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Regulation of *C. elegans* Longevity by Specific Gustatory and Olfactory Neurons

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Summary

The life span of *C. elegans* is extended by mutations that inhibit the function of sensory neurons. In this study, we show that specific subsets of sensory neurons influence longevity. We find that certain gustatory neurons inhibit longevity, whereas others promote longevity, most likely by influencing insulin/IGF-1 signaling. Olfactory neurons also influence life span, and they act in a distinct pathway that involves the reproductive system. In addition, we find that a putative chemosensory G protein-coupled receptor that is expressed in some of these sensory neurons inhibits longevity. Together our findings imply that the life span of *C. elegans* is regulated by environmental cues and that these cues are perceived and integrated in a complex and sophisticated fashion by specific chemosensory neurons.

Introduction

The *C. elegans* hermaphrodite has 302 neurons, 60 of which have cilia that are exposed to the environment (White et al., 1986). The cilia contain putative sensory receptors (Sengupta et al., 1996; Dwyer et al., 1998) and other components of the animal's sensory transduction pathways (Coburn and Bargmann, 1996; Komatsu et al., 1996; Colbert et al., 1997; Roayaie et al., 1998). Mutations in genes required for cilium formation or in sensory signal transduction not only inhibit the animal from responding normally to sensory stimuli (Perkins et al., 1986; Tabish et al., 1995; Coburn and Bargmann, 1996; Komatsu et al., 1996; Cole et al., 1998; Collet et al., 1998) but also extend its life span, suggesting that life span is influenced by the perception of an environmental signal or signals (Apfeld and Kenyon, 1999).

The longevity of *C. elegans* sensory mutants has been proposed to result from a decrease in the activity of the animals' DAF-2 insulin/IGF-1 pathway (Apfeld and Kenyon, 1999), which has previously been shown to regulate the life span of the animal (Kenyon et al., 1993; Larsen et al., 1995; Kimura et al., 1997; Lin et al., 1997; Ogg et al., 1997). Reduction-of-function mutations in *daf-2*, a homolog of the insulin/IGF-1 receptor (Kimura et al., 1997), extend life span (Kenyon et al., 1993; Larsen et al., 1995), and this life span extension requires the FOXO family transcription factor DAF-16 (Kenyon et al., 1993; Lin et al., 1997; Ogg et al., 1997). Likewise, the longevity of sensory mutants also requires *daf-16* activity (Apfeld and Kenyon, 1999), and, like *daf-2* mutations, sensory mutations cause nuclear localization of DAF-16 (Lin et al., 2001). In addition, sensory mutations do not extend the life span of *daf-2* mutants (Apfeld and Kenyon, 1999).

The genome of *C. elegans* contains more than 30 insulin/IGF-1-like genes (Pierce et al., 2001; Li et al., 2003), many of which are expressed in sensory neurons. Several of these genes have been implicated in the regulation of longevity (Kawano et al., 2000; Pierce et al., 2001; Li et al., 2003; Murphy et al., 2003). Thus, it is possible that these or other putative DAF-2 ligands are released from sensory neurons in response to environmental signals (Apfeld and Kenyon, 1999; Guarente and Kenyon, 2000).

The different sensory neurons of *C. elegans* can recognize different types of environmental cues (reviewed in Bargmann and Mori, 1997; Driscoll and Kaplan, 1997). Therefore, it is important to know whether only certain sensory neurons might influence life span. This is a possibility, since some long-lived sensory mutants have defects only in specific subsets of neurons (Apfeld and Kenyon, 1999). Many sensory neurons are situated in a pair of bilaterally symmetric sensory organs, called amphids, which are located in the head. Each amphid contains 12 sensory neurons that function in taste reception (Bargmann and Horvitz, 1991a), olfaction (Bargmann et al., 1993), mechanosensation (Kaplan and Horvitz, 1993), and thermal (Mori and Ohshima, 1995) and pheromone perception (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). Inhibiting amphid function by laser ablation of the amphid sheath cells is sufficient to extend the life span of *C. elegans* by approximately 35%, suggesting that at least some of the sensory neurons whose activities regulate life span are amphid neurons (Apfeld and Kenyon, 1999). In this study, we have used laser microsurgery and genetics to identify individual pairs of amphid neurons that affect longevity.

Results

Sensory Neurons that Regulate Dauer Formation Affect Life Span

Certain amphid neurons are known to regulate an alternative developmental pathway known as dauer formation (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). The dauer is an alternative, developmentally arrested L3 larval state specialized for long-term survival. Dauer formation is induced under unfavorable environmental conditions by a pheromone that is produced by the animal (Golden and Riddle, 1982, 1984). Like the sensory regulation of life span (Apfeld and Kenyon, 1999), dauer formation is mediated, in part, by the *daf-2* pathway (reviewed by Riddle and Albert, 1997). Strong *daf-2* pathway mutants never reach adulthood but arrest as dauers even under favorable environmental conditions (Riddle and Albert, 1997). The DAF-2 pathway functions during development, prior to the L3 stage, to regulate dauer formation, and later, during adulthood, to regulate adult life span (Dillin et al., 2002). Thus, it

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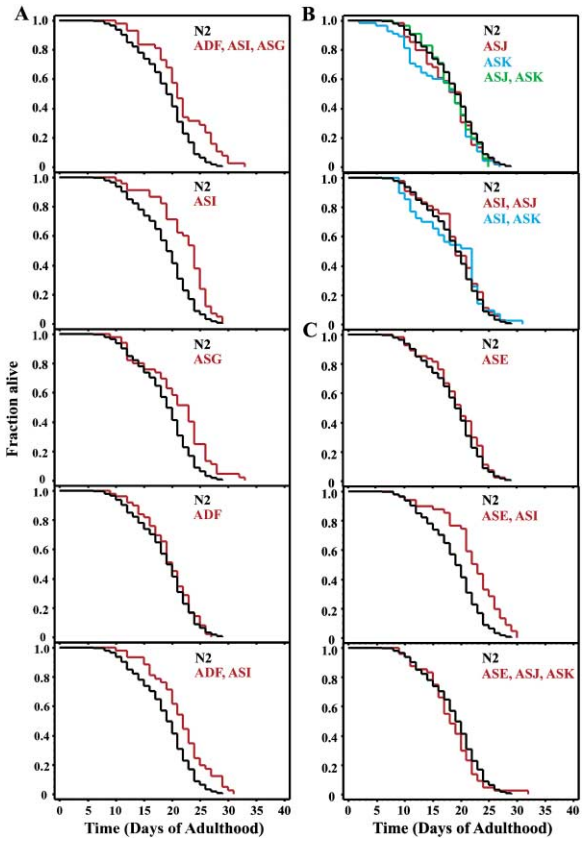

Figure 1. Gustatory Neurons Regulate Life Span

(A–C) The fraction of animals that survive is plotted against time. Black curves represent life span of control animals. Colored curves (in this and subsequent figures) represent life span of treated animals, and the corresponding treatment, such as the pairs of neurons that have been ablated, is indicated in the same color in the upper right hand corner of each panel. Life span of ablated or mock-ablated wild-type (N2) worms was determined at 20°C.

seemed possible that, by influencing the DAF-2 pathway, the same neurons that regulate the formation of dauers during development might also regulate the longevity of the adult.

