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Chemosensory Neurons Function in Parallel to Mediate a Pheromone Response in C. elegans

Wendy S. Schackwitz, Takao Inoue, and James H. Thomas
Department of Genetics
University of Washington
Seattle, Washington 98195

Summary

Formation of the C. elegans dauer larva is repressed by the chemosensory neurons ADF, ASI, and ASG. Mutant analysis has defined two parallel genetic pathways that control dauer formation. By killing neurons in these mutants, we show that mutations in one of these genetic pathways disrupt dauer repression by ADF, ASI, and ASG. One gene in this pathway is *daf-7*, which encodes a TGFβ-related protein. We find that *daf-7::GFP* fusions are expressed specifically in ASI and that expression is regulated by dauer-inducing sensory stimuli. We also show that a different chemosensory neuron, ASJ, functions in parallel to these neurons to induce dauer formation. Mutations in the second genetic pathway activate dauer formation in an ASJ-dependent manner. Thus, the genetic redundancy in this process is reflected at the neuronal level.

Introduction

Nervous systems process complex sensory information to generate appropriate responses to environmental stimuli. To acquire and integrate this information, several sensory systems, including olfaction, taste, and vision, use parallel neural circuitry. Since most such systems are very complex, it has been difficult to identify specific sets of genes and corresponding neurons that mediate complex sensory functions. Chemosensory response to dauer-inducing stimuli in Caenorhabditis elegans provides an opportunity to gain a detailed understanding of such neuronal processing at the genetic and cellular levels. This is possible because response to dauer-inducing stimuli is specific and easily assayed, there are many identified genes that mediate the process, and specific neurons in which these genes function to regulate dauer formation can be identified.

Environmental signals that influence C. elegans larval development are sensed and interpreted by the nervous system (reviewed by Thomas, 1993). Under favorable conditions, C. elegans progresses through four larval stages (L1–L4) to become an adult. Under less favorable conditions, C. elegans forms a developmentally arrested third-stage larva called the dauer larva. The dauer larva does not feed and is resistant to harsh conditions (Cassada and Russell, 1975). When conditions improve, the dauer larva recovers and resumes its life cycle (Cassada and Russell, 1975). The primary environmental signal that induces dauer formation is a pheromone (Golden and Riddle, 1984a), which is stable, nonvolatile, and constitutively produced (Golden and Riddle, 1982). Thus, the concentration of dauer-inducing pheromone in the environment reflects population density. Other environmental signals such as scarce food and high temperature also encourage dauer formation (Golden and Riddle, 1984b).

Response to dauer pheromone requires a pair of bilaterally symmetrical sensory organs called amphids (Albert et al., 1981; Perkins et al., 1986; Shakir et al., 1993; Vowels and Thomas, 1994). Each amphid is composed of twelve sensory neurons, eight of which are directly exposed to the external environment through a pore in the cuticle (Perkins et al., 1986). These exposed endings are ciliated, as are vertebrate olfactory neurons (Ache, 1991). Three amphid neuron classes, ADF, ASI, and ASG, repress dauer formation under non-dauer-inducing conditions (Bargmann and Horvitz, 1991).

Many genes that regulate dauer formation have been identified. Mutations in most of these genes result in one of two phenotypes: dauer formation–defective (Daf-d) mutations cause failure to form dauers under dauer-inducing conditions, and dauer formation–constitutive (Daf-c) mutations cause dauers to form even under non-inducing conditions. These genes have been ordered in a complex genetic pathway based largely on gene interactions (Riddle et al., 1981; Vowels and Thomas, 1992; Thomas et al., 1993; Gottlieb and Ruvkun, 1994). These genetic studies suggest that sensory transduction of dauer-inducing signals is mediated by two parallel branches as shown in Figure 1 (Thomas et al., 1993; Malone and Thomas, 1994). We refer to genes in the upper branch of this genetic pathway as group 1 and genes in the lower branch as group 2. One large class of genes, called cilium-structure genes, appear to function in both the group 1 and group 2 branches of the pathway. Mutations in these genes cause the ciliated sensory endings to be structurally abnormal, causing a Daf-d phenotype (Perkins et al., 1986). The group 2 branch of the pathway includes five Daf-c genes, at least three of which encode components of a transforming growth factor β (TGFβ)-related transduction pathway: *daf-7* encodes a TGFβ-related protein (Lim, 1993), and *daf-1* and *daf-4* encode proteins similar to TGFβ receptor subunits (Georgi et al., 1990; Estevez et al., 1993).

In this study, we address how the parallel genetic pathways relate to neuronal regulation of dauer formation. To determine the neurons in which different parts of the genetic pathway function, we killed specific neurons in the wild type and in various mutants to determine the effect on dauer formation. We find that one set of sensory neurons represses dauer formation, while a distinct set promotes dauer formation. We also find that *daf-7* fusions to green fluorescent protein (GFP) are expressed specifically in ASI, one of the dauer-repressing neurons. Our data indicate that the two branches of the genetic pathway perturb sensory function differently and that they may function in parallel sets of sensory neurons.

