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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Environmental information is acquired by the peripheral sensory system and relayed to multiple circuits to mediate developmental and behavioral changes. The functions of these sensory pathways can be altered in response to changing environmental conditions or in specific life stages. Mechanisms of short-term sensory plasticity have been shown to include the reversible modification of signal transduction molecules in sensory neurons, whereas mechanisms of long-term plasticity involve changes in gene expression, leading to alterations in synaptic strengths (Bailey and Chen 1988; Yin et al. 1994; Finkbeiner and Greenberg 1998; Impey et al. 1998; Pham et al. 1999; Zufall and Leinders-Zufall 2000; Fain et al. 2001). However, for maximum efficiency, it is likely that different sensory modalities use distinct mechanisms to effect sensory plasticity.

Animals sense chemicals in the environment by using their olfactory and gustatory sensory systems. Although olfactory plasticity regulated by environmental conditions and developmental cues has been described in both insects and vertebrates (Kendrick et al. 1992; Wang et al. 1993; Dubin et al. 1995; Brennan and Keverne 1997; Morgan et al. 1998; Shaver et al. 1998), the underlying molecular mechanisms have not been fully defined. The nematode *Caenorhabditis elegans* provides an excellent model system in which to study the mechanisms by which olfactory behaviors are modulated. Nematodes exhibit robust and sensitive olfactory responses to multiple chemicals using a small and well-defined number of chemosensory neurons (Bargmann and Mori 1997; Troemel 1999b). Chemosensory responses are altered under environmental conditions of overcrowding or starvation (Colbert and Bargmann 1997), long-term exposure to a chemical (Colbert and Bargmann 1995; Bernhard and van der Kooy 2000; L'Etoile and Bargmann 2000), or pairing of a normally attractive chemical with aversive stimuli (Wen et al. 1997; Morrison et al. 1999). Moreover, sensory responses are dramatically changed in specific developmental stages. Under favorable growth conditions in early larval stages, the DAF-7 TGF-β (transforming growth factor-β) ligand and insulin-like peptides are secreted by the ASI chemosensory and other unidentified neurons, respectively (Ren et al. 1996; Schackwitz et al. 1996; Pierce et al. 2001). DAF-7 binds the widely expressed DAF-1 and DAF-4 type I and type II TGF-β receptors to promote reproductive growth (Georgi et al. 1990; Estevez et al. 1993). However, under adverse environmental conditions, such as high concentrations of a constitutively secreted pheromone, *daf-7* expression is

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reduced, and down-regulation of TGF-β and insulin signaling triggers entry into the alternative third larval dauer stage (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).

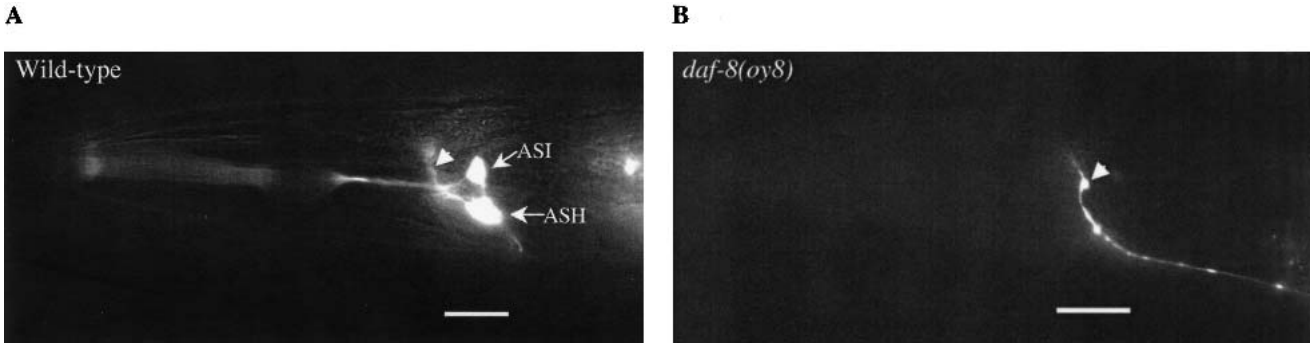

Figure 1. Expression of an *sra-6*::*gfp* transgene is reduced in the ASH and ASI sensory neurons of *daf-8(oy8)* mutants. (*A*) *sra-6*::*gfp* is expressed in the ASH and ASI sensory neurons in the head of an adult wild-type animal. This transgene also drives expression in the PVQ interneurons whose 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.

sra-6 expression was affected similarly in an additional *daf-8* allele (Table 1).