The amphid neurons ADF, ASI, and ASG inhibit dauer formation (Bargmann and Horvitz, 1991b). As expected, ablation of these neurons soon after hatching caused 75% of the animals to form transient dauers. We found that it also extended their adult life span (Figure 1A; Table 1). Next, we asked which of these three neurons influenced longevity. In dauer formation, the ADF and ASI neurons share a redundant function, whereas the ASG neurons play a minor role (Bargmann and Horvitz, 1991b). Ablation of any single pair of these neurons does not cause dauer formation (Bargmann and Horvitz, 1991b). However, this was not the case for life span. We found that the ASI and ASG neurons but not the ADF neurons appear to influence adult life span. Ablation of either the ASI or ASG neurons alone extended life span, and ablation of ADF neurons did not (Figure 1A; Table 1). In addition, although killing ADF caused ASI-ablated animals to form transient dauers (at a frequency of 38%), it did not enhance the longevity of ASI-ablated animals (Figure 1A; Table 1).

In contrast to the dauer-inhibiting neurons ADF, ASI, and ASG, the amphid neurons ASJ and ASK promote dauer formation (Schackwitz et al., 1996). We found that ablating the ASJ and/or ASK neurons did not affect life span (Figure 1B; Table 1). However, ablating either ASJ or ASK neurons completely suppressed the life span extension produced by ablation of ASI neurons (Figure 1B; Table 1). Together, these findings indicate that a number of the neurons that influence dauer formation do influence life span and that some of these neurons promote, whereas others inhibit, longevity.

Dauer Pheromone Does Not Extend Adult Life Span

Sensory neurons are thought to regulate dauer formation in response to dauer pheromone (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996). Dauer pheromone is a fatty acid-like substance produced constitutively by *C. elegans* throughout its life (Golden and Riddle, 1982). Dauer pheromone is both necessary and sufficient to induce dauer formation, though its effectiveness is enhanced by increased temperature and decreased food levels (Golden and Riddle, 1982, 1984). Dauer pheromone has been proposed to repress the dauer-inhibiting activities of ADF, ASI, and ASG and to activate the dauer-promoting activities of ASJ and ASK (Bargmann and Horvitz, 1991b; Schackwitz et al., 1996).

It was curious that only some of the neurons that regulate dauer formation also regulated adult longevity. This suggested that the cues that regulate dauer formation might be different from those that influence life span. To test directly whether dauer pheromone was able to extend life span, we added it to the culture medium of a temperature-sensitive sterile strain, *fer-15(b26)*; *fem-1(hc17)* (Garigan et al., 2002), whose adult life span can also be extended significantly by the ablation of the ASI neurons (Figure 2A; Table 1). We found that dauer pheromone had no effect on adult longevity (Figure 2B; Table 1), even at concentrations that induced 33% of the animals to form dauers. Because mutations in the *daf-2* pathway that cause this frequency of dauer formation invariably extend adult life span dramatically (Kenyon et al., 1993; Larsen et al., 1995; Kimura et al., 1997; Gems et al., 1998), these findings suggest that dauer pheromone plays little or no role in the control of adult longevity.

It is possible that these neurons regulate life span by sensing other environmental cues. ASI, ASG, and ASK, which influenced life span, have also been shown to function as gustatory neurons that sense food or its breakdown products, such as amino acids, in the environment (Bargmann and Horvitz, 1991a). The amphids contain other food-sensing gustatory neurons, such as ADF and ASE (Bargmann and Horvitz, 1991a); however, as with ADF, we found that ablating the ASE neurons (either singly or in combination with other neurons) did not affect life span (Figure 1C; Table 1). Together these findings suggest that only a specific subset of gustatory neurons may influence life span.

Olfactory Neurons Also Influence Longevity

The amphids also contain olfactory neurons that sense food-derived substances (Bargmann et al., 1993). Unlike the gustatory neurons described above, the olfactory

Table 1. Adult Life Span of Treated Animals

Strain/Treatment	Mean Life Span ± SEM (Days)	75th Percentile (Days)	Number of Animals Observed/Total Initial Animals	% Control	p Value Against Control	p Value Against Specified Groups
N2						
Mock ablated control	18.7 ± 0.2	22	496/628 (8)			
Gustatory neurons ablated						
ADF, ASI, ASG	21.5 ± 0.9	26	39/56 (1)	**+15**	<0.0001	0.83‡
ASI	22.2 ± 0.7	25	44/60 (1)	**+19**	<0.0001	
ASG	20.9 ± 0.9	24	46/59 (1)	**+12**	0.0002	
ADF	19.5 ± 0.6	23	49/60 (1)	+4	0.56	
ADF, ASI	21.6 ± 0.8	24	41/61 (1)	**+16**	<0.0001	0.83‡
ASJ	17.9 ± 0.7	22	53/60 (1)	−4	0.26	
ASK	16.8 ± 0.8	21	49/60 (1)	−10	0.07	
ASJ, ASK	18.4 ± 0.6	22	50/59 (1)	−2	0.37	
ASI, ASJ	19.2 ± 0.8	23	38/61 (1)	+3	0.49	0.003‡
ASI, ASK	18.3 ± 0.9	23	44/60 (1)	−2	0.52	0.005‡
ASE	19.3 ± 0.7	23	53/61 (1)	+3	0.37	
ASE, ASI	22.0 ± 0.8	26	46/60 (1)	**+18**	<0.0001	0.68‡
ASE, ASJ, ASK	18.2 ± 0.7	21	45/48 (1)	−3	0.31	
Olfactory neurons ablated						
AWA	20.8 ± 0.7	24	50/60 (1)	**+11**	0.002	
AWC	18.9 ± 0.7	22	47/60 (1)	+1	0.55	
AWA, AWC	23.6 ± 1.0	27	30/51 (1)	**+26**	<0.0001	0.02*
Gustatory and olfactory neurons ablated						
ASI, AWA	22.2 ± 0.9	28	45/60 (1)	**+19**	<0.0001	0.29‡
ASI, AWC	22.1 ± 0.7	25	43/58 (1)	**+18**	<0.0001	0.77‡
ASI, AWA, AWC	24.9 ± 1.3	29	30/50 (1)	**+33**	<0.0001	0.002‡
AWA, AWC, ASJ, ASK	21.6 ± 0.9	27	36/55 (1)	**+16**	<0.0001	0.17§
CF512 (25.5°C)						
Mock-ablated control	16.9 ± 0.5	20	76/76 (1)			
ASI ablated	19.8 ± 0.8	25	50/51 (1)	**+17**	0.0001	
(−) pheromone	15.0 ± 0.6	18	35/40 (1)			
(+) pheromone	14.3 ± 0.7	18	31/40 (1)	−5	0.43	
N2#	19.3 ± 0.4	23	118/130 (2)			
Olfaction mutants						
odr-7(ky4)	21.3 ± 0.4	24	107/130 (2)	**+10**	0.0009	
odr-1(n1936)	18.4 ± 0.5	22	56/60 (1)	**−5**	0.02	
odr-7(ky4) odr-1(n1936)	22.9 ± 0.6	25	56/70 (1)	**+19**	<0.0001	0.006‖
odr-2(n2145)	23.6 ± 0.6	26	49/60 (1)	**+22**	<0.0001	
odr-3(n1605)	21.2 ± 0.6	25	104/300 (2)	**+10**	<0.0001	
odr-10(ky225)	19.8 ± 1.0	24	41/50 (1)	+3	0.05	
daf-16(mu86)						
Mock-ablated control	14.9 ± 0.3	17	114/125 (2)			
ASI	15.1 ± 0.5	18	51/59 (1)	+1	0.78	
ASJ, ASK	14.8 ± 0.5	17	46/51 (1)	−1	0.87	
AWA, AWC	16.8 ± 0.6	19	40/50 (1)	**+13**	0.004	
daf-2(e1370)						
Mock-ablated control	49.9 ± 1.9	58	62/74 (1)			
ASI	47.4 ± 2.5	58	37/47 (1)	−5	0.46	
ASJ, ASK	40.4 ± 2.4	54	40/49 (1)	**−20**	0.004	
rrf-3(pk1426)						
Control RNAi‡‡	11.6 ± 0.5	16	98/119 (1)			
str-2 RNAi‡‡	14.8 ± 0.5	18	94/127 (1)	**+28**	0.0001	
osm-3 RNAi‡‡	15.9 ± 0.9	19	35/40 (1)	**+37**	0.0009	
Control RNAi**	12.7 ± 0.5	17	128/140 (1)			
str-2 RNAi**	15.0 ± 0.4	19	133/140 (1)	**+18**	0.0003	