Results

Our goal in this study was to define the sensory neuronal pathways that regulate dauer formation and to relate

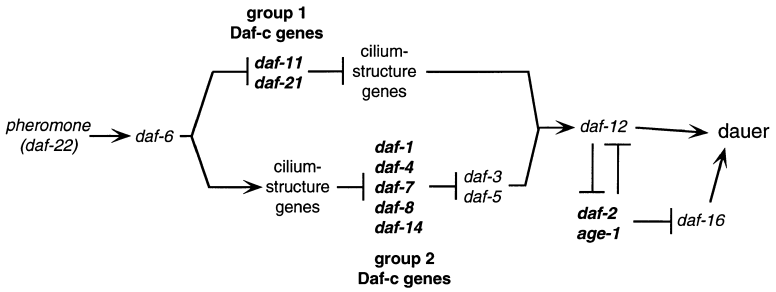

Figure 1. Parallel Branches in the Genetic Pathway for Dauer Formation

Daf-c genes are shown in bold, and Daf-d genes are shown in plain text. The Daf-c genes in the upper branch are called group 1, and the Daf-c genes in the lower branch are called group 2. Arrows indicate positive regulatory interactions, and lines ending in bars indicate negative interactions.

these neuronal pathways to the described genetic pathway for dauer formation. Sensory neurons could regulate dauer formation either by repressing or by promoting dauer formation. Dauer-repressing neurons would actively repress dauer formation under noninducing conditions and derepress in response to dauer-inducing environmental conditions. Dauer-promoting neurons would be inactive under noninducing conditions and activate dauer formation in response to dauer-inducing conditions. Killing a dauer-repressing neuron would result in inappropriate derepression of dauer formation (Daf-c), whereas killing a dauer-promoting neuron would inhibit dauer formation (Daf-d).

Bargmann and Horvitz (1991) have shown that ADF, ASI, and ASG are dauer-repressing neurons. When ADF and ASI were killed together, a Daf-c phenotype resulted. (Because the amphids are bilaterally symmetrical, in their study and throughout this study, the corresponding neurons in both amphids were killed, except where noted.) When ASG was killed in addition to ADF and ASI, the penetrance of the Daf-c phenotype increased. No effect on dauer formation was seen when either ADF or ASI remained intact. The Daf-c phenotype caused by killing ADF, ASI, and ASG was suppressed by group 2 Daf-d mutations in *daf-3* and *daf-5*, but was not suppressed by mutations in the cilium-structure genes. These data are consistent with ADF, ASI, and ASG mediating the group 2 branch of the genetic pathway (see Figure 1). However, mutations in *daf-3* and *daf-5* partially suppress group 1 Daf-c mutations (Vowels and Thomas, 1992; Thomas et al., 1993), making the assignment of ADF, ASI, and ASG to the group 2 branch of the pathway inconclusive.

Group 2 Daf-c Mutations Disrupt Dauer Repression by ADF, ASI, and ASG

To relate the function of ADF, ASI, and ASG to the genetic pathway, we tested whether group 1 or group 2 Daf-c mutations could disrupt dauer repression by these neurons. To perform this test, we conducted a series of neuron isolation experiments, in which all exposed amphid neurons except ADF, ASI, and ASG were killed in the wild type and in Daf-c mutants. We reasoned that some Daf-c mutations might disrupt the capacity of these neurons to repress dauer formation, while other Daf-c mutations might not. We killed AFD and all exposed amphid neurons except for ADF, ASI, and ASG in the wild type and in Daf-c mutants and determined whether dauer formation was still repressed (Table 1). Though not exposed to the environment, AFD was included because it regulates response to temperature (Mori and Ohshima, 1995), an important modulator of dauer formation (Golden and Riddle, 1984a). As previously reported (Bargmann and Horvitz, 1991), we found that isolated ADF, ASI, and ASG neurons were sufficient to prevent dauer formation in the wild type. These neurons were also sufficient to prevent dauer formation in two *daf-11* mutants, suggesting that *daf-11*

Table 1. Isolation of ADF, ASI, and ASG Neurons in the Wild-Type and Mutants

Strain	Amphid Neurons Killed	Exposed Amphid Neurons Left Intact	Number of Dauers/Total
Wild type	None	All (ASE, ADF, ASG, ASH, ASI, ASJ, ASK, ADL)	0/(>100)
Wild type	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	0/5
daf-7(e1372)	None	All	45/45
daf-7(e1372)	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	6/6
daf-7(e1372)	AWA, AWB, AWC, AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	2/2
daf-1(sa184)	None	All	23/23
daf-1(sa184)	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	4/4
daf-11(sa195)	None	All	121/126
daf-11(sa195)	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	0/5
daf-11(m87)	None	All	14/15
daf-11(m87)	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	1/6
daf-21(p673)	None	All	71/76
daf-21(p673)	AFD, ASE, ASH, ASJ, ASK, ADL	ADF, ASI, ASG	4/6

Experiments were done at 25°C. Controls in which no cells were killed were otherwise treated identically to the kills (see Experimental Procedures). Exposed amphid sensory neurons: ASE, ADF, ASG, ASH, ASI, ASJ, ASK, and ADL. Unexposed amphid sensory neurons: AFD, AWA, AWB, and AWC.

does not disrupt ADF, ASI, or ASG function. In contrast, these neurons were not sufficient to prevent dauer formation in *daf-1* or *daf-7* mutants (Table 1), indicating that *daf-1* and *daf-7* are required for dauer repression by ADF, ASI, and ASG. All evidence to date suggests that only exposed amphid neurons mediate response to dauer-inducing conditions (Bargmann and Horvitz, 1991; Vowels and Thomas, 1992, 1994). However, to test further whether group 2 Daf-c mutations might affect dauer formation through unexposed amphid neurons, all exposed and unexposed amphid neurons except ADF, ASI, and ASG were killed in a *daf-7* mutant (Table 1). These animals still formed dauers, ruling out the possibility that *daf-7* mutations require unexposed amphid neurons to promote dauer formation. We conclude that Daf-c mutations in the group 2 branch of the genetic pathway disrupt dauer repression by ADF, ASI, and ASG. Since mutations in *daf-11* did not perturb dauer repression by ADF, ASI, and ASG, we hypothesize that the *daf-11* branch of the genetic pathway functions through other neurons. The group 1 mutation *daf-21(p673)* partially disrupted dauer repression by ADF, ASI, and ASG, suggesting that *daf-21* may function both in ADF, ASI, and ASG and in additional neurons (see below and Discussion).

