regulate receptor expression and uncover a previously unknown role of this pathway in maintaining chemoreceptor gene expression in the adult. Additionally, we show that distinct mechanisms regulate chemoreceptor gene expression in the ASI and ASH sensory neurons. These findings suggest that the precise regulation of chemoreceptor genes by multiple mechanisms enables C. elegans to exhibit sensory behaviors appropriate for its developmental stage and environmental conditions.

Results

Expression of a subset of chemoreceptor genes is altered in daf-7 TGF-β pathway mutants

The promoter of the candidate chemosensory receptor gene sra-6 drives expression of a GFP reporter in the ASH and ASI sensory neurons in the head and in the PVQ interneurons in the tail [Troemel et al. 1995]. In a genetic screen, we isolated a mutant oy8, in which expression of an sra-6::gfp transgene (henceforth referred to as expression of sra-6) was strongly reduced in both the ASH and ASI neurons but was unaffected in the PVQ neurons [Fig. 1]. Subsequent mapping and complementation experiments demonstrated that oy8 is allelic to the gene daf-8, which encodes a SMAD protein [Riddle and Albert 1997].

Figure 1. Expression of an sra-6::gfp transgene is reduced in the ASH and ASI sensory neurons of daf-8(oy8) mutants. (A) sra-6::gfp is expressed in the ASH and ASI sensory neurons in the head of an adult wild-type animal. This transgene also drives expression in the PVQ interneurons whose cell bodies are present in the tail (not shown). Arrowhead points to the axonal process of the PVQ interneurons in the nerve ring. (B) No or weak sra-6::gfp expression is observed in the ASH and ASI neurons of daf-8(oy8) adult animals. Expression in the PVQ interneurons is unaffected (arrowhead). Lateral view; anterior is at left. Bar, 20 μm.
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| Receptor^a | Neuron       | Wild type | daf-7^b | daf-1^b | daf-4^b | daf-8^b | daf-14^b |
|------------|--------------|-----------|---------|---------|---------|---------|----------|
| stra-6 larvae | ASH          | 100       | 100     | —       | 100     | 100     | 100      |
| stra-6    | ASH/ASI      | 100       | 100     | —       | 100     | 100     | 100      |
| str-2     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-3     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-1     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-1     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-1     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-1     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |
| str-1     | ASI          | 100       | 100     | —       | 100     | 100     | 100      |

Expression was observed at 400x magnification in all cases. n > 70 for each with the exception of daf-4, Ex[arg-13::gfp] animals (n = 58). — indicates not done.

*Expression was examined in strains containing integrated copies of receptor::gfp fusion genes or extrachromosomal arrays [arg-8 and arg-13] grown at 20°C. For arrays, numbers shown are from the same array examined in wild-type and mutant animals.

*Alleles used were daf-7(e1372), daf-1(m20), daf-4(m63), daf-8(e1393), and daf-14(m77).

*L1/early L2 larvae were examined. All other animals examined were young adults.

*In wild-type animals, 100% of the animals expressed GFP in a total of four cells [two ASH and two ASI]. In daf-7 and daf-4 mutants, 1% expressed GFP in one neuron, in daf-1 mutants, 12% expressed GFP in one or two neurons; in daf-8 mutants, 12% expressed GFP in one, 60% in two, and 1% in three neurons; in daf-14 mutants, 6% expressed GFP in one and 18% in two neurons.

*Under normal growth conditions, str-2 is expressed weakly or undetectably in the ASI neurons. Expression is scored as bright GFP expression in both ASI neurons.
correct expression of a subset of chemosensory receptor genes.

Chemosensory receptor gene expression is differentially regulated in wild type and in daf-7 TGF-β pathway mutant dauer animals

Whereas wild-type animals enter into the dauer stage only on exposure to adverse environmental stimuli (Golden and Riddle 1984; Riddle and Albert 1997), daf-7 pathway mutants form dauers constitutively [Daf-c]. The expression patterns of str-2 and srd-1 are altered in wild-type dauer animals (Peckol et al. 2001). To examine whether the expression of these chemoreceptor genes is altered similarly in TGF-β pathway mutant dauers, we compared the expression of str-6, str-2, str-3, and srd-1 in wild-type and daf-4 dauer animals (Table 2).

str-2 expression was up-regulated and srd-1 expression was down-regulated in the ASI neurons of both wild-type and daf-4 dauer animals (Table 2). However, while only 32% of wild-type dauers retained str-2 expression in a single AWC neuron, ~73% of daf-4 dauers continued to express str-2 in an AWC neuron. We found that the expression of sra-6 and str-3 was regulated differently in wild-type and daf-4 dauer animals. Although sra-6 expression is unaltered in wild-type dauers, str-6 expression was abolished in the ASI, but not in the AHS neurons in daf-4 dauers (Table 2). Similarly, although str-3 is expressed at high levels in the ASI neurons in wild-type dauers, 98% of daf-4 dauer animals failed to express str-3 (Table 2). These results are summarized in Table 3.

Dauer entry is accompanied by remodeling of multiple tissues, including the nervous system (Albert and Riddle 1983; Riddle and Albert 1997). The ciliary endings of chemosensory neurons such as ASI retract, so that they are no longer exposed to the external environment via the amphid pore and consequently fail to uptake lipophilic dyes such as DiI (Albert and Riddle 1983; Herman and Hedgecock 1990; Peckol et al. 2001). This remodeling has been proposed to play a role in the altered expression of chemosensory receptor genes such as str-2 and str-3 in wild-type dauers (Peckol et al. 2001). The observed differences in chemoreceptor expression patterns such as that of str-3 between wild-type and TGF-β pathway mutant dauers could arise in part from the failure of the ASI neurons to remodel correctly in the absence of TGF-β signaling or could reflect a direct requirement of TGF-β signaling for gene expression. To address this issue, we examined the dye-filling ability of the ASI neurons in wild-type and daf-4 mutant dauers. We found that overall, similar numbers of wild-type and daf-4 mutant dauers failed to dye-fill the ASI neurons [96% wild-type versus 99% daf-4 dauers; n > 95 for each], suggesting that the ASI neurons are remodeled similarly. However, TGF-β pathway mutant dauers recover more slowly than do wild-type dauers (Vowels and Thomas 1992), implying functional differences between these two types of dauers. These results suggest that TGF-β signaling is required for the expression of a subset of chemosensory receptor genes in the ASI neurons in dauer animals.