Control and experimental animals were assayed in parallel. We show cumulative statistics in the figures and tables because experimental animals compared to their respective controls assayed at the same time behaved similarly as when experimental animals were compared to the cumulative control. The mean life span and 75th percentiles of the different groups of animals in the second and third columns, respectively, were determined using the Statview 4.5 (SAS) software. The 75th percentile is the age when the fraction of animals alive in each group is 0.25. The first number in the fourth column equals the number of animals observed as having died, whereas the second number in the fourth column equals the number of animals observed plus the number of animals censored. Animals that crawled off the plate, exploded, or bagged were censored at the time of the event, which allowed these worms to be incorporated into the data set until the censor date and thus avoid loss of information. The number of independent experiments in which life span was determined is in parenthesis. The % difference between treated or mutant animals and their respective control, which are in boldface type and underlined if significant, is indicated in the fifth column. The logrank (Mantel-Cox) test (Lawless, 1982) was used to determine p values and if the life span of the different groups are similar. p values less than 0.05 are considered statistically significant. The first set of p values compare the difference between treated or mutant animals and their

(*continued*)

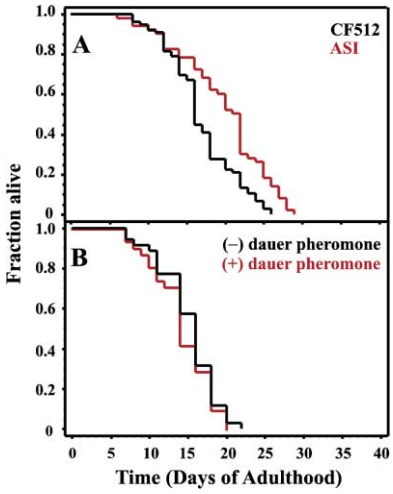

Figure 2. Dauer Pheromone Does Not Affect Adult Life Span

(A–B) Life span of CF512 [*fer-15(b26)*; *fem-1(hc17)*] worms was measured at 25.5°C. (A) The survival curve of ASI-ablated worms was compared to that of mock-ablated worms. (B) The life span of worms exposed to dauer pheromone throughout their lives was compared to that of untreated worms.

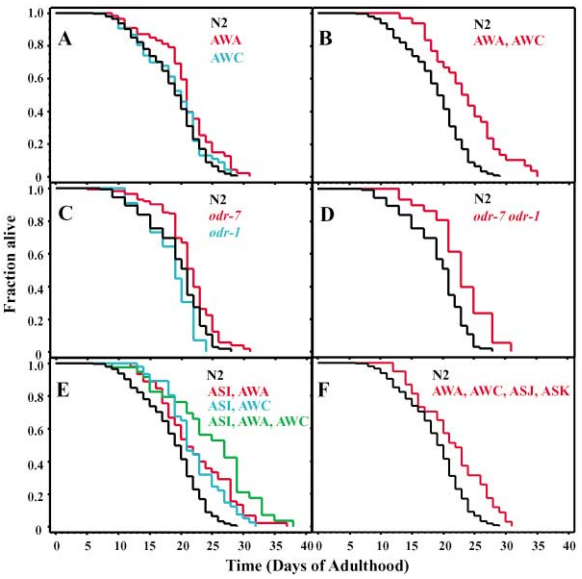

Figure 3. The Olfactory Neurons AWA and AWC Inhibit Longevity

(A, B, E, and F) Life span of ablated N2 worms was compared to that of mock-ablated N2 worms.

(C) Life span of olfaction mutants *odr-7(ky4)* and *odr-1(n1936)* was compared to that of N2.

(D) Life span of the double mutant CX2336 [*odr-7(ky4) odr-1(n1936)*] (Chou et al., 2001) was compared to that of N2.

neurons do not appear to influence dauer formation (Bargmann and Horvitz, 1991a, 1991b; Bargmann et al., 1993). Two well-characterized pairs of olfactory neurons are called AWA and AWC neurons (Bargmann et al., 1993). We found that ablation of AWA neurons extended adult life span, whereas ablation of AWC had no effect (Figure 3A; Table 1). However, ablation of both the AWA and AWC neurons extended adult life span to a greater extent than did ablation of the AWA neurons alone (Figure 3B; Table 1), suggesting that the neurons AWA and AWC inhibit longevity in a partly redundant fashion.