Because DAF-8 functions in the DAF-7 TGF-β-mediated signaling pathway (Malone and Thomas 1994), we determined whether additional components of this pathway are also required for *sra-6* expression. Mutations in the *daf-7* TGF-β ligand (Ren et al. 1996; Schackwitz et al. 1996), the *daf-1* type I TGF-β receptor (Georgi et al. 1990), the *daf-4* type II TGF-β receptor (Estevez et al. 1993), and the *daf-14* SMAD (Inoue and Thomas 2000) genes also abolished *sra-6* expression in the ASH and ASI neurons but not in the PVQ neurons (Table 1). *daf-8* and *daf-14* have been shown to act partly redundantly to regulate multiple processes (Inoue and Thomas 2000). Consistent with this, we found that although only 27% and 73% of *daf-8* and *daf-14* single mutants, respectively, showed a complete loss of *sra-6* expression in the head (Table 1), 100% of *daf-8*; *daf-14* double mutants failed to express *sra-6* in both the ASH and ASI neurons (n = 71). Interestingly, in all cases, expression of *sra-6* was unaffected in L1 and early L2 stages, indicating that TGF-β signaling is required for maintenance but not for initiation of *sra-6* expression (Table 1).

The *C. elegans* genome is predicted to encode >600 chemosensory receptors of which the functions of only one have been defined (Troemel et al. 1995; Sengupta et al. 1996; Robertson 1998, 2000). The expression patterns of a fraction of these genes have been examined to date and shown to be expressed in small sets of chemosensory neurons (Troemel et al. 1995, 1997, 1999; Sengupta et al. 1996; Troemel 1999a; Peckol et al. 2001). To determine whether TGF-β signaling is required for the expression of additional chemoreceptor genes, we examined the expression of receptor::*gfp* transgenes in *daf-7* TGF-β pathway mutants. Expression of three chemoreceptor genes was dramatically altered in the ASI chemosensory neurons, whereas the expression of additional receptors was largely unaffected or only weakly altered in other neuron types (Table 1). In wild-type animals cultured under favorable growth conditions, *str-3* and *srd-1*::*gfp* fusion genes are expressed strongly in the ASI neurons (Peckol et al. 2001), whereas *str-2*::*gfp* is expressed strongly in one of the two AWC olfactory neurons and weakly in the two ASI neurons (Troemel et al. 1999; Peckol et al. 2001). In TGF-β pathway mutants, expression of both *str-3* and *srd-1* was abolished, whereas expression of *str-2* was strongly up-regulated in the ASI neurons. Expression of *str-2* in the AWC neurons was not significantly affected (data not shown). Unlike *sra-6* expression, the effects on *str-3*, *srd-1*, and *str-2* expression were observed at all stages of postembryonic development. Consistent with previous reports (Vowels and Thomas 1994), we did not detect any gross defects in the ability of *daf-7(e1372)* or *daf-4(m63)* mutants to respond to multiple sensory stimuli, including a panel of volatile attractants and repellents (data not shown).

Two additional TGF-β pathways are known to function in *C. elegans*. The DBL-1 pathway regulates body size (Suzuki et al. 1999), whereas the UNC-129 TGF-β ligand is required for axon guidance and cell migration (Colavita et al. 1998). Mutations in *dbl-1* or *unc-129* did not affect chemosensory receptor gene expression (data not shown). These results indicate that DAF-7 but not UNC-129 or DBL-1 TGF-β signaling is required for the

Table 1. *Expression of a subset of chemoreceptor genes is altered in DAF-7 TGF-β pathway mutants*

		% animals expressing GFP					
Receptor[a]	Neuron	Wild type	*daf-7*[b]	*daf-1*[b]	*daf-4*[b]	*daf-8*[b]	*daf-14*[b]
sra-6 larvae[c]	ASH	100	100	—	100	100	100
sra-6	ASH/ASI	100[d]	1[d]	12[d]	1[d]	73[d]	24[d]
str-2	ASI[e]	0	100	100	100	59	95
str-3	ASI	100	0	1	0	0	0
srd-1	ASI	99	0	14	0	—	—
str-1	AWB	100	100	—	100	100	100
odr-10	AWA	100	100	—	—	100	—
sre-1	ADL	98	100	97	100	—	—
srg-8	ASK	87	98	—	99	—	—
T08G3.3	ADF	100	100	—	—	100	100
srg-13	PHA	97	70	—	80	—	—

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

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

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

[c]L1/early L2 larvae were examined. All other animals examined were young adults.

[d]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.