Table 2. Chemosensory receptor genes are differentially expressed in wild-type and daf-4 dauers

| Receptor | % dauers expressing GFP | daf-4<sup>a</sup> |
|----------|-------------------------|-----------------|
|          | Neuron                  | Wild type       |               |
| sra-6    | ASH                     | 100             | 98            |
| str-6    | ASI                     | 97              | 2             |
| str-3    | ASI                     | 100             | 77            |
| srd-1    | ASI                     | 10              | 2             |

n > 65 in each case.

<sup>a</sup>Integrated receptor::gfp fusion genes were examined. Expression was scored as expression in one or both neurons.

<sup>b</sup>Allele used was daf-4(m63).

daf-3 SMAD mutations suppress daf-7-induced but not pheromone-induced alterations in chemoreceptor gene expression patterns

The daf-7 TGF-β pathway functions in developmental processes other than dauer formation. When allowed to bypass the dauer stage, daf-7 pathway mutant adults exhibit increased social behavior, decreased rates of egg-laying, and a darkened intestine (Trent et al. 1983; Thomas et al. 1993). All phenotypes, including the Daf-c phenotype, are suppressed by mutations in the daf-3 SMAD and daf-5 genes (Vowels and Thomas 1992; Thomas et al. 1993). To determine whether mutations in daf-3 also suppress the receptor gene expression defects, we examined sra-6 and str-2 expression in daf-7, daf-3 double mutants. As shown in Table 4, daf-3(mgD90) fully suppressed the gene expression defects of str-2 and sra-6 in daf-7 mutants.

Exposure to high concentrations of pheromone results in down-regulation of daf-7 expression and subsequent dauer entry (Ren et al. 1996; Schackwitz et al. 1996). However, exposure to concentrations of pheromone that are insufficient to either grossly affect daf-7 expression or induce dauer entry has been shown to be sufficient to down-regulate str-3, srd-1, and str-2 expression in the ASI neurons (Peckol et al. 2001). We found that pheromone also down-regulates str-6 expression in the ASI but not the AHS neurons (Fig. 2A; Table 3; data not shown). To determine whether pheromone-mediated receptor regulation functions via down-regulation of TGF-β signaling, we examined receptor expression in daf-3 mutants on the addition of low levels of pheromone. Mutations in daf-3 failed to suppress the pheromone-mediated down-regulation of str-3, str-2, or sra-6 expression in the ASI neurons (Fig. 2A–C). This suggests that pheromone, at subdauer-inducing concentrations, acts via a TGF-β-independent pathway to regulate receptor expression. However, because dauer pheromone is a complex mixture of fatty acids, we cannot exclude the possibility that different components of pheromone are active at different concentrations.
Dauer entry is regulated in parallel by the *daf-7* TGF-β and the *daf-2* insulin signaling pathways (Gottlieb and Ruvkun 1994; Riddle and Albert 1997). We determined whether insulin signaling also plays a role in chemosensory receptor gene expression. We found that expression of chemoreceptor genes examined was unaltered in *daf-2* mutants (Table 4; data not shown), indicating that expression of these chemoreceptor genes does not require insulin signaling via DAF-2.

The *daf-12* nuclear hormone receptor gene is required for chemoreceptor regulation in the ASI but not the ASH neurons

In one model, the observed defects in chemoreceptor gene expression could arise solely as a consequence of defects in TGF-β signaling in the context of dauer formation. Alternatively, these defects could reflect a requirement for TGF-β signaling independent of its role in the dauer pathway. The *daf-7* and *daf-2*-regulated pathways converge at the *daf-12* nuclear hormone receptor gene to regulate dauer formation. Mutations in *daf-12* suppress the *daf-c* but not the adult-specific phenotypes of *daf-7* pathway mutants (Thomas et al. 1993; Riddle and Albert 1997). Thus, the adult-specific phenotypes are regulated by TGF-β signaling via a *daf-12*-independent and hence a dauer pathway-independent mechanism. We examined receptor gene expression in *daf-7; daf-12* double mutants to determine whether mutations in *daf-12* suppress the gene expression defects of TGF-β pathway mutants.

Unexpectedly, we found that mutations in *daf-12* alone affected chemoreceptor gene expression in the ASI neurons. Expression of *stra-6* was strongly down-regulated, and expression of *str-2* was up-regulated in the ASI neurons and was unaffected in the ASH and AWC neurons in *daf-12(rh61rh411) null mutants (Table 4; data not shown), suggesting that *DAF-12* function is required for the correct expression of these receptors only in the ASI neurons. Interestingly, *str-2* expression in the ASI neurons of *daf-7; daf-12* double mutants was more variable than that of either *daf-7* or *daf-12* mutants alone. It is possible that in the absence of both TGF-β signaling and *daf-12* function, additional mechanisms for *str-2* regulation in the ASI neurons are revealed. Moreover, *daf-12(rh61rh411)* failed to suppress the *stra-6* expression defect in the ASH neurons of *daf-7* mutants (Table 4), suggesting that the requirement for TGF-β signaling in *stra-6* regulation in the ASH neurons is independent of its role in dauer formation. These results imply that the TGF-β pathway may function through or in parallel to *daf-12* to regulate chemoreceptor gene expression in the ASI but not in the ASH neurons, thus revealing cell-specific mechanisms of chemoreceptor gene regulation.