We also examined mutants with specific defects in olfactory neurons. Consistent with our observation that AWA plays a role in life span regulation, animals harboring a null mutation in *odr-7*, a gene that encodes a nuclear hormone receptor specifically required for AWA function (Sengupta et al., 1994; Sagasti et al., 1999), lived longer than wild-type (Figure 3C; Table 1). Furthermore, as we had observed with the ablation of AWC neurons alone, mutations in *odr-1*, a gene that encodes a transmembrane guanylyl cyclase required for AWC function (L'Etoile and Bargmann, 2000), did not extend adult life span (Figure 3C; Table 1). However, *odr-1* mutations did enhance the life span extension observed in *odr-7* mutants (Figure 3D; Table 1), just as ablation of AWC neurons enhanced the life span extension seen in AWA-ablated animals.

Gustatory and Olfactory Neurons Act in Parallel to Influence Life Span

Next, we asked whether gustatory and olfactory neurons were likely to act in the same or different pathways to affect life span. To do this, we killed combinations of olfactory and gustatory neurons in individual animals. We found that ablation of either the olfactory AWA or AWC neurons alone did not enhance the life span extension produced by ablating the gustatory ASI neurons (Figure 3E; Table 1). However, ablation of both AWA and

Table 1. continued

respective control, whereas the second set of p values compare the difference between certain treated or mutant animals and other treated or mutant animals specified by the superscripted symbols. The superscripted symbols indicate the following: ‡, compared with ASI-ablated N2 worms; *, compared with AWA-ablated N2 worms; §, compared with AWA- and AWC-ablated N2 worms; #, since the olfaction mutants we studied came from Cori Bargmann's lab at the University of California, San Francisco, we compared their life span to that of N2 from her lab; ||, compared with *odr-7(ky4)*; ‡‡, *rrf-3(pk1426)* L1 larvae grown on the specified *E. coli* clone were shifted between 22°C and 25.5°C (see Experimental Procedures); and **, *rrf-3(pk1426)* worms fed on the specified *E. coli* clone were grown continuously at 22°C. CF512 [*fer-15(b26)*; *fem-1(hc17)*] is a temperature-sensitive sterile strain (Garigan et al., 2002). The p values comparing mean life span of the same strain in different experiments were p = 0.11 for N2 mock-ablated animals (mean life span of animals tested in each of eight independent experiments [see supporting online material (Figure S1 at http://www.neuron.org/cgi/content/full/41/1/45/DC1)]: 1, 18.0 ± 0.5 [n = 53/66]; 2, 18.3 ± 0.7 [n = 45/58]; 3, 19.0 ± 0.5 [n = 80/100]; 4, 18.1 ± 1.1 [n = 33/45]; 5, 18.2 ± 0.6 [n = 76/91]; 6, 19.0 ± 0.5 [n = 107/129]; 7, 19.2 ± 0.8 [n = 41/55]; and 8, 19.2 ± 0.6 [n = 61/84]); p = 0.56 for N2 animals from Cori Bargmann's lab (mean life span of animals tested in each of two experiments: 19.1 ± 0.6 [n = 56/60] and 19.4 ± 0.6 [n = 62/70]); p = 0.53 for *odr-7(ky4)* (mean life span tested in each of two experiments: 21.4 ± 0.7 [n = 48/60] and 21.2 ± 0.5 [n = 59/70]); p = 0.99 for *odr-3(n1605)* (mean life span tested in each of two experiments: 21.2 ± 1.1 [n = 29/100] and 21.2 ± 0.7 [n = 75/200]); and p = 0.61 for *daf-16(mu86)* (mean life span tested in each of two experiments: 14.6 ± 0.5 [n = 55/57] and 15.1 ± 0.4 [n = 59/68]).

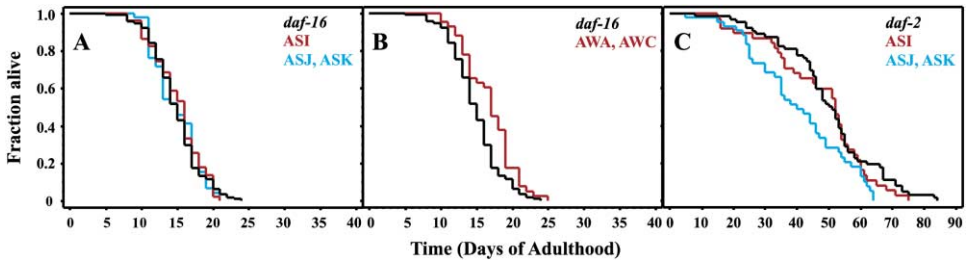

Figure 4. Gustatory Neurons Appear to Act through the *daf-2* Signaling Pathway

(A and B) Life span of ablated and mock-ablated *daf-16(mu86)* worms was assayed.

(C) Life span of ablated and mock-ablated *daf-2(e1370)* worms was determined. Although ablation of ASI did not affect the lifespan of *daf-2* mutants, it did enhance dauer formation by inducing 15% of the animals (8 dauers/55 total larvae) to form dauers at 20°C.

AWC did enhance the life span extension of animals lacking the ASI neurons (Figure 3E; Table 1). Animals in which all three pairs of neurons were ablated lived 32% longer than wild-type (Table 1). Hence, defects in several specific sensory neurons, but not necessarily in every neuron, can produce relatively large increases in animal life span (compare ASI-, AWA-, and AWC-ablated versus ADF-, ASI-, and ASG-ablated versus ASE-, ASJ-, and ASK-ablated animals in Figures 3E, 1A, and 1C, respectively; Table 1).

We also asked whether ablation of ASJ and ASK, the neurons required for the extended longevity of ASI-ablated animals, could suppress the life span extension seen in AWA- and AWC-ablated animals. We found that this did not appear to be the case, since the ablation of the olfactory neurons AWA and AWC still enhanced longevity even when both the ASJ and ASK neurons had been ablated (Figure 3F; Table 1). Together these findings suggest that olfactory and gustatory neurons function in at least partially distinct pathways to control life span.

Gustatory Neurons Appear to Regulate Longevity through the *daf-2* Pathway

Sensory mutations are thought to influence life span, at least in part, by perturbing the insulin/IGF-1 *daf-2* signaling pathway (Apfeld and Kenyon, 1999). To determine whether individual neurons might affect life span by modulating insulin/IGF-1 signaling, we asked whether they could influence the life span of *daf-16* and *daf-2* mutants. We found that the life span extension caused by ablating the gustatory ASI neurons was completely dependent on *daf-16* (Figure 4A; Table 1). In addition, ablating ASI neurons in *daf-2* hypomorphic mutants did not further increase their life span (Figure 4C; Table 1). Therefore, ASI seems likely to affect life span by modulating the *daf-2* pathway.

Next, we ablated ASJ and ASK in these mutants. We found that, as with wild-type, ablation of the ASJ and ASK neurons did not shorten the life span of *daf-16* null mutants (Figure 4A; Table 1). Interestingly, however, ablation of the ASJ and ASK neurons did shorten the life span of *daf-2* hypomorphic mutants (Figure 4C; Table 1; see Discussion).