[e]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 *sra-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, *sra-6* expression was abolished in the ASI, but not in the ASH 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. *Chemoreceptor genes are differentially expressed in wild-type and* daf-4 *dauers*

		% dauers expressing GFP	
Receptor[a]	Neuron	Wild type	*daf-4*[b]
sra-6	ASH	100	98
sra-6	ASI	97	2
str-2	ASI	100	77
str-3	ASI	100	2
srd-1	ASI	10	6

n > 65 in each case.
[a]Integrated receptor::*gfp* fusion genes were examined. Expression was scored as expression in one or both neurons.
[b]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(mgDf90)* 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 *sra-6* expression in the ASI but not the ASH 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.

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

Receptor	Neuron	GFP expression under various conditions				
		WT adults	*daf-4* adults	WT dauers	*daf-4* dauers	+ pheromone
sra-6	ASH	ON	OFF	ON	ON	ON
sra-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[a]

[a]Data from Peckol et al. 2001.

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 *sra-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 *sra-6* expression defect in the ASH neurons of *daf-7* mutants (Table 4), suggesting that the requirement for TGF-β signaling in *sra-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.

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 *sra-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 *sra-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 *sra-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 *sra-6* phenotype as adults (Fig. 3A). However, animals shifted at late L2 stages or later exhibited the wild-type pattern of *sra-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 *sra-6* expression.

To examine whether DAF-4 function is also required in the adult to maintain receptor gene expression, we

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

		% mutants expressing the wild-type pattern[a]					
Receptor	Neuron	*daf-7*[b]	*daf-3*[b]	*daf-12*[b]	*daf-2*[b]	*daf-7; daf-3*[b]	*daf-7; daf-12*[b,c]
sra-6	ASH	0	100	100	99	100	6
	ASI	0	100	0	99	100	0
str-2	ASI	0	100	0	95	100	45[d]

n > 95 for each.
[a]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.
[b]Alleles used were *daf-7(el372), daf-3(mgDf90), daf-12(rh61rh411),* and *daf-2(el370).*
[c]*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).
[d]The intensity of GFP expression and the number of animals expressing GFP in the ASI neurons was variable both within and between independent experiments.

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 perdures (Li et al. 1998; Corish and Tyler-Smith 1999), it is possible that endogenous *sra-6*