daf-7::GFP Is Expressed in ASI

To test molecularly whether the group 2 genes mediate the function of ADF, ASI, and ASG, we determined where *daf-7::GFP* fusions are expressed (Chalfie et al., 1994). Of the genes in this branch of the pathway, *daf-7* seemed most likely to be expressed in the sensory neurons because it encodes a TGFβ-related protein (Lim, 1993), which could function as a dauer-repressing signal. We generated two constructs in which GFP was fused in frame at amino acid 197 of *daf-7* (see Experimental Procedures). Each fusion contained 4.8 kb of DNA upstream and 1.3 kb downstream of the *daf-7* start codon. One fusion had a nuclear localization signal, and the other did not. We generated transgenic strains with fusions integrated into the chromosome (Mello et al., 1991). Animals with either construct expressed *daf-7::GFP* in the cell body of one pair of bilaterally symmetric neurons in the lateral ganglia. Based on their positions in the L1 (Sulston et al., 1983), we identified these as the two ASI neurons (Figure 2). GFP was consistently expressed starting in the L1, and expression became stronger in the L2, the stages during which dauer formation is controlled (Swanson and Riddle, 1981). Since GFP acquires its fluorescence slowly (Heim et al., 1994), this pattern is consistent with the report that levels of *daf-7* mRNA peak in the L1 (Lim, 1993). After the L2, GFP fluorescence became progressively weaker and, by adulthood, was nearly undetectable. Since the fusion protein may persist after mRNA degradation, these results are consistent with the absence of *daf-7* mRNA after the L2 stage (Lim, 1993). Rarely, other cells showed weak *daf-7::GFP* expression. When seen, there was only one such cell, and its identity varied from animal to animal. To corroborate that the fusion is expressed predominantly in ASI, we killed both ASI neurons in *daf-7::GFP*-containing animals. In four of five animals, we detected no GFP in the

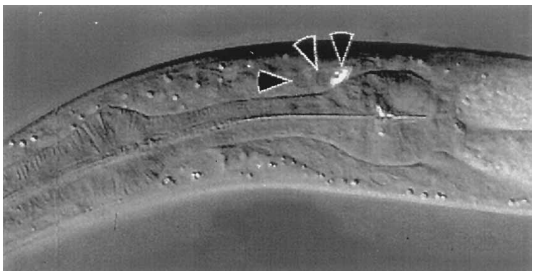

Figure 2. GFP Fluorescence in a *daf-7::GFP* Strain

Combined epifluorescence and Nomarski photograph of the strain JT8459 *lin-15(n765ts); sals8*, which carries an integrated *daf-7::GFP* fusion. The view is of the lateral ganglion of an L2 animal in the focal plane of ASI on the right side (ASIR). The positions of ASKR, ADLR, and ASIR (from left to right) are indicated by arrowheads. The epifluorescence and Nomarski images were photographed separately and were superimposed using Adobe Photoshop.

L1 and L2, and the fifth animal weakly expressed GFP in one unidentified cell in the lateral ganglion (not in the position of ASI). These results indicate that ASI is the only cell that consistently expresses *daf-7::GFP* at detectable levels in larvae. The occasional expression in other neurons may be due to an incomplete promoter or from transcriptional influences at the site of integration of the transgene. Interestingly, the strain with the highest GFP expression (carrying *sals8*) has a Daf-c phenotype (49% dauers at 25°C; n = 169). One possibility is that this phenotype is due to competition for expression or secretion of the wild-type *daf-7* gene product. The *sals7* strain produced less than 1% dauers at any temperature.

Dauer formation is induced by high levels of dauer pheromone. To test whether this induction might result in part from regulation of the expression of *daf-7* protein, we observed expression of the *daf-7::GFP* fusion in animals exposed to various pheromone levels. When exposed to even low concentrations of pheromone, *daf-7::GFP* was undetectable throughout the L1 and L2 stages (Table 2). This result suggests that the activity state of the ASI neurons regulates *daf-7* expression. Such regulation could account for the action of dauer pheromone through this branch of the dauer pathway. Similar results on the expression of *daf-7::GFP* in ASI and its

Table 2. Fraction of ASI Neurons Expressing *daf-7::GFP* When Exposed to Pheromone

Strain	Larval Stage	Units of Pheromone 0	0.5	1	2
sals8	L1	17/24	0/24	0/22	2/28[a]
	L2	28/34	0/34	0/46	0/36
sals7	L1	18/66	0/20	0/22	0/52
	L2	26/40	2/28	0/32	0/42

Assays were performed at 15°C. Similar assays of *sals8* at 20°C gave similar results (data not shown). As a control, *edls1*, an integrated *unc-119::GFP* fusion that is expressed in many neurons, was tested, and the pattern and level of expression were not detectably affected by pheromone (data not shown).

[a] These two cells were very faint and were probably not ASI, but the animal was twisted, making positive identification difficult.

Table 3. Strength of *daf-7::GFP* Fluorescence at Different Temperatures

Temperature	Larval Stage	*daf-7::GFP* Fluorescence		
		None	Weak	Strong
15°C	L1	82	13	11
	L2	10	8	24
20°C	L1	48	3	1
	L2	54	8	10
25°C	L1	32	0	0
	L2	12	0	0

Assays were with *sals7* on uncrowded standard NG agar plates (non-dauer-inducing conditions). Cells were qualitatively divided into those in which no fluorescence was observed, those with fluorescence comparable to the typical strong expression, and those with detectable but clearly less intense fluorescence (weak). An integrated *unc-119::GFP* fusion (*edls1*, provided by D. Pilgrim) and an extrachromosomal array of *sra-6::GFP* (*saEx163*, fusion DNA provided by C. Bargmann) were observed as controls, and no difference in frequency, pattern, or intensity of fluorescence was observed (at least 15 animals for each fusion at each temperature).

regulation by pheromone have been obtained by P. Ren and D. Riddle (personal communication).