We also asked whether the life span extension produced by ablation of the olfactory neurons required *daf-16*. We found that, unlike the gustatory neurons, the activities of the olfactory AWA and AWC neurons were only partly dependent on *daf-16* (Figure 4B; Table 1). Again, this suggests that olfactory and gustatory neurons act on different, or at least partially nonoverlapping, pathways to influence life span.

Olfactory Neurons Influence the Ability of the Somatic Gonad to Affect Life Span

The reproductive system of *C. elegans* influences its life span (Hsin and Kenyon, 1999). Ablation of the germline precursor cells extends life span approximately 60%, and this life span extension is completely dependent on *daf-16* (Hsin and Kenyon, 1999). The long life span of germline-ablated animals requires the activity of the somatic gonad, since this extended longevity can be suppressed entirely by killing the somatic gonad precursor cells (Hsin and Kenyon, 1999; Figure 5A; Table 2). Interestingly, *daf-16* null mutants in which the somatic gonad precursor cells are ablated have shorter life spans than *daf-16* mutants with intact gonads, indicating that *daf-16* mutants can still respond to the effects of somatic gonad ablations (Hsin and Kenyon, 1999). Together these findings have suggested the model that the germline influences life span via a *daf-16*-dependent pathway, whereas the somatic gonad influences life span via a counterbalancing pathway that is at least partly *daf-16* independent (Hsin and Kenyon, 1999).

Surprisingly, the function of sensory neurons is required in order for somatic gonad ablation to suppress the life span extension caused by germline ablation (Apfeld and Kenyon, 1999). Ablation of the germline invariably extends the life span of sensory mutants further; however, in some sensory mutants, this life span extension cannot be suppressed by somatic gonad ablations (Apfeld and Kenyon, 1999). To ask which sensory neurons might be responsible for this effect, we killed the germ cells and the whole gonad in animals lacking individual pairs of neurons. We found that animals lacking the gustatory ASI neurons responded like wild-type to germline and whole-gonad ablations (Figures 5A and 5B; Table 2). In contrast, *odr-7* mutants, which specifically lack functional olfactory AWA neurons, did not (Figure 5C; Table 2). As with wild-type, ablating the germline precursors of *odr-7* animals further extended their life span, but this effect was only partially suppressed by ablation of their somatic gonad precursors. Thus, the requirement for sensory function in the regula-

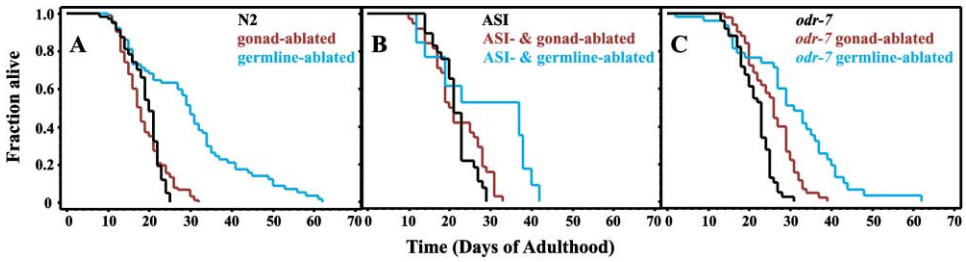

Figure 5. Olfactory Neurons Are Required for Somatic Gonad Ablation to Suppress the Extended Life Span of Germline-Ablated Animals

The fraction of germline- and gonad-ablated N2 (A) or ASI-ablated animals (B) or of germline- and gonad-ablated *odr-7(ky4)* mutants (C) remaining alive is plotted against animal age. Because the germline does not develop in the absence of the somatic gonad (Kimble and White, 1981), ablation of the somatic gonad precursors effectively removes the entire gonad.

tion of life span by the somatic gonad resides, at least in part, in the olfactory AWA neurons.

Long-Lived Sensory-Impaired Worms Are Not Calorically Restricted

The life span of *C. elegans* can be extended by caloric restriction (Klass, 1977). Because sensory neurons mediate chemotaxis toward components of food products, one can imagine that sensory mutants are unable to locate food and, consequently, are calorically restricted. Calorically restricted animals develop more slowly than normal, are thin, and have reduced fecundity (Klass, 1977). Previously, we showed that long-lived sensory mutants exhibit normal feeding behavior, develop at the same rate as wild-type, are not thin, and have normal fecundity (Apfeld and Kenyon, 1999). We also tested the long-lived animals in which specific gustatory and/or olfactory neurons had been ablated for phenotypes consistent with caloric restriction. We found that, unlike food-restricted animals, these ablated animals developed normally and were not thin. They also had wild-type brood sizes (299 ± 34, n = 20 control animals; 286 ± 29, n = 14 ASI-ablated animals; 292 ± 9, n = 3 AWA- and AWC-ablated animals; and 300 ± 18, n = 9 ASI-, AWA-, and AWC-ablated animals; we note that the life span extension seen in these latter animals was equivalent to that produced by caloric restriction [Klass, 1977]). Furthermore, the long life span of calorically restricted animals does not require *daf-16* (Lakowski and Hekimi, 1998; Houthoofd et al., 2003), whereas the extended life span of sensory-impaired animals does (Apfeld and Kenyon, 1999; Figures 4A and 4B). Thus, neither the gustatory nor olfactory neurons are likely to influence life span by affecting food consumption.

Sensory Signaling Molecules that Influence Life Span

Previously, we showed that mutations in a cyclic nucleotide-gated ion channel required for sensory signal transduction (Komatsu et al., 1996) increased life span (Apfeld and Kenyon, 1999). We tested additional sensory transduction mutants and found that a hypomorphic mutation in *odr-2*, which encodes a GPI-linked protein required in olfaction and is expressed in many neurons, including interneurons onto which the AWA and AWC neurons synapse (Chou et al., 2001), extended adult life span (Figure 6A; Table 1). In addition, we found that null mutations in *odr-3*, which encodes a Gα protein that mediates

Table 2. Adult Life Spans of Germline-Ablated and Gonad-Ablated Animals

Strain/Treatment	Mean Life Span ± SEM (Days)	75th Percentile (Days)	Number of Animals Observed/Total Initial Animals	% Control	p Value Against Control	p value against specified groups
N2 mock-ablated control[#]	19.1 ± 0.4	22	99/120 (2)			
N2 germline-ablated	29.8 ± 1.8	36	60/73 (2)	**+56**	<0.0001	
N2 gonad-ablated	18.6 ± 0.6	22	92/94 (2)	−3	0.81	
ASI-ablated control	21.6 ± 0.8	23	28/35 (1)	**+13**[‡]		0.002[‡]
ASI- and germline-ablated	28.3 ± 3.3	38	12/14 (1)	**+31**	0.01	0.73*
ASI- and gonad-ablated	21.9 ± 1.1	28	38/39 (1)	+1	0.29	0.004[‖]
odr-7(ky4) mock-ablated control	21.5 ± 0.6	25	47/62 (1)	**+13**[‡]		<0.0001[‡]
odr-7(ky4) germline-ablated	30.5 ± 1.9	39	35/57 (1)	**+42**	<0.0001	0.87*
odr-7(ky4) gonad-ablated	25.6 ± 0.8	30	47/55 (1)	**+19**	<0.0001	0.0007[§]