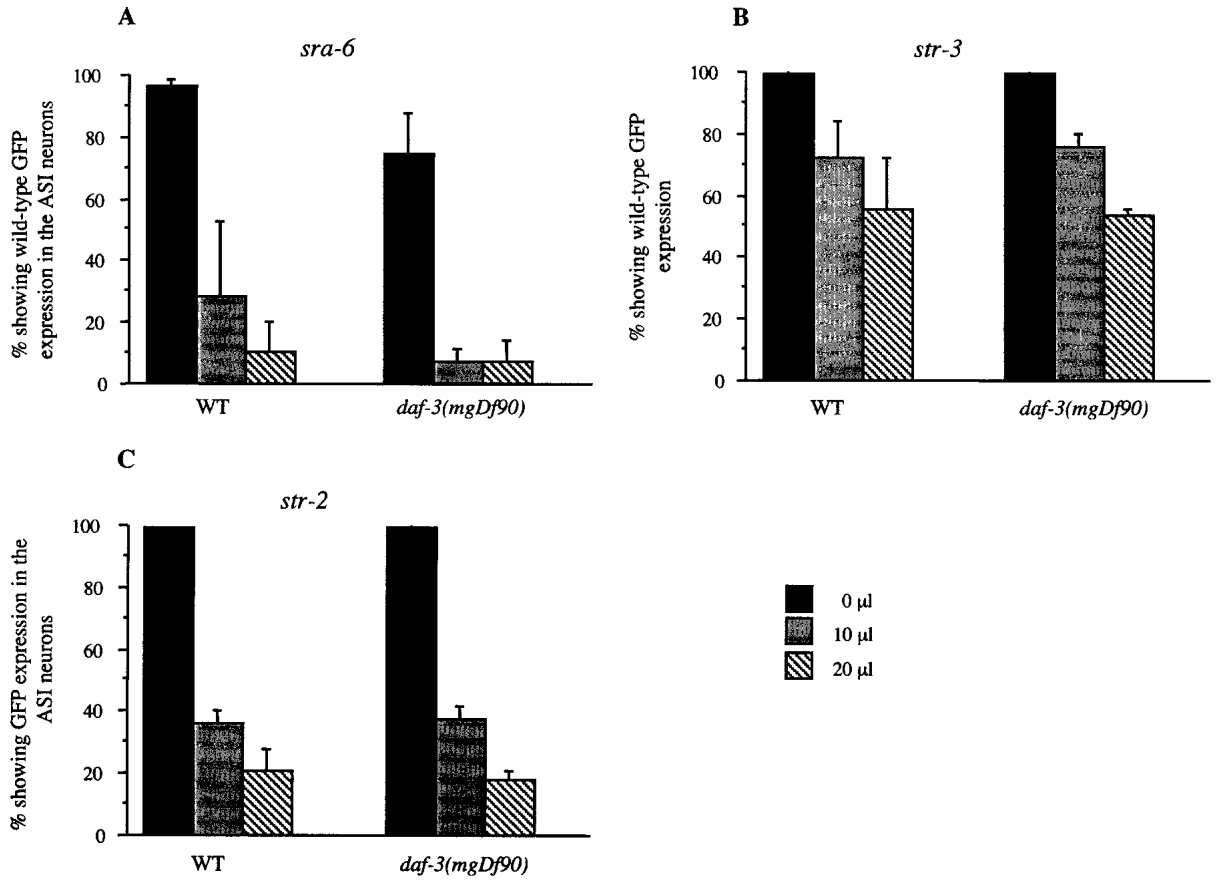

Figure 2. *daf-3(mgDf90)* fails to suppress the pheromone-mediated alteration of chemoreceptor expression in the ASI neurons. Shown are the percentages of wild-type or *daf-3(mgDf90)* animals expressing *sra-6* (*A*), *str-3* (*B*), or *str-2* (*C*) in both ASI neurons on exposure to 0 µL (solid bars), 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.