### Table 3. Summary of expression patterns of chemoreceptor genes

| Receptor | Neuron | GFP expression under various conditions |
|----------|--------|----------------------------------------|
|          |        | WT adults | *daf-4* adults | WT dauer | *daf-4* dauer | + pheromone |
| *stra-6* | ASH    | ON        | OFF | ON | ON | ON |
| *stra-6* | ASI    | ON        | OFF | ON | OFF | OFF |
| *str-2*  | ASI    | weak/OFF  | ON | ON | ON | OFF |
| *str-3*  | ASI    | ON        | OFF | ON | OFF | OFF |
| *srd-1*  | ASI    | ON        | OFF | OFF | OFF | OFF* |

*Data from Peckol et al. 2001.

**DAF-4 function is required both early and late in development to regulate chemoreceptor gene expression**

daf-7 mRNA levels have been shown to peak in the L1 larval stage, and are down-regulated in subsequent stages [Ren et al. 1996]. Because TGF-β pathway mutants exhibit a number of adult phenotypes, it is possible that TGF-β signaling early in development is sufficient to regulate adult stage-specific characteristics. Alternatively, TGF-β signaling may be required at multiple developmental stages, including the adult stage. To distinguish between these possibilities, we examined the temporal requirement for DAF-4 type II receptor function in the regulation of *stra-6* expression.

Animals carrying the *daf-4(m592)* mutation are temperature-sensitive: When grown at the permissive temperature of 15°C, they exhibit wild-type body size and male tail morphology [Baird and Ellazar 1999]. To determine when DAF-4 function is required for receptor gene regulation, we grew *daf-4(m592)* animals expressing the *stra-6::gfp* fusion gene at 15°C or 25°C, and shifted animals between these temperatures at different developmental stages. Adult animals were then examined for their *stra-6* expression pattern 24 h after the final molt. Animals shifted from the permissive to restrictive temperature prior to late L2 stages exhibited a mutant *stra-6* phenotype as adults [Fig. 3A]. However, animals shifted at late L2 stages or later exhibited the wild-type pattern of *stra-6* expression. In converse downshift experiments, we found that animals shifted to the permissive temperature prior to the L3 stage exhibited the wild-type expression pattern as adults [Fig. 3B]. These experiments suggest that DAF-4 function is required during the late L2/early L3 stage to regulate *stra-6* expression.

To examine whether DAF-4 function is also required in the adult to maintain receptor gene expression, we
temperature shifted daf-4(m592) mutant adult animals for various lengths of time and examined sra-6 expression. When daf-4(m592) mutant animals were grown at 15°C until 24 h after the final molt, and then shifted to 25°C for 72 h, we observed reduced levels of GFP expression with further reduction in expression after 96 h (Fig. 3C). Because GFP perdure (Li et al. 1998; Corish and Tyler-Smith 1999), it is possible that endogenous sra-6...

Table 4. daf-3 suppresses the expression defects of chemoreceptor genes in daf-7 mutants

| Receptor | Neuron | daf-7b | daf-3b | daf-12b | daf-2b | daf-7b; daf-3b | daf-7b; daf-12b,c |
|----------|--------|--------|--------|---------|--------|---------------|------------------|
| sra-6    | ASH    | 0      | 100    | 100     | 99     | 100           | 6                |
| sra-6    | ASI    | 0      | 100    | 0       | 99     | 100           | 0                |
| str-2    | ASI    | 0      | 100    | 0       | 95     | 100           | 45d              |

n > 95 for each.

Wild-type sra-6 expression is defined as GFP expression in two ASH or ASI neurons. Wild-type str-2 expression is defined as weak or undetectable GFP expression in both ASI neurons.

Alleles used were daf-7(el372), daf-3(mgDf90), daf-12(rh61rh411), and daf-2(el370).

*daf-12(rh61rh411) fully suppressed the Daf-c phenotype, but not the small body size or Egl phenotypes of daf-7 mutants (data not shown).

The intensity of GFP expression and the number of animals expressing GFP in the ASI neurons was variable both within and between independent experiments.

Figure 2. daf-3(mgDf90) fails to suppress the pheromone-mediated alteration of chemoreceptor expression in the ASI neurons. Shown are the percentages of wild-type or daf-3(mgDf90) animals expressing sra-6 [A], str-3 [B], or str-2 [C] in both ASI neurons on exposure to 0 µL (solid bars), 10 µL (shaded bars), or 20 µL (hatched bars) of pheromone. str-2 is expressed weakly in the ASI neurons and is further reduced on pheromone addition. Pheromone did not induce dauer formation at these concentrations. Larval-stage animals were examined 24 h after hatching from eggs laid on plates containing the indicated concentrations of pheromone. Expression in adult animals examined 48 h after hatching on pheromone were also similar in wild-type and daf-3 mutants (data not shown). Data shown are the mean of at least three independent experiments, and error bars represent S.E.M.; n > 50 for each column.
expression is reduced earlier on temperature shift from 15°C to 25°C. However, complete restoration of GFP expression to wild-type levels was not observed in animals shifted as adults from 25°C to 15°C, even after 96 h. Wild-type and daf-4(m63) mutant animals grown continuously at either 15°C or 25°C retained their expected expression patterns. These results indicate that DAF-4 function is required both before and during the adult stage to maintain sra-6 expression.

The ASI neurons are required in the larval but not in the adult stages to regulate sra-6 expression

Because neither daf-7 mRNA nor an ASI-expressed DAF-7::GFP fusion protein is detected in adult animals [Ren et al. 1996, Schackwitz et al. 1996], it is possible that DAF-4 may be activated by a different TGF-β ligand or via a novel mechanism in adults. To determine whether the ASI neurons are also the source of a DAF-4 ligand in adult animals, we killed both the ASI neurons in L1 larvae and adult wild-type animals and examined sra-6::gfp expression in the ASH neurons. Killing the ASI neurons in L1 larvae resulted in the expected loss of sra-6 expression in the ASH neurons of young adult animals [Table 5], whereas killing both the ASI neurons in adult animals did not significantly affect sra-6 expression in the ASH neurons, even after 4 d. These results suggest that DAF-4-mediated maintenance of sra-6 expression in adult animals may require a ligand secreted from a cell[s] in addition to or other than the ASI neurons. However, we are unable to exclude the possibility that ablation of the ASI nuclei in adult animals does not lead to a complete elimination of ASI function [Chalfie and Sulston 1981].