The analyses performed on these experiments are as described in the legend of Table 1. The first set of p values compare the difference between treated animals and their respective control, whereas the second set of p values compare the difference between certain treated animals and other treated animals specified by the superscripted symbols. The superscripted symbols indicate the following: [#], that the N2 used in these experiments was from Cori Bargmann's lab since the *odr-7* allele came from her lab; [‡], compared with N2 mock-ablated worms; *, compared with N2 germline-ablated worms; [‖], compared with ASI- and germline-ablated worms; and [§], compared with *odr-7(ky4)* germline-ablated worms. The p values comparing mean life span of the same strain in different experiments were p = 0.34 for N2 animals (mean life span of animals tested in each of two experiments: 19.3 ± 0.6 [n = 44/54] and 18.9 ± 0.5 [n = 55/66]); p = 0.65 for germline-ablated N2 animals (mean life span tested in each of two experiments: 30.6 ± 2.2 [n = 42/51] and 28.2 ± 3.1 [n = 18/22]); and p = 0.64 for gonad-ablated N2 animals (mean life span of animals tested in each of two experiments: 19.0 ± 0.7 [n = 50/51] and 18.2 ± 0.9 [n = 42/43]).

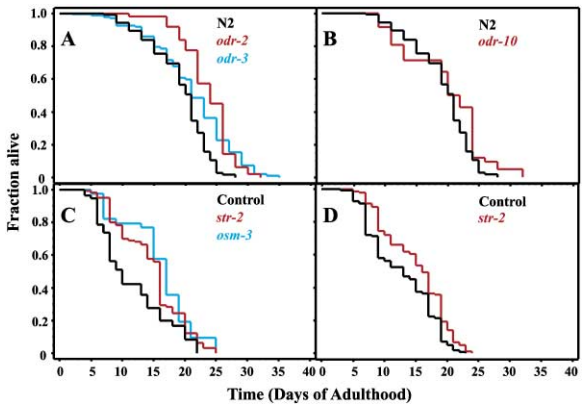

Figure 6. A Putative Chemosensory Receptor Inhibits Longevity

(A and B) Life span of olfaction mutants *odr-2(n2145)*, *odr-3(n1605)*, and *odr-10(ky225)* was compared to that of N2.

(C and D) Life span of RNAi-treated *rrf-3(pk1426)* animals. *rrf-3(pk1426)* animals were fed on the designated *E. coli* clone and were either shifted between 22°C and 25.5°C (C) or grown continuously at 22°C (D). RNAi of the *osm-3* gene was done as a positive control for RNAi of neuronal genes that influence life span.

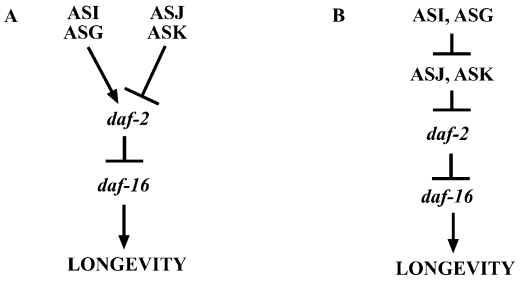

Figure 7. Models of Life Span Regulation by Gustatory Neurons

(A) The longevity-inhibiting ASI and ASG neurons as well as the longevity-promoting ASJ and ASK neurons act directly on the DAF-2 pathway; alternatively, (B) ASI and ASG influence the activities of ASJ and ASK, which in turn promote longevity by inhibiting the DAF-2 pathway.

the function of sensory neurons (Roayaie et al., 1998), increased life span (Figure 6A; Table 1), suggesting that a G protein-coupled receptor might sense an environmental cue that inhibits longevity.

The *odr-10* gene encodes an olfactory G protein-coupled receptor that senses diacetyl, an odorant sensed by AWA neurons (Sengupta et al., 1996). We found that *odr-10* null mutants were not long-lived (Figure 6B; Table 1), implying that neither diacetyl nor its receptor regulates life span. In contrast, we observed that decreasing the mRNA levels of the putative chemosensory G protein-coupled receptor *str-2* (Troemel et al., 1999), through RNA-mediated interference (RNAi [Timmons and Fire, 1998]), extended life span (Figures 6C and 6D; Table 1). This suggests that *C. elegans*' life span is influenced by its perception of an environmental cue—as yet unidentified—that is sensed by STR-2.

Discussion

In this study, we have asked which sensory neurons influence longevity. Our findings reveal that the sensory control of life span does not reside in a single type of neuron, or in all neurons equally, but instead is distributed across a specific set of gustatory and olfactory neurons. These neurons, in turn, appear to function in different ways to influence the life span of the animal.

Gustatory Neurons Exert Opposing Effects on Life Span

Our previous finding that sensory mutations extend life span (Apfeld and Kenyon, 1999) implied the existence of neurons that inhibit longevity. Thus, it was surprising to find that whereas some gustatory neurons, such as ASI and ASG, do inhibit longevity, other gustatory neurons, such as ASK, promote longevity. One might imagine that these two classes of neurons exert their effects on life span by affecting the same downstream target in opposite ways (Figure 7A). However, this model seems unlikely, since ablation of ASJ and ASK alone did not shorten the life span of wild-type animals. Instead, our findings suggest models in which, directly or indirectly, ASI and ASG shorten life span by inhibiting ASJ and ASK activities (Figure 7B). In these models, when ASI and ASG are silenced, either by ablation or by an environmental cue, ASJ and ASK can promote longevity. The fact that the ability of the ASI neurons to influence life span depends on the presence of the ASJ and ASK neurons raises the possibility that these neurons might regulate life span by affecting each other's activity within a neural circuit.

The combined ablation of certain gustatory and olfactory neurons produced a relatively large increase in life span, comparable to the life span extension produced by killing the amphid sheath cells. However, many of our ablation experiments, which involved one or a few pairs of neurons, produced relatively small increases in longevity. The finding that certain neurons act to promote longevity raises the possibility that one might achieve larger increases in life span if one could silence all of the neurons that inhibit longevity without affecting the neurons that promote longevity. Because of the large number of sensory neurons in the animal, examining all possible combinations of laser-ablated animals is not feasible. However, it could be that by affecting specific subsets of neurons, certain environmental cues might be able to elicit large increases in life span.