High growth temperature also promotes dauer formation (Golden and Riddle, 1984b). To test whether this temperature effect might also reflect regulation of *daf-7* expression, we observed *daf-7::GFP* expression in animals grown at various temperatures. We found that expression was strongest in animals grown at 15°C, intermediate at 20°C, and weakest at 25°C (Table 3). Expression of two control GFP fusions was not detectably affected by temperature. These results suggest that part of the temperature sensitivity of dauer formation is accounted for by changes in *daf-7* expression. *daf-7* expression is unlikely to account fully for this temperature sensitivity, since dauer formation remains temperature sensitive in all group 2 Daf-c single and double mutants (Golden and Riddle, 1984a; Vowels and Thomas, 1992; Thomas et al., 1994).

ASJ Is Required for the Daf-c Phenotype of a *daf-11* Mutant

Since no amphid neurons other than ADF, ASI, and ASG are required to repress dauer formation, we hypothesized that the Daf-c phenotype of *daf-11* results from the activity of dauer-promoting neurons and that loss of *daf-11* function inappropriately activates these neurons, causing constitutive dauer formation. This hypothesis predicts that killing these dauer-promoting neurons would suppress the Daf-c phenotype of *daf-11* mutants. The experiments in Table 1 showed that killing all of the exposed amphid neurons and AFD suppresses dauer formation in *daf-11*, suggesting that *daf-11* functions in a subset of these neurons. To determine which amphid neurons were responsible for the suppression of *daf-11*, each neuron class was killed individually in a *daf-11* mutant (Figure 3A). Only killing ASJ resulted in statistically significant suppression of the *daf-11* Daf-c phenotype ($p < 0.0001$). Since killing ASJ did not completely suppress *daf-11*, we tested whether the suppression could be strengthened by killing other neurons in addition to ASJ (Figure 3B). Suppression of *daf-11* was probably stronger when ASJ and ASK ($p = 0.03$) or ASJ and ADL ($p = 0.01$) were killed together. Killing the thermosensory neuron AFD may also enhance the ASJ kill, though the change was marginally significant ($p = 0.07$). All other amphid neurons were killed in combination with ASJ and none enhanced (ASH and ASE data are shown as an example in Figure 3B). These results indicate that extraneous laser-induced damage is not the cause of enhanced suppression by ASK and ADL and that no other amphid neuron contributes significantly to the *daf-11* Daf-c phenotype. We conclude that the Daf-c phenotype of *daf-11* is dependent on ASJ, with ASK and ADL probably also playing a minor role.

To test whether ASJ alone could mediate the Daf-c phenotype of a *daf-11* mutant, we killed all exposed and unexposed amphid neurons except for ASJ and ASI in one experiment, and all but ASJ and ADF in a second experiment. It is not possible to interpret an experiment in which only ASJ is left intact, because killing ADF and ASI causes a Daf-c phenotype (Bargmann and Horvitz, 1991). In both experiments, the Daf-c phenotype of *daf-11* was not suppressed (Figure 3C). As a control, the same kills were performed in the wild type, and 0 out of 2 dauers were formed in each case. These data suggest that ASJ is sufficient among exposed amphid neurons to mediate the Daf-c phenotype of *daf-11(sa195)*.

To determine whether function of both the left and right ASJ neurons is required to induce dauer formation in *daf-11* mutants, we killed ASJ in only one amphid (Figure 3C). This unilateral kill reduced dauer formation of a *daf-11* mutant regardless of whether the right or left neuron was killed (side not shown), suggesting that the ASJ neurons from both sides of the animal contribute to dauer formation. Similar results were obtained when ASJ, ASK, and ADL were killed together in only one amphid (Figure 3C).

ASJ Is Required for the Group 1 but Not the Group 2 Daf-c Phenotype

The experiments in the previous section were performed with one *daf-11* mutation. To determine whether suppression of the Daf-c phenotype was specific to *daf-11(sa195)*, we tested whether killing ASJ alone or killing ASJ, ASK, and ADL together suppresses other Daf-c mutations. Killing ASJ suppressed dauer formation for two additional alleles of *daf-11* (*m84* and *m87*), as well as for *daf-21(p673)*, another group 1 Daf-c mutation (Figure 4A). In contrast, even weak group 2 Daf-c mutations such as *daf-8(sa234)* and *daf-14(m77)* were not significantly suppressed by killing ASJ (Figure 4B). In addition, a Daf-c mutation in *daf-2*, a gene implicated in a downstream step of the pathway (Figure 1), was not suppressed by killing ASJ (Figure 4B). Similar results were obtained when ASJ, ASK, and ADL were killed together (Figures 4A and 4B). These results show that killing ASJ specifically suppresses group 1 Daf-c mutations.