Figure 3. DAF-4 function is required in the larval and adult stages for sra-6 expression. (A,B) daf-4(m592); oyIs14 animals were raised at 15°C [A] or 25°C [B] and shifted to the restrictive [25°C] or permissive [15°C] temperatures, respectively, at 12-h intervals. The developmental stage at the time of shift is indicated. The sra-6 expression pattern was examined in young adult animals 24 h after the L4/adult transition. Wild-type expression was defined as expression in at least three neurons in the head. Data shown are from at least three independent experiments. A total of >95 animals were examined at each point for the upshift experiments [A]; >70 animals were examined per time point for the downshift experiments [B]. (C) daf-4(m592); oyIs14 animals were grown at either the permissive or restrictive temperatures until the adult stage. Adult animals were then temperature-shifted for the indicated times, and the sra-6 expression pattern was examined. Wild-type expression was defined as above. Data shown are from at least two independent experiments with >70 animals examined at each time point.
The DAF-4 type II TGF-β receptor acts cell autonomously to regulate chemosensory receptor gene expression and non-cell-autonomously to regulate dauer formation

In contrast to the restricted expression pattern of daf-7, the daf-1 and daf-4 receptor genes and the daf-14 SMAD gene are expressed broadly in many tissue types, including the nervous system [Georgi et al. 1990; Estevez et al. 1993; Inoue and Thomas 2000]. DAF-4 functions non-cell-autonomously in the nervous system to regulate dauer formation [Inoue and Thomas 2000], but specific sites of action within the nervous system have not been identified.

To investigate where DAF-4 acts to regulate chemosensory receptor expression, we expressed a daf-4 cDNA by using the odr-4 and osm-10 promoters and determined whether sra-6 and str-2 expression was rescued in daf-4 mutants. The odr-4 promoter drives expression primarily in 10 pairs of chemosensory neurons in the head and two pairs of chemosensory neurons in the tail [Dwyer et al. 1998], whereas the osm-10 promoter drives expression strongly in the ASH and weakly in the ASI neurons, as well as in the two phasmid neurons in the tail [Hart et al. 1999]. We found that odr-4::daf-4 expression was sufficient to fully rescue the sra-6 and weakly rescue the str-2 expression defects (Table 6). However, expression of daf-4 under the myo-3 promoter, which drives expression in body wall muscles [Hsieh et al. 1999], did not restore sra-6 expression. The Daf-c phenotype of daf-4 mutants was also weakly rescued by expression of daf-4 under the odr-4 promoter (Table 6), suggesting that DAF-4 may act partly in the chemosensory neurons to regulate dauer formation.

Expression of daf-4 under the osm-10 promoter also rescued the sra-6 gene expression defects in the ASH neurons, whereas sra-6 expression defects in the ASI neurons were only weakly rescued (Table 6). Although these results are likely due to the stronger expression driven by the osm-10 promoter in the ASH compared with the ASI neurons [Hart et al. 1999], it is also possible that the low levels of daf-4 expression in the ASI neurons in these animals is sufficient to regulate sra-6 expression in the ASH neurons. To address this possibility, we killed the ASI neurons in adult daf-4 transgenic animals expressing osm-10::daf-4 and examined sra-6 expression in the ASH neurons. We found that killing both the ASI neurons did not significantly affect maintenance of sra-6 expression in the ASH neurons (Table 6), suggesting that DAF-4 functions cell-autonomously to regulate sra-6 expression. In similar experiments, we found that the DAF-3 SMAD protein may also act cell-autonomously to antagonize the daf-7 TGF-β pathway in chemoreceptor gene regulation and non-cell-autonomously in the nervous system to regulate dauer formation [Supplementary Table 1].

Discussion

Multiple mechanisms regulate the expression of individual chemoreceptor genes

We have shown that the DAF-7 TGF-β pathway regulates the expression of all known chemoreceptor genes in

### Table 6. daf-4 acts cell-autonomously to regulate chemoreceptor gene expression and non–cell-autonomously to regulate dauer formation

| Strain | % animals expressing<sup>a</sup> | % dauers formed<sup>b</sup> |
|--------|-------------------------------|-----------------------------|
|        | sra-6::gfp | str-2::gfp |        |        |
| WT     | 100 | 0 | 0 |        |
| daf-4(m63) | 4<sup>c</sup> | 100 | 100 |        |
| daf-4(m63); Ex[odr-4::daf-4] | 93<sup>d</sup> | 23 | 76 |        |
| daf-4(m63); Ex[osm-10::daf-4] | 91<sup*e</sup> | — | 85 |        |
| daf-4(m63); Ex[myo-3::daf-4] | 3 | — | — |        |
| daf-4(m63); Ex[osm-10::daf-4] | 81 | — | — |        |
| ASI killed (adults)<sup>†</sup> | 100 | — | — |        |
| mock ablated (adults)<sup>†</sup> | 100 | — | — |        |

All strains contain the pRF4 coinjection marker with the exception of ASI-killed and mock-ablated animals which contain the unc-122::gfp coinjection marker [Miyabayashi et al. 1999]. Data shown are from two independent lines, each of which showed similar levels of rescue. n > 95 for each.

<sup>a</sup>Expression was scored as sra-6::gfp expression in one to four neurons in the head. In ASI-killed animals, expression was scored as expression in both ASH neurons. Expression was scored as weak str-2::gfp expression in one or two ASI neurons.

<sup>b</sup>Dauer formation was examined at 25.5°C as described previously [Lanjuin and Sengupta 2002].