Specific Sensory Cues Are Likely to Influence Life Span

Sensory mutations affect longevity without affecting feeding (Apfeld and Kenyon, 1999; this study), suggesting that the animal's perception of environmental cues affects its life span. This idea is supported by our discovery that inhibiting a putative chemosensory receptor expressed in ASI and AWC, two longevity-inhibiting neurons, lengthens lifespan. What might this cue (or cues) be? Our observation that not all food-sensing gustatory neurons affect longevity suggests that the animal does not adjust its life span in response to all food-related signals but rather to more specific sensory cues. Our findings provide valuable information about the possible nature of these signals. First, al-

though dauer pheromone seemed like a good candidate for a longevity signal, we find that it is unlikely to play a major role in the regulation of longevity.

Second, our studies suggest that volatile, as well as soluble, compounds influence longevity, since the olfactory AWA and AWC neurons affect longevity. Furthermore, the finding that a Gα protein is required for the life-shortening function of olfactory neurons implies that an olfactory G protein-coupled receptor might sense an odorant that inhibits longevity. One such receptor in AWA neurons, the diacetyl receptor ODR-10 (Sengupta et al., 1996), does not appear to influence life span, again suggesting that only specific sensory cues (or combinations of cues) affect longevity.

It is possible that these cues influence life span, at least in part, by activating the putative chemosensory G protein-coupled receptor STR-2, which we found to affect life span. Although the ligand that activates STR-2 is not known, STR-2::GFP fusions are expressed in one AWC neuron and in two ASI neurons (Troemel et al., 1999). Thus, this receptor may allow one or both types of neurons to sense cues that affect longevity.

Gustatory Neurons May Influence Life Span by Regulating Endocrine Signaling

Our findings suggest that gustatory neurons are likely to influence life span by perturbing the insulin/IGF-1 pathway. One possibility is that these neurons sense cues that regulate the release of insulin/IGF-1-like hormones that influence DAF-2 activity. The *C. elegans* genome contains more than 30 insulin/IGF-1 homologs, and several of these are expressed in gustatory neurons (Pierce et al., 2001). As described above, we favor the model that longevity-inhibiting ASI and ASG neurons exert their effects on life span by inhibiting the activities of the ASJ and ASK neurons. Thus, one possibility is that the ASI and ASG neurons prevent the longevity-promoting ASJ and ASK neurons from producing a DAF-2 antagonist. It is intriguing that double mutants that have defects in proteins thought to be required for neuronal insulin secretion and in *daf-2* activity have an intermediate life span between those of the corresponding neurosecretory single mutants and *daf-2* hypomorphic single mutants (Ailion et al., 1999). This finding is consistent with the idea that some sensory neurons might secrete DAF-2 antagonists.

A number of insulin-like peptides have now been implicated in the regulation of aging. One such candidate DAF-2 antagonist is *ins-1*, but this gene is expressed not only in longevity-promoting neurons but also in longevity-inhibiting neurons (Pierce et al., 2001). At this point, the information we have about specific insulin-like peptides does not suggest simple models that explain our data. However, this may change as more is learned about the functions of these proteins. For example, it is possible that other insulin/IGF-1-like peptides function as antagonists in the ASJ and ASK neurons but not in the ASI and ASG neurons, since other insulin/IGF-1-like peptides are expressed in ASJ (Li et al., 2003; J.A. and C.K., unpublished data).

Olfactory Neurons Act in Association with the Reproductive System to Affect Life Span

Our observations suggest that olfactory neurons act in a distinct regulatory pathway from gustatory neurons to affect life span. First, the combined ablation of the gustatory ASI and olfactory AWA and AWC neurons increases life span more than does ablation of either ASI or of AWA and AWC neurons alone (Figures 1A and 3; Table 1). Second, killing ASJ and ASK suppresses the longevity of ASI-ablated animals but not that of olfactory neuron-ablated animals. Finally, the life span extension produced by killing gustatory neurons is completely *daf-16* dependent, whereas the life span extension produced by killing olfactory neurons is only partially *daf-16* dependent.

Olfactory neurons may influence life span by perturbing an endocrine signaling pathway that involves the reproductive system. Previous findings have suggested that the germline of *C. elegans* generates a signal that inhibits longevity and is counterbalanced by a signal from the somatic gonad that promotes longevity (Hsin and Kenyon, 1999). Like the olfactory neurons we characterized, the somatic gonad of *C. elegans* was previously shown to affect life span, at least in part, in a *daf-16*-independent fashion (Hsin and Kenyon, 1999). In addition, we found that olfactory neurons are required for the somatic gonad to influence life span. In wild-type animals, killing the somatic gonad precursors completely prevents germline ablation from extending life span, but in animals lacking olfactory neurons, it does not. One possibility is that olfactory neurons regulate the release of a hormone that allows the somatic gonad to influence longevity. If this model is correct, then it implies that, under some environmental conditions, the somatic gonad signal is silenced and may no longer be able to counterbalance the signals from the animal's germline (see Apfeld and Kenyon, 1999). Alternatively, these olfactory neurons could produce a longevity signal in response to a different signal from the somatic gonad. The somatic gonad appears to regulate a pathway that involves DAF-2 (Hsin and Kenyon, 1999). Thus, as with the gustatory neurons, it is possible that the olfactory neurons influence longevity by regulating the release of insulin-like peptides.

Why Might Sensory Neurons Influence Longevity?

One environmental condition, food limitation, is known to have a dramatic effect on life span in many organisms (reviewed by Koubova and Guarente, 2003). Caloric restriction extends life span and also delays reproduction (Klass, 1977; Koubova and Guarente, 2003). When ample food is restored to calorically-restricted rats, they can reproduce, even at a time when the age-matched controls are post-reproductive or dead (Weindruch and Walford, 1988). Thus, this response to caloric restriction has obvious survival value, since it allows animals to postpone reproduction until conditions improve. Dauer formation, which is regulated, at least in part, by sensory cues, serves the same function in *C. elegans*—it allows animals to postpone reproduction under harsh environmental conditions (reviewed by Riddle and Albert, 1997). We did not observe obvious changes in the timing of reproduction in our neuron-ablated animals; however, it is possible that the environmental cues that influence the activities of these neurons in nature also influence other neurons that control reproduction. In this way, sensory cues could affect life span and reproduction coordinately. Alternatively, certain environmental condi-

tions could favor a shorter post-reproductive life span to prevent the aging animals from competing for resources with their progeny. A population of worms that lacks parental competition for resources should, over time, develop a significant advantage relative to populations in which such competition takes place. The identification of sensory cues that influence life span, such as those sensed by STR-2, should make it possible to address this interesting question experimentally.

Is the Sensory Control of Life Span Conserved in Other Organisms?