Killing ADF and ASI Enhances Group 1 Daf-c Mutations

Genetic analysis has shown that Daf-c mutations in the two parallel branches of the dauer pathway are syner-

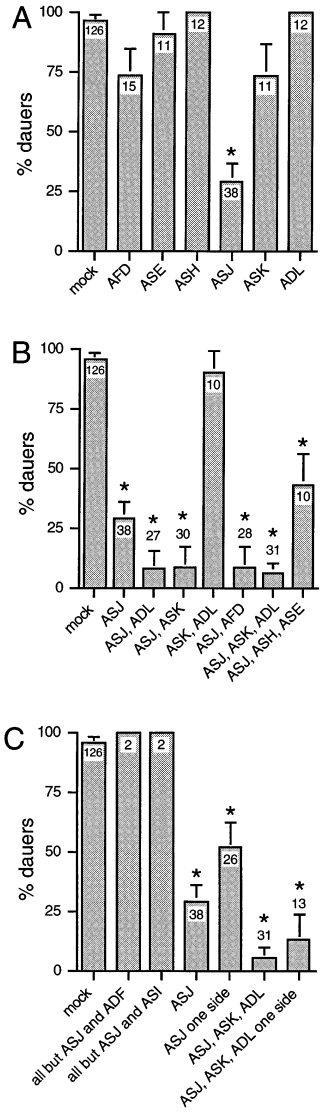

Figure 3. Effects of Killing Amphid Neurons in a *daf-11(sa195)* Mutant

Experiments were conducted at 25°C. The number of animals tested is indicated in each bar. An asterisk indicates a highly significant difference from the mock-kill control ($p < 0.0001$). The error bars show the standard error of the mean. For cases in which all animals formed dauers, the standard error cannot be meaningfully calculated.

(A) Kills of single neuron pairs. Only killing ASJ strongly affected dauer formation. Killing ASK or AFD resulted in moderately significant suppression ($p = 0.008$ and 0.02, respectively).

(B) Kills of multiple neuron pairs. Killing certain other neurons in addition to ASJ may have increased suppression compared with killing ASJ alone, as follows: ADL ($p = 0.01$), ASK ($p = 0.03$), ASK and ADL ($p = 0.03$), and AFD ($p = 0.07$). Each other amphid neuron was killed together with ASJ, and none showed significantly enhanced suppression (data not shown).

(C) Isolation experiments and kills of neurons on one side only. In the animals for the second and third bars (isolation experiments), all of the exposed and unexposed amphid neurons were killed except for the two noted. The same two isolation experiments were performed on wild-type animals to test whether killing these neurons would generate a Daf-c phenotype. In each case, 4 out of 4 animals formed a non-dauer.

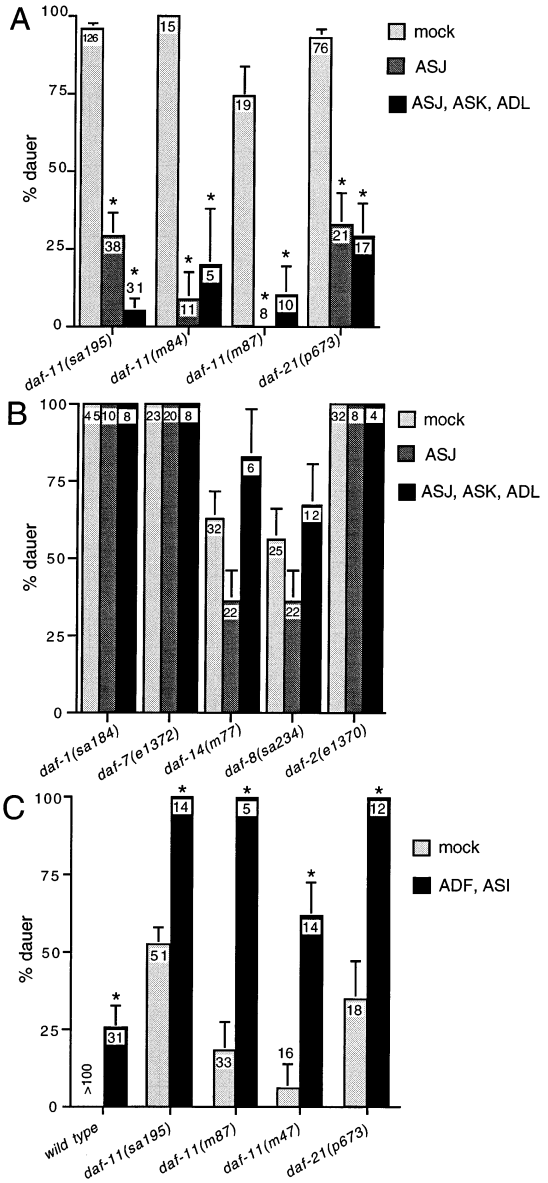

Figure 4. Effects of Killing ASJ, ASK, and ADL in Other Daf-c Mutants and of Killing ADF and ASI in Group 1 Daf-c Mutants

Experiments in (A) and (B) were at 25°C, and experiments in (C) were at 20°C. An asterisk indicates that a result is significantly different from mock-kill controls. The number of animals tested is indicated for each bar. Error bars show the standard error of the mean.

(A) Kills in other *daf-11* mutants and in *daf-21(p673)*. For each strain, killing ASJ significantly suppressed the Daf-c phenotype ($p < 0.001$) and killing ASJ, ASK, and ADL together significantly suppressed the Daf-c phenotype ($p < 0.002$).

(B) Kills in group 2 and *daf-2* Daf-c mutants. Killing ASJ or ASJ, ASK, and ADL together did not significantly alter the Daf-c phenotype of these mutants (*daf-14(m77)*, J kill, $p = 0.10$; *daf-8(sa234)*, J kill, $p = 0.24$; all other p values were larger).