<sup>†</sup>3.1% of animals expressed GFP in one cell, and 1% expressed it in two cells.

<sup>c</sup>2% expressed GFP in one cell, 33% expressed GFP in two cells, 11% expressed GFP in three cells, and 45% expressed GFP in four cells.

<sup>d</sup>10% expressed GFP in one cell, 52% expressed GFP in two cells, 12% expressed GFP in three cells, and 17% expressed GFP in four cells. In animals in which GFP was expressed in two cells, both cells were identified as ASH by dye-filling (100%, n = 31).

<sup>e</sup>Data shown for ASI-killed animals are from one line; n = 11 for ASI-killed animals; n = 22 for mock ablated animals. ASI neurons were killed in young adult animals, and GFP expression was scored after 4 d at 25°C.
the ASI neurons and of the sra-6 chemoreceptor gene in the ASH neurons, as determined by examining changes in the expression of receptor ::gfp transgenes. Chemosensory neurons such as ASI and ASH are born embryonically [Sulston et al. 1983], and the expression of chemosensory signal transduction genes can be detected by late embryonic stages [Troemel et al. 1999; P. Sengupta, unpubl.]. Transcription factors required for the developmental specification of individual sensory neuron types have been described [Sengupta et al. 1994; Baran et al. 1999; Sagasti et al. 1999; Sarafi-Reinach and Sengupta 2000; Pierce-Shimomura et al. 2001; Sarafi-Reinach et al. 2001; Satterlee et al. 2001]. Mutations in these factors result in the altered expression of most, if not all, sensory neuron-specific genes encoding chemoreceptors, channels, and guanylyl cyclases, resulting in strong behavioral phenotypes [Sengupta et al. 1994; Baran et al. 1999; Sagasti et al. 1999; Satterlee et al. 2001]. In contrast, daf-7 signaling pathway mutants exhibit wild-type responses to known odors, suggesting that although the expression of a subset of chemoreceptors is altered, the expression of other receptors and downstream signal transduction components is largely unaffected. In previous work, we described the Ser/Thr kinase gene kin-29, mutations in which also result in altered expression of a partly overlapping group of chemoreceptors, but not of additional signaling components [Lanjuin and Sengupta 2002]. These results imply that in addition to developmentally hard-wired mechanisms, individual chemoreceptor genes are subject to additional modes of regulation.

Figure 4. Model for regulation of chemoreceptor expression. (A) In the ASI neurons, DAF-7 TGF-β signaling maintains the expression of sra-6, promotes the expression of str-3 and srd-1, and represses expression of str-2. High levels of dauer pheromone repress daf-7 expression and promote dauer entry. Sub-dauer-inducing concentrations of pheromone [indicated by the dashed line] repress the expression of sra-6, str-3, str-2, and presumably srd-1 (in box) via a DAF-7-independent pathway. In addition, the DAF-12 nuclear hormone receptor is required to promote sra-6 expression and to repress str-2 expression in the ASI neurons. srd-1 expression also requires neuronal activity [Peckol et al. 2001]. (B) In the ASH neurons, DAF-7 signaling via the DAF-3 inhibitory SMAD but not DAF-12 maintains sra-6 expression.

The expression of chemosensory receptors in the ASI neurons in particular appears to be subject to multiple modes of regulation [Fig. 4]. Non-dauer-inducing levels of pheromone repress the expression of all ASI-expressed receptors [Peckol et al. 2001, the present study]. In addition, srd-1 expression in the ASI neurons requires neuronal activity [Peckol et al. 2001]. In addition to these regulatory mechanisms, we find that TGF-β signaling regulates receptor expression in the ASI neurons. DAF-7 signaling is required to promote the expression of str-3, sra-6, and srd-1 and to repress the expression of str-2 in the ASI neurons. We suggest that during reproductive growth in wild-type animals, the balance of DAF-7 signaling and levels of pheromone regulates receptor expression in the ASI neurons. Very low levels of pheromone may be sufficient to down-regulate str-2 expression in the ASI neurons, without affecting the expression of additional receptors [Peckol et al. 2001]. Higher, yet non-dauer-inducing levels of pheromone repress the expression of all ASI-expressed receptors via a DAF-7-independent pathway. It is interesting to note that the pheromone-mediated repression of ASI-expressed chemoreceptor genes has been observed at all developmental stages [Peckol et al. 2001; the present study]. Moreover, pheromone has also been shown to modulate the responses of adult animals to volatile anesthetics via a DAF-7-independent pathway [Van Swinderen et al. 2002].

On entry into the dauer stage, withdrawal of the ASI cilia from the amphid pore results in derepression from the effects of pheromone, resulting in restoration of expression of str-2, str-3, and sra-6. However, lack of neuronal activity prevents expression of srd-1 [Peckol et al. 2001]. DAF-7 signaling may also be required to regulate receptor expression in the ASI neurons during the dauer stage. Alternatively, altered chemoreceptor expression in the dauer stage in TGF-β pathway mutants may simply reflect a failure to activate or repress receptor expression in earlier larval stages. Thus, at least three pathways of pheromone, TGF-β signaling, and neuronal activity regulate the expression of individual chemoreceptors in the ASI neurons. Curiously, to date, only ASI-expressed receptors have been shown to be regulated by pheromone, suggesting that modulation of ASI function by environmental conditions may be critical for behavioral or developmental plasticity.