Mutations that affect insulin/IGF-1 signaling have been shown to extend life span not only in worms but also in flies and mammals (Clancy et al., 2001; Kenyon, 2001; Tatar et al., 2001; Blüher et al., 2003; Holzenberger et al., 2003). Interestingly, insulin-producing cells in *Drosophila* extend processes to the subesophageal ganglion (Rulifson et al., 2002), which contains a gustatory center. Thus, the sense of taste might regulate insulin secretion in flies. In vertebrates, the hormone leptin, which controls body mass and inhibits taste receptor cells, also inhibits insulin secretion (reviewed by Lindemann, 2001). Furthermore, the smell of food has been reported to increase insulin levels in humans (reviewed by Brand et al., 1982). Thus, the two requirements for sensory regulation of life span—sensory control of endocrine signaling and endocrine control of life span—are already known to be in place in mammals. Given the many striking similarities between the biology of *C. elegans* and that of other animals, it would not surprise us if at least some aspects of this interesting sensory system prove to influence longevity in other organisms as well.

Experimental Procedures

Laser Ablations

L1 larvae were mounted on 2% agarose pads containing an anesthetic (3 mM NaN_3) within 6 hr after hatching at 20°C. Amphid neurons and gonad precursor cells were visualized using Nomarski microscopy and identified by their positions and morphology (Sulston et al., 1983). The cells were ablated with a laser microbeam as described previously (Bargmann and Avery, 1995). Several sets of different neuronal ablations were performed in parallel over a period of several weeks. For each set of neuronal ablations, the animals were generated over a period of several days. In conjunction with the ablated animals, a similar number of mock-ablated animals were treated identically over the same period, but no cells were killed. Ablated and mock-ablated animals were then kept at 20°C, unless otherwise indicated, and monitored for dauer formation, if applicable, until they reached the young adult stage, at which time life span analysis was initiated. Dauer formation did not extend adult life span, since ablated animals that formed transient dauers, like ADF-, ASI-, and ASG-ablated worms, did not live longer than ablated animals that did not form dauers, like ASI-ablated worms (Figure 1A; Table 1).

The life spans of ablated and mock-ablated animals were measured in parallel. We showed cumulative data and statistics in all the figures and tables, since the ablated animals behaved similarly when compared either to their respective controls or to the cumulative control. The variation in the life span of the different mock-ablated control populations are shown in Supplemental Figure S1 (available online at http://www.neuron.org/cgi/content/full/41/1/45/DC1), and their mean life spans are listed in the legend of Table 1.

Some sets of neuronal ablations were performed in more than one strain and/or at different temperatures. For example, ASI ablations were performed in two different wild-type strains at 20°C (Figure 1A; Tables 1 and 2) and in the CF512 [*fer-15(b26)*; *fem-1(hc17)*] strain at 25.5°C (Figure 2; Table 1). In all cases, ablation of this pair of neurons extended the life span of the animal. The effects of certain sets of neuronal ablations were also confirmed with existing mutations that affect the function of these neurons, as in the case of the olfactory neurons.

Life Span Analysis

The life span of treated or untreated animals was assayed at 20°C, with the exception of the dauer pheromone experiment, which was carried out at 25.5°C, and the RNAi experiments (see below). We initiated life span analysis on the first day of adulthood of all animals. Animals were transferred away from progeny to new plates every day or every other day until animals stopped laying eggs. We used the Statview 4.5 (SAS) software to perform statistical analysis and to determine mean life span. Censored animals (see legend of Table 1) were incorporated into the data set as described (Lawless, 1982).

Dauer Pheromone Treatment

A volume of 100 μl dauer pheromone (gift of Piali Sengupta, Brandeis University, MA) was added to a 35 mm plate containing 4 ml NG agar. This concentration of pheromone caused 33% of CF512 L1 larvae to form dauers (16 dauers/48 total worms) at 25.5°C. To preclude the need to separate the adult worms from their progeny, thereby facilitating the assay for life span, 20 CF512 animals in the third larval stage (L3), which are sterile at the restrictive temperature of 25.5°C due to a defect in spermatogenesis (Garigan et al., 2002), were added to each plate, either containing or lacking dauer pheromone. To each plate containing pheromone, 15 eggs were added at the same time as the L3 larvae to ensure that the amount of pheromone in each plate could induce dauer formation. The eggs were monitored for dauer formation or development to the L3 stage and were subsequently removed from the plates, whereas the L3 larvae were monitored for development to adulthood, after which their life span was assayed. To confirm that the pheromone remained active, batches of 15 eggs were added to each pheromone-containing plate at different intervals during the course of the life span analysis, assayed for dauer formation and subsequently removed. The pheromone was able to induce 28% of the eggs to form dauers throughout the course of the life span assay (34 dauers/120 total worms). Animals treated with pheromone were also transferred twice to new pheromone plates during the course of the life span assay at intervals of 6–7 days. Control CF512 animals were treated similarly, but no pheromone was added to their plates. All plates were monitored daily.

RNAi Experiments

The RNAi-hypersensitive *rrf-3(pk1426)* animals (Simmer et al., 2002) were fed *E. coli* (HT115) that contain the control vector pAD12 (Dillin et al., 2002) alone or *E. coli* that express double-stranded RNA of *str-2*, *srd-1*, or *osm-3* (Fraser et al., 2000; Kamath et al., 2003) for three generations at 22°C. Each generation of animals was transferred to new plates. In the first trial, third generation (F2) L1 larvae were shifted from 22°C to 25.5°C to induce sterility, since *rrf-3(pk1426)* animals have little or no progeny at the higher temperature (Simmer et al., 2002). After 2 days at 25.5°C, they were shifted back to 22°C and their life span was measured. In the second trial, *rrf-3* animals were kept at 22°C throughout three generations, and the life span of the third generation was measured at 22°C as soon as they reached the first day of adulthood.

osm-3 is required for sensory function (Tabish et al., 1995), and mutations within this gene extend life span (Apfeld and Kenyon, 1999). Feeding-induced RNAi of this gene was performed as a positive control for RNAi of neuronally expressed genes that affect life span. Like *str-2*, *srd-1* encodes a putative chemosensory G protein-coupled receptor (Troemel et al., 1995). Unlike *str-2*, feeding-induced RNAi of *srd-1* had no effect on the life span of *rrf-3* animals (data not shown). Since RNAi only reduces gene function (Timmons and Fire, 1998), it is possible that mutations that completely abolish *srd-1* activity would affect life span. Alternatively, unlike *str-2*, the putative chemosensory receptor *srd-1* might not influence life span. The *osm-3*, *srd-1*, and *str-2* RNAi clones (Fraser et al., 2000; Kamath et al., 2003) were sequenced to ensure that they correspond to their respective ORFs (Tabish et al., 1995; Troemel et al., 1995, 1999). The *str-2* clone includes the 231 base pairs upstream of the *str-2* translational start site and is missing the last 208 bp of its mRNA.

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