(C) Kills of ADF and ASI in group 1 Daf-c mutants. P values for the kills compared with the mock kills in each strain are as follows: *daf-11(sa195)* $p < 0.0001$, *daf-11(m87)* $p = 0.0016$, *daf-11(m47)* $p = 0.021$, and *daf-21(p673)* $p = 0.0006$.

gistic, since double mutants between genes in each branch of the pathway are more strongly Daf-c than any of the single mutants or double mutants within the same

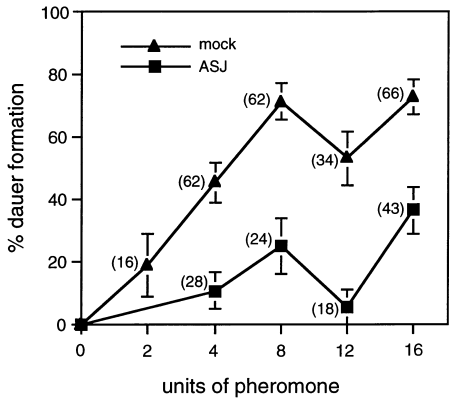

Figure 5. Killing ASJ in the Wild Type Impairs Pheromone Response
Experiments were conducted at 25°C. Error bars show the standard error of the mean. Units of pheromone are as defined by Thomas et al. (1993). Numbers in parentheses are the number of animals tested for each condition. P values for the comparison of mock and ASJ kills at each pheromone concentration were $p < 0.001$, except for the four unit point ($p = 0.004$).

pathway (Thomas et al., 1993). If ADF, ASI, and ASG are specific to the group 2 branch of the pathway, as our results suggest, then the Daf-c phenotype that is generated by killing these neurons should enhance the Daf-c phenotype of mutations in the group 1 branch of the pathway. To make this potential enhancement more apparent, we wanted to test conditions under which the group 1 mutation and the dauer-repressing neurons each make weak contributions to the Daf-c phenotype. Because dauer formation is a temperature-sensitive process, mutations that cause a Daf-c phenotype are more penetrant at higher temperatures (Golden and Riddle, 1984a). Therefore, a weak Daf-c phenotype from group 1 mutations was achieved by growing the animals at a low temperature (15°C). A weak Daf-c contribution from the dauer-repressing neurons was accomplished by killing only ADF and ASI (Bargmann and Horvitz, 1991). We tested whether killing ADF and ASI enhanced the Daf-c phenotype of three different *daf-11* mutations and the one existing *daf-21* mutation. A stronger Daf-c phenotype was observed when the kill and mutation were combined than in either single condition (Figure 4C). We conclude that the genetically defined enhancement of *daf-11* and *daf-21* by group 2 Daf-c mutations results from their effects on the ADF or ASI neuronal pathway.

ASJ Contributes to Pheromone Response in the Wild Type

It remained possible that *daf-11* and *daf-21* mutations generate a state not reflected in wild-type regulation of dauer formation. If ASJ is involved in transducing pheromone response in the wild type, then killing ASJ might impair response to pheromone. We killed ASJ in the wild type and exposed the animals to several different concentrations of pheromone. At each pheromone concentration, killing ASJ significantly impaired the ability of wild-type animals to form dauers (Figure 5). We conclude that the activity of ASJ contributes to the activation of dauer formation in the wild type.

Discussion

Our results suggest that two parallel genetic pathways function in different sets of amphid sensory neurons to control dauer formation. We showed that group 2 but not *daf-11* Daf-c mutations disrupt the repression of dauer formation by ADF, ASI, and ASG, suggesting that ADF, ASI, and ASG mediate the function of the group 2 genetic pathway. We also showed that *daf-7*, one of the group 2 Daf-c genes, is specifically expressed in ASI, consistent with the group 2 genetic pathway functioning as part of the ADF, ASI, and ASG neuronal pathway. We propose that the TGFβ-related protein encoded by *daf-7* is produced and secreted by ASI to inhibit dauer formation. We also identified a dauer-promoting neuron, ASJ, that is required for the Daf-c phenotype of *daf-11* and *daf-21* but not group 2 Daf-c mutations, indicating that ASJ is required for the function of the *daf-11* and *daf-21* genetic pathway. We also showed that ASJ is required in the wild type for full dauer–pheromone response.

The Parallel Genetic Pathways That Mediate Dauer Formation Are Reflected in Parallel Neuronal Pathways

Based on our results, we present a model for sensory regulation of dauer formation (Figure 6). The dauer-repressing neurons ADF and ASI mediate the group 2 genetic pathway, whereas the dauer-promoting neuron ASJ is required for activation of dauer formation by *daf-11* and *daf-21*. Additional neurons ASG, ASK, and ADL may make smaller contributions to dauer regulation (not shown in figure). In the absence of dauer pheromone, the dauer-repressing neurons ADF and ASI are active and repress dauer formation, and the dauer-promoting neuron ASJ is inactive. When pheromone is present at levels sufficient to promote dauer formation, the dauer-repressing neurons ADF and ASI are inactivated and ASJ is activated. We have arbitrarily described these neuronal activities as regulating dauer formation, but they can also be described as regulating L3 formation. For example, we have described ASJ as promoting dauer formation, but we are currently unable to distinguish this from ASJ repressing L3 formation. The key distinction between ASJ and the ADF and ASI group is whether the neurons are active in the presence of pheromone (ASJ, inducing dauer or repressing L3) or in the absence of pheromone (ADF and ASI, repressing dauer or inducing L3).