Although sra-6 expression in the ASH neurons is also regulated by the daf-7 pathway, receptor expression in the ASI and ASH neurons appear to be regulated by distinct mechanisms [Fig. 4; Table 3]. First, sra-6 expression in the ASH neurons is unaffected by pheromone. Second, although the expression of both sra-6 and str-2 in the ASI neurons is also regulated by daf-12, sra-6 expression in the ASH neurons is unaffected in daf-12 null mutants. Third, TGF-β signaling appears to be required for sra-6 expression in the ASI, but not the ASH, neurons in dauer animals. The differential regulation of receptor genes in individual neuron types under different environmental and developmental conditions may allow animals to more precisely modulate their sensory behaviors.
The DAF-4-mediated TGF-β pathway functions in adult animals to maintain sra-6 expression

We have defined a new role for the daf-7 TGF-β pathway in maintaining sra-6 expression in adult animals. Mutations in components of this signaling pathway result in adult-specific phenotypes, including increased social behavior and defective egg-laying (Trent et al. 1983; Thomas et al. 1993). Although these phenotypes could result solely from a lack of TGF-β signaling in larval stages, we have shown that in addition to being required during the late L2/early L3 stage, the function of the DAF-4 type II TGF-β receptor is also required in the adult to maintain sra-6 expression in the ASI and ASH neurons. Because this regulation occurs at developmental stages after the stage at which animals make the decision to form dauer larvae, this result indicates that the regulation of receptor expression by the daf-7 pathway is independent of the function of this pathway in dauer formation. This hypothesis is further supported by our finding that mutations in daf-12 do not suppress the ASH-specific sra-6 expression defects of daf-7 pathway mutants.

The finding that DAF-4 signaling is required in adults is unexpected because it has been previously shown that daf-7 mRNA levels peak in the L1 larval stage and are severely reduced in the dauer and later developmental stages (Ren et al. 1996). A daf-7::gfp fusion gene appears to be expressed primarily in the ASI neurons in larvae (Ren et al. 1996; Schackwitz et al. 1996), and we have shown that killing the ASI neurons in L1 animals results in a loss of sra-6 expression in the ASH neurons in adults. However, the ASI neurons appear not to be the sole source of ligand for maintenance of sra-6 expression in adult animals. It is possible that daf-7 is expressed at low levels in the adult in additional neurons. Alternatively, DAF-4 could be activated by a distinct TGF-β ligand. The DBL-1 TGF-β ligand also acts through DAF-4 to regulate body size (Suzuki et al. 1999). However, mutations in dbi-1 or unc-129 do not affect chemoreceptor expression. At least one additional TGF-β-like ligand is predicted to be encoded by the C. elegans genome (Ruskun and Hobert 1998). It is possible that DAF-4 could be activated by other ligands to regulate receptor expression in the adult.

Regulation of chemosensory receptor expression may contribute to sensory plasticity

The combinatorial control of chemoreceptor gene expression by multiple pathways may enable the expression of these genes to be finely adjusted as in a rheostat. Different subsets of receptors in particular neuron types may be regulated by different developmental and environmental signals. By integrating information acquired via distinct pathways to alter chemoreceptor gene expression, animals can exhibit the responses most optimal for development and survival. Other organisms also modulate chemosensory receptor expression in response to developmental or environmental cues. Drosophila exhibits distinct chemosensory responses and expresses different sets of chemosensory receptor genes in the larval and adult stages (Dubin et al. 1995; Shaver et al. 1998; Scott et al. 2001). Olfactory receptors are also expressed in a temporally regulated manner in zebrafish, Xenopus laevis, and Drosophila during development and may contribute to stage-specific sensory behaviors (Barth et al. 1996; Clyne et al. 1999; Mezler et al. 1999). The expression of an olfactory receptor has been shown to be down-regulated after a blood meal in the mosquito Anopheles gambiae and may be causal to their reduced responses to human odors during this period (Fox et al. 2001). Thus, modulation of receptor expression may be a general mechanism contributing to chemosensory plasticity.

Materials and methods

Strains

Worms were grown by using standard methods (Brenner 1974); all animals were grown at least one generation poststarvation at 20°C prior to analysis unless otherwise noted. Strains were obtained from the Caenorhabditis Genetics Center with the exception of daf-4(m592), which was kindly provided by D. Riddle (University of Missouri-Columbia, Columbia, MS; Baird and El-lazar 1999). Strains containing integrated transgenes were obtained as indicated previously (Lanjuin and Sengupta 2002). Double and triple mutant strains were constructed by using standard methods and confirmed by complementation tests or by sequencing. Details of strain construction are available on request.

Isolation of daf-8(oy8)

A strain carrying integrated copies of sra-6::gfp (oyIs14) was mutagenized with EMS by using standard protocols. oy8 was identified in a screen of ~13,000 haploid genomes for alterations in sra-6::gfp expression by using a dissection microscope equipped with epifluorescence. oy8 was mapped to LG I and fine-mapped with respect to deficiencies, genetic markers, and polymorphisms. oy8 fails to complement daf-8(e1393) for the sra-6 expression defect and the Daf-c phenotype.

Expression constructs and generation of transgenic animals

daf-4 expression constructs were generated by fusing odr-4 (Dwyer et al. 1998), osm-10 (Hart et al. 1999), or myo-3 (Hsieh et al. 1999) promoters to a daf-4 cDNA kindly provided by J. Thomas (University of Washington, Seattle, WA; Inoue and Thomas 2000). Amplified products were sequenced to confirm the absence of errors. Transgenic animals were generated by using the dominant co-injection markers pRF4 rol-6(su1006) or unc-122::gfp at 100 ng/μL. All other plasmids were injected at 30 ng/μL.

Laser ablations

ASI neurons in oyIs14 animals were identified by DI filling prior to ablation. Ablations were carried out using a Micropoint laser system (Photonic Instruments) essentially as previously described (Avery and Horvitz 1989). Successful killing of ASI was determined 24 h postablation by confirming loss of oyIs14 expression in the ASI neurons. In daf-4(m63), oyIs14 [Ex osm-10::daf-4, unc-122::gfp] animals, the ASI neurons were killed
only in those transgenic animals in which the expression of oyIs14 was restored in both the ASH and ASI neurons. All phenotypes were scored 4 d postablation at 25°C. Mock controls were treated identically to ablated animals, except that no ablations were performed.