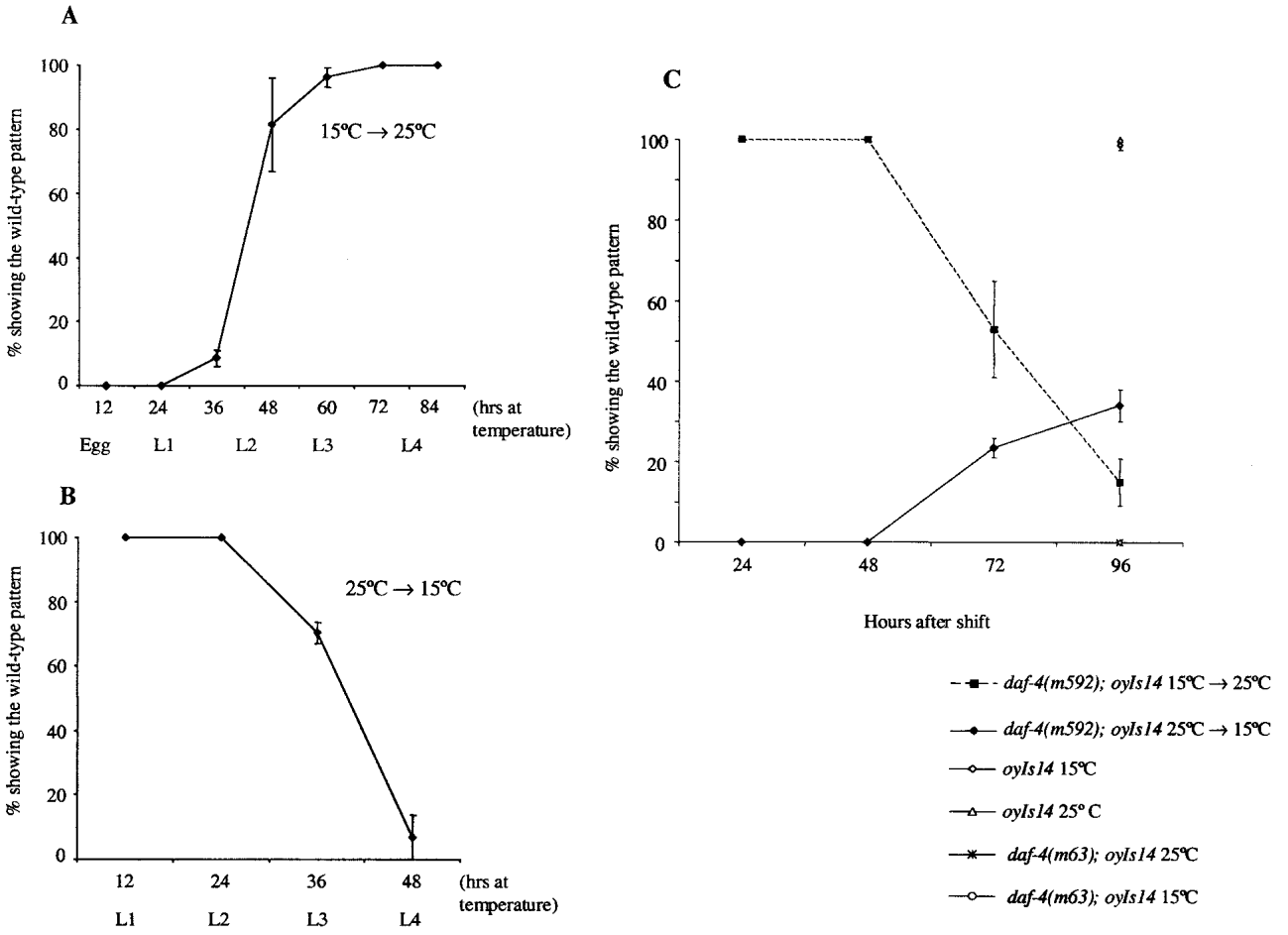

Figure 3. DAF-4 function is required in the larval and adult stages for *sra-6* expression. (*A*,*B*) *daf-4(m592); oyIs14* animals were raised at 15°C (*A*) or 25°C (*B*) and shifted to the restrictive (25°C) or permissive (15°C) temperatures, respectively, at 12-h intervals. The developmental stage 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.

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).

Table 5. *The ASI neurons are required in larval but not in adult stages to maintain* sra-6 *gene expression in the ASH neurons*

Developmental stage of ASI ablation	% expressing *sra-6::gfp* in the ASH neurons (n)[a]
L1	6.6 (15)
L1 mock ablation	100 (25)
Young adult	81 (16)
Young adult mock ablation	100 (23)

All experiments were carried out in wild-type animals carrying integrated copies of the *sra-6::gfp (oyIs14)* fusion gene. Animals were grown at 25°C postablation.
GFP expression in the ASH neurons was examined 4 d postablation.
[a]n = number of animals in which both ASI neurons were killed.

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[a] *sra-6::gfp*	% animals expressing[a] *str-2::gfp*	% dauers formed[b]
WT	100	0	0
daf-4(m63)	4[c]	100	100
daf-4(m63); Ex[*odr-4::daf-4*]	93[d]	23	76
daf-4(m63); Ex[*osm-10::daf-4*]	91[e]	—	85
daf-4(m63); Ex[*myo-3::daf-4*]	3	—	—
daf-4(m63); Ex[*osm-10::daf-4*] ASI killed (adults)[f]	81	—	—
daf-4(m63); Ex[*osm-10::daf-4*] mock ablated (adults)[f]	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.
— indicates not done.
[a]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.
[b]Dauer formation was examined at 25.5°C as described previously (Lanjuin and Sengupta 2002).
[c]3.1% of animals expressed GFP in one cell, and 1% expressed it in two cells.
[d]2% expressed GFP in one cell, 32% expressed GFP in two cells, 14% expressed GFP in three cells, and 45% expressed GFP in four cells.
[e]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).
[f]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; Pierce-Shimomura et al. 2001; Satterlee et al. 2001). In contrast, *daf-7* signaling pathway mutants exhibit wild-type responses to known odorants, 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.

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

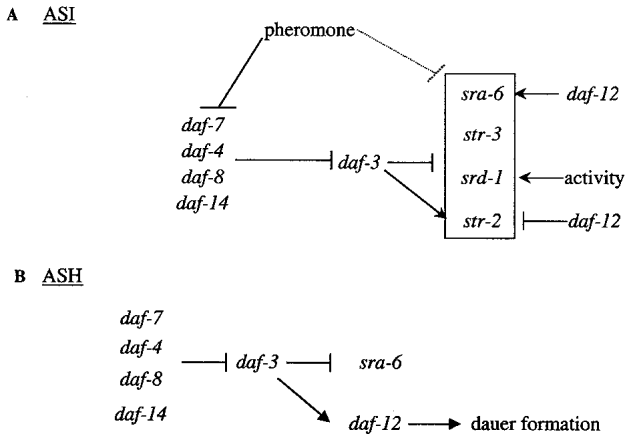

Figure 4. Model for regulation of chemoreceptor expression. (*A*) In the ASI neurons, DAF-7 TGF-β signaling maintains the expression of *sra-6*, promotes the expression of *str-3* and *srd-1*, and represses expression of *str-2*. High levels of dauer pheromone repress *daf-7* expression and promote dauer 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 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 dauers, 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 *dbl-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 (Ruvkun 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 Ellazar 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(su006)* 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 DiI 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 nondauer 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° 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 until the adult stage. Animals were then shifted to the restrictive or permissive temperatures, and GFP expression was scored every 24 h under 400× magnification. Statistical analysis was performed using Statview 4.5.

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