Interesting parallels with the sensory regulation of dauer formation are found in vertebrate retinal bipolar cells (Tessier-Lavigne, 1991). There are two classes of bipolar cells, called on-center and off-center. In response to an on-center light stimulus to their receptive field, on-center bipolar cells are excited and off-center bipolar cells are inhibited. In response to the opposite sensory stimulus (off-center light), on-center bipolar cells are inhibited and off-center bipolar cells are excited. The resulting changes in bipolar cell synaptic activity are passed on to the brain by ganglion cells. Studies with selective inhibitors of on-center bipolar cell activity suggest that most aspects of visual function are redundantly encoded by these two parallel classes of

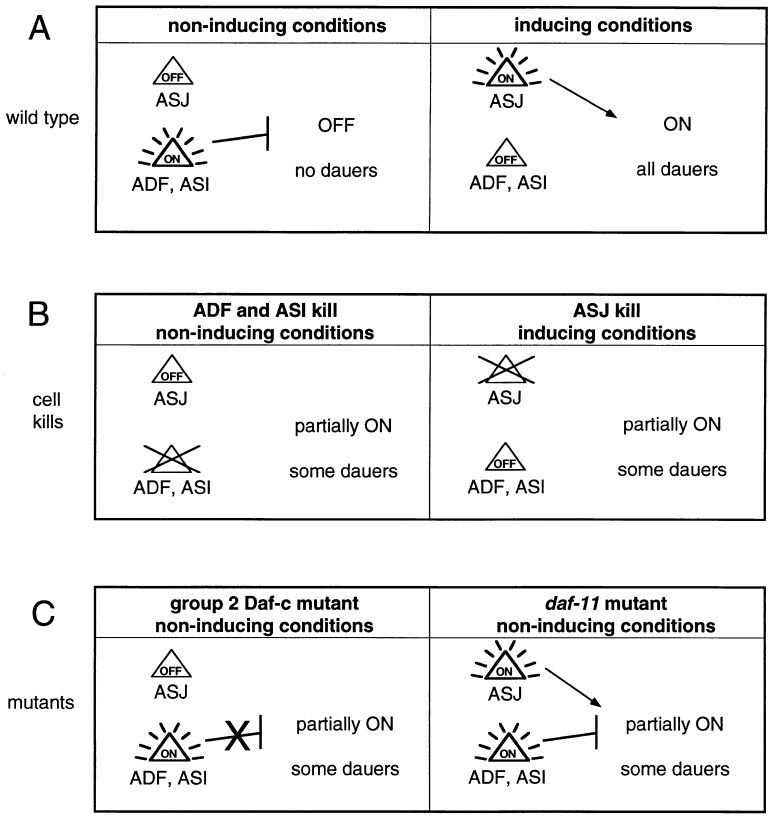

Figure 6. Combined Cellular and Genetic Model for Dauer Formation

Triangles represent amphid neurons. Arrows indicate dauer-promoting signals, and lines ending in bars indicate dauer-repressing signals.

(A) The effects of non-dauer-inducing and dauer-inducing conditions on the activity of dauer-inducing and dauer-repressing neurons. Dauer-inducing conditions activate the dauer-promoting neuron ASJ and inactivate the dauer-repressing neurons ADF and ASI. There may be minor involvement of ASK and ADL (dauer inducing) and ASG (dauer repressing).

(B) The effects on dauer formation of killing dauer-repressing or dauer-inducing neurons. The effect of killing ADF and ASI is apparent under non-dauer-inducing conditions because they normally repress dauer formation under these conditions. Dauer formation is only partially activated because the dauer-regulating functions of other neurons (including ASJ) remain intact. The effect of killing ASJ is apparent only under inducing conditions because ASJ is active only under these conditions. Dauer formation in response to pheromone is only partially blocked by killing ASJ because the dauer-regulating functions of other neurons (including ADF and ASI) remain intact.

(C) The effects of *daf-11* or group 2 Daf-c mutations on the activity of dauer-repressing and dauer-inducing neurons under non-dauer-inducing conditions. Group 2 Daf-c mutations block the capacity of ADF or ASI to repress dauer formation, but ASJ remains unactivated. In *daf-11* mutants, ASJ inappropriately promotes dauer formation, but ADF and ASI remain activated to repress dauer formation. The result in each case is partial activation of dauer formation. The defect in the other group 1 mutant, *daf-21*, is probably quite similar to *daf-11*, but genetic analysis and the experiments in Table 1 suggest that *daf-21* interpretation is less clear.

bipolar cell (Schiller, 1982). It is thought that parallel pathways function primarily to enhance contrast sensitivity and to respond rapidly to light changes (Schiller et al., 1986).

Molecular cloning of group 2 Daf-c genes has suggested that they affect dauer formation by altering either neuronal development or neuronal signal transduction. In accord with the identity of *daf-7*, the products of *daf-1* and *daf-4* are members of the TGFβ receptor family (Georgi et al., 1990; Estevez et al., 1993). These identities indicated that the *daf-7* gene product might be a ligand that is released by ADF, ASI, and ASG in the absence of pheromone and that acts on the gene products of *daf-1* and *daf-4* to repress dauer formation. In this model, when *daf-7* or its receptor are mutated, this repression would fail, resulting in a Daf-c phenotype (Figure 6). Our expression data suggest that *daf-7* is expressed only in ASI. This may indicate that an additional pathway functions in ADF and ASG, or it may reflect an incomplete expression pattern from the *daf-7::GFP* fusion gene. Expression of a Daf-c gene only in ASI is surprising, because killing only ASI does not cause a Daf-c phenotype (Bargmann and Horvitz, 1991; our data not shown). It is possible that mutating *daf-7* leaves ASI in a state that promotes dauer formation, for example by releasing a different transmitter. Alternatively, killing ASI in the early L1 may not fully eliminate neuronal function by the time of dauer formation is regulated (though killing ASI did eliminate detectable *daf-7::GFP* expression). An alternative model for the function of the group 2 Daf-c genes is that they regulate neuronal development. In this model, mutations in the group 2 Daf-c genes result in failure of ADF, ASI, and ASG to develop into dauer-repressing neurons, thus causing a Daf-c phenotype. Expression of *daf-7* only in ASI is difficult to reconcile with this model.