**Dauer assays**

Adults were allowed to lay ∼50 to 75 eggs/6-cm worm growth plate at room temperature. Parents were then removed, and the plates were shifted to 25.5°C. The number of dauer and non-dauer animals was counted 48 h later. Data presented in tables are from a single experiment, with all strains assayed in parallel. Experiments were repeated a minimum of three times on independent days, and the data showed similar relative differences. For pheromone exposure experiments, animals were allowed to lay eggs for 2 h at room temperature on dauer agar plates containing pheromone (Vowels and Thomas 1994), parents were removed, and the plates were placed at 25.5°C. GFP expression was scored in larvae 24 h later and in young adults after 48 h. No dauers were formed under these conditions; however, 38% to 48% of kin-29(oy39) mutants formed dauers in the presence of 10 or 20 µL pheromone (Lanjuin and Sengupta 2002).

**Temperature-shift experiments**

daf-4(m592), oyIs14 were grown for at least one generation at either 15°C or 25°C prior to the experiment. Adults were allowed to lay eggs for 2–4 h at room temperature, the parents were removed, and the plates were placed at either 15°C or 25°C. Plates were then shifted to the restrictive (25°C) or permissive (15°C) temperature every 12 h, and the stage of the animals at the time of shift was noted. GFP expression was scored 24 h after the L4/adult transition under 400× magnification. In the case of adult shifts, animals were raised at either 15°C or 25°C prior to the experiment. Adults were allowed to lay eggs for 2–4 h at room temperature, the parents were removed, and the plates were placed at 25°C. GFP expression was scored in larvae 24 h later and in young adults after 48 h. No dauers were formed under these conditions; however, 38% to 48% of kin-29(oy39) mutants formed dauers in the presence of 10 or 20 µL pheromone (Lanjuin and Sengupta 2002).

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**References**

Albert, P.S. and Riddle, D.L. 1983. Developmental alterations in sensory neuroanatomy of the Caenorhabditis elegans dauer larva. J. Comp. Neurol. 219: 461–481.

Albert, P.S., Brown, S.J., and Riddle, D.L. 1981. Sensory control of dauer larva formation in Caenorhabditis elegans. J. Comp. Neurol. 198: 435–451.

Avery, L. and Horvitz, H.R. 1989. Pharyngeal pumping continues after laser killing of the pharyngeal nervous system of C. elegans. Neuron 3: 473–485.

Bailey, C.H. and Chen, M. 1988. Long-term memory in Aplysia modulates the total number of varicosities of single identified sensory neurons. Proc. Natl. Acad. Sci. 85: 2373–2377.

Baird, S.E. and Elazar, S.A. 1999. TGF-β-like signaling and spicule development in Caenorhabditis elegans. Dev. Biol. 212: 93–100.

Baran, R., Aronoff, R., and Garriga, G. 1999. The C. elegans homedomain gene unc-42 regulates chemosensory and glutamate receptor expression. Development 126: 2241–2251.

Bargmann, C.I. and Mori, I. 1997. Chemotaxis and thermotaxis in C. elegans II (eds. D.S. Riddle et al.), pp. 717–737. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Barth, A.L., Justice, N.J., and Ngi, J. 1996. Asynchronous onset of odorant receptor expression in the developing zebrafish olfactory system. Neuron 16: 23–34.

Bernhard, N. and van der Kooy, D. 2000. A behavioral and genetic dissection of two forms of olfactory plasticity in Caenorhabditis elegans: Adaptation and habituation. Learn. Mem. 7: 199–212.

Brennan, P.A. and Keverne, E.B. 1997. Neural mechanisms of mammalian olfactory learning. Prog. Neurobiol. 51: 457–481.

Brenner, S. 1974. The genetics of Caenorhabditis elegans. Genetics 77: 71–94.

Chalfie, M. and Sulston, J. 1981. Developmental genetics of the mechanosensory neurons of Caenorhabditis elegans. Dev. Biol. 82: 358–370.

Chess, A., Simon, I., Cedar, H., and Axel, R. 1994. Allelic inactivation regulates olfactory receptor gene expression. Cell 78: 832–834.

Clyne, P.J., Warr, C.G., Freeman, M.R., Lessing, D., Kim, J., and Carlson, J.R. 1999. A novel family of divergent seven-transmembrane proteins: Candidate odorant receptors in Drosophila. Neuron 22: 327–338.

Coburn, C. and Bargmann, C.I. 1996. A putative cyclic nucleotide-gated channel is required for sensory development and function in C. elegans. Neuron 17: 695–706.

Colavita, A., Krishna, S., Zheng, H., Padgett, R.W., and Cullotta, J.G. 1998. Pioneer axon guidance by UNC-129, a C. elegans TGF-β. Science 281: 706–709.

Colbert, H.A. and Bargmann, C.I. 1995. Odorant-specific adaptation pathways generate olfactory plasticity in C. elegans. Neuron 14: 803–812.

Corish, P. and Tyler-Smith, C. 1999. Attenuation of green fluorescent protein half-life in mammalian cells. Protein Eng. 12: 1035–1040.

Dubin, A.E., Heald, N.L., Cleveland, B., Carlson, J.R., and Harris, G.L. 1995. Scutoid mutation of Drosophila melanogaster specifically decreases olfactory responses to short-chain carboxylic esters and ketones. J. Neurobiol. 28: 214–233.

Dwyer, N.D., Troemel, E.R., Sengupta, P., and Bargmann, C.I. 1998. Odorant receptor localization to olfactory cilia is me-
Hsieh, J., Liu, J., Kostas, S.A., Chang, C., Sternberg, P.W., and Herman, R.K., and Hedgecock, E.M. 1990. Limitation of the size of the vulval primordium of Caenorhabditis elegans by neuronal expression of a TGF-β homolog. Science 217: 192–204.

Kendrick, K.M., Levy, F., and Keverne, E.B. 1992. Changes in the sensory processing of olfactory signals induced by birth in sheep. Science 256: 833–836.

Komatsu, H., Mori, I., and Ohshima, Y. 1996. Mutations in a cyclic nucleotide-gated channel lead to abnormal thermosensation and chemosensation in C. elegans. Neuron 17: 707–718.

Lanjuin, A. and Sengupta, P. 2002. Regulation of chemosensory receptor expression and sensory signaling by the KIN-29 Ser/Thr kinase. Neuron 33: 369–381.

L’Etoile, N.D. and Bargmann, C.I. 2000. Olfaction and odor discrimination are mediated by the C. elegans guanylyl cyclase ODR-1. Neuron 25: 575–586.

Li, X., Zhao, X., Fang, Y., Jiang, X., Duong, T., Fan, C., Huang, C.C., and Kain, S.R. 1998. Generation of destabilized green fluorescent protein as a transcription reporter. J. Biol. Chem. 273: 34970–34975.

Malnic, B., Hiro, J., Sato, T., and Buck, L.B. 1999. Combinatorial receptor codes for odors. Cell 96: 713–723.

Malone, E.A. and Thomas, J.H. 1994. A screen for nonconditional dauer-constitutive mutations in Caenorhabditis elegans. Genetics 136: 879–886.

Mezler, M., Konzelmann, S., Freitag, J., Rossler, P., and Breer, H. 1999. Expression of olfactory receptors during development in Xenopus laevis. J. Exp. Biol. 202: 365–376.

Miyabayashi, T., Palfreyman, M.T., Sluder, A.E., Slack, F., and Sengupta, P. 1999. Expression and function of members of a divergent nuclear receptor family in Caenorhabditis elegans. Dev. Biol. 215: 314–331.

Morgan, S.M., Butz Huryn, V.M., Downes, S.R., and Mercer, A.R. 1998. The effects of queenlessness on the maturation of the honey bee olfactory system. Behav. Brain. Res. 91: 115–126.

Morrison, G.E., Wen, J.Y., Runciman, S., and van der Kooy, D. 1999. Olfactory associative learning in Caenorhabditis elegans is impaired in lrn-1 and lrn-2 mutants. Behav. Neurosci. 113: 358–367.

Peckol, E.L., Troemel, E.R., and Bargmann, C.I. 2001. Sensory experience and sensory activity regulate chemosensory receptor gene expression in C. elegans. Proc. Natl. Acad. Sci. 98: 11032–11038.

Plam, T.A., Impey, S., Storm, D.R., and Stryker, M.P. 1999. CRE-mediated gene transcription in neocortical neuronal plasticity during the developmental critical period. Neuron 22: 63–72.

Pierce, S.B., Costa, M., Wisotzkey, R., Devadhar, S., Homburger, S.A., Buchman, A.R., Ferguson, K.C., Heller, J., Platt, D.M., Pasquinelli, A.A., et al. 2001. Regulation of DAF-2 receptor signaling by human insulin and ins-1, a member of the unusually large and diverse C. elegans insulin gene family. Genes & Dev. 15: 672–686.

Pierce-Shimomura, J.T., Faumont, S., Gaston, M.R., Pearson, B.J., and Lockery, S.R. 2001. The homeobox gene daf-16 mediates dietary control in C. elegans. Proc. Natl. Acad. Sci. 98: 368–373.

Ren, P., Lim, C.S., Johnsen, R., Albert, P.S., Pilgrim, D., and Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. 1998. The Ga protein ODR-3 mediates olfactory and nociceptive signaling in Caenorhabditis elegans. Neuron 20: 55–67.

Riddle, D.L. and Albert, P.S. 1997. Genetic and environmental regulation of dauer larva development. In C. elegans II (eds. D.S. Riddle et al.), pp. 739–768. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Roayaie, K., Crump, J.G., Sagasti, A., and Bargmann, C.I. 1998. The Ga protein ODR-3 mediates olfactory and nociceptive function and controls cilium morphogenesis in C. elegans olfactory neurons. Neuron 20: 55–67.

Robertson, H.M. 1998. Two large families of chemoreceptor genes in the nematodes Caenorhabditis elegans and Caenorhabditis briggsae reveal extensive gene duplication, diversification, movement, and intron loss. Genome Res. 8: 449–463.

—. 2000. The large srh family of chemoreceptor genes in Caenorhabditis briggsae reveals processes of genome evolution involving large duplications and deletions and intron gains and losses. Genome Res. 10: 192–203.

Ruvkun, G. and Hobert, O. 1998. The taxonomy of developmental control in Caenorhabditis elegans. Science 282: 2033–
TGF-β signaling regulates chemoreceptor expression

C.M. 2002. A Caenorhabditis elegans pheromone antagonizes volatile anesthetic action through a Gα-coupled pathway. Genetics 161: 109–119.

Vossall, L.B., Amrein, H., Morozov, P.S., Rzhetsky, A., and Axel, R. 1999. A spatial map of olfactory receptor expression in the Drosophila antenna. Cell 96: 725–736.

Vowels, J.J. and Thomas, J.H. 1992. Genetic analysis of chemosensory control of dauer formation in Caenorhabditis elegans. Genetics 130: 303–316.

Wang, H.-W., Wysocki, C.J., and Gold, G.H. 1993. Induction of olfactory receptor sensitivity in mice. Science 260: 998–1000.

Wen, J.Y., Kumar, N., Morrison, G., Rambaldini, G., Runciman, S., Rousseau, J., and van der Kooy, D. 1997. Mutations that prevent associative learning in C. elegans. Behav. Neurosci. 111: 354–368.

Yin, J.C., Wallach, J.S., Del Vecchio, M., Wilder, E.L., Zhou, H., Quinn, W.G., and Tully, T. 1994. Induction of a dominant negative CREB transgene specifically blocks long-term memory in Drosophila. Cell 79: 49–58.

Zulall, F. and Leinders-Zulall, T. 2000. The cellular and molecular basis of odor adaptation. Chem. Senses 25: 473–481.
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