The specifics of gene function in the group 1 branch of the pathway await the molecular characterization of *daf-11* and *daf-21*. Because mutations that affect the structure of the amphid sensory endings suppress the Daf-c phenotype of *daf-11* and *daf-21* mutants, it has been inferred that the *daf-11* and *daf-21* gene products function in these sensory endings (Vowels and Thomas, 1992; Thomas et al., 1993). It seems likely that the *daf-11* gene product functions in the sensory ending of the dauer-promoting neuron ASJ to keep ASJ inactive. When *daf-11* is mutant, ASJ is inappropriately activated, causing a Daf-c phenotype. It is also possible that the activity of ASJ is indirectly required for activation of dauer formation by *daf-11*. For example, *daf-11* might function in a different neuron whose dauer-regulating function depends on the presence of ASJ. The results in Table 1 and genetic analysis (Thomas et al., 1993; E. A. Malone, personal communication) suggest that *daf-21(p673)* does not fully activate the group 1 genetic pathway and that it may also affect other pathways that

are relatively ASJ independent. In addition, *daf-21(p673)* may not cause a simple loss of gene function (E. A. Malone, personal communication), making further interpretation of *daf-21* difficult at this time.

Killing ASJ did not completely suppress *daf-11* or *daf-21* mutations. This may indicate that activity of ASJ is not fully required for dauer activation by *daf-11* and *daf-21* function. For example, *daf-11* might be expressed in both ASJ and other neurons, perhaps ASK and ADL. Alternatively, laser-damaged ASJ neurons may not lose function immediately. This explanation is particularly plausible in this case because the critical time period for dauer formation starts about 10 hr after the neurons are killed (Swanson and Riddle, 1981; Golden and Riddle, 1984b).

ASJ Mediates Dauer Formation and Dauer Recovery

In addition to playing a role in dauer formation, ASJ promotes dauer recovery (Bargmann and Horvitz, 1991). Interestingly, most *daf-11* mutations and *daf-21(p673)* also cause severe dauer recovery defects (Vowels and Thomas, 1994), suggesting that these genes are key regulators of dauer recovery as well as dauer formation. However, inappropriate activation of ASJ by mutations in *daf-11* and *daf-21* during dauer recovery would be expected to promote, rather than inhibit, recovery. One possible explanation of this contradiction is that a developmental change occurs in ASJ in the dauer larva, such that *daf-11* and *daf-21* mutations now inactivate the neuron. Alternatively, *daf-11* and *daf-21* might not function in ASJ during dauer recovery.

Why Is There More Than One Pathway?

Our results indicate that two sets of molecularly distinct amphid neurons function in parallel to regulate dauer formation. In an animal with so few neurons at its disposal, why would C. elegans devote two sets of amphid neurons to this purpose? One possibility is that two pathways allow refined precision or fidelity of response to unpredictable environmental conditions, in a manner similar to the function of parallel pathways in retinal bipolar cells. The decision of whether to become a dauer is critical. If an animal fails to form a dauer appropriately, the result may be starvation and death. On the other hand, dauer formation and recovery are time-consuming and presumably energy-consuming processes, so forming a dauer inappropriately will confer a reproductive disadvantage. Thus, there should be strong selective pressure to perfect the decision to form a dauer. Integrating signals from two independent pathways might contribute to a precise and robust decision. For example, there might be two pheromones, each of which can partially induce dauer formation and both of which are present in our crude preparations of pheromone. An example of such a system exists in Bacillus subtilis, in which two convergent pathways mediate the response to two pheromones that induce genetic competence (Solomon et al., 1995). Two pheromones acting synergistically might reduce problems of noise that are inevitable in high gain sensory transduction. Alternatively, two pheromones might carry distinct environmental information (Solomon et al., 1995). Another possibility is that the two pathways might respond to signals other than pheromone. Though both pathways appear to mediate pheromone response, they may also integrate response to food, temperature, or other unknown sensory factors. Having two pathways might enrich the sophistication with which these multiple cues can be effectively integrated.

Experimental Procedures

Strains

C. elegans strains were maintained as described by Brenner (1974). The following strains were used: wild-type strain N2, JT195 *daf-11 (sa195)*, DR87 *daf-11(m87)*, JT5936 *daf-11(m84)*, DR47 *daf-11(m47)*, JT6130 *daf-21(p673)*, JT184 *daf-1(sa184)*, JT6918 *daf-7(e1372)*, JT6919 *daf-14(m77)*, JT234 *daf-8(sa234)*, CB1370 *daf-2(e1370)*, JT6273 *daf-21(p673); daf-3(e1376)*, JT6556 *daf-5(e1385); daf-11 (sa195)*, JT6640 *daf-5(e1385); daf-21(p673)*, JT6548 *daf-11(sa195); daf-3(e1376)*, JT5852 *daf-5(e1385); daf-11(m87)*, JT5877 *daf-11 (m87); daf-3(e1376)*, JT8458 *lin-15(n765ts); saIs7*, JT8459 *lin-15 (n765ts); saIs8*, and JT8454 *lin-15(n765ts); saEx128*.

Laser Kills and Pheromone Assays

All 2 × 2 contingency table statistics were performed using Fisher's exact test, run using Instat 2.01 for the Macintosh. Neurons were killed as described by Avery and Horvitz (1989), except that kills were confirmed 2–5 hr after operation. Control animals, referred to as "mock," were treated identically to operated animals, but no neurons were killed. For tests at 25°C, parent animals were transferred from 15°C to 25°C 2 days prior to the experiment to ensure that larvae were exposed to this temperature before fertilization. This was not possible for *daf-21(p673)*, which is nearly sterile at 25°C; this strain was shifted to 23°C 1 day prior to experiments. Eggs were picked, and larvae that hatched less than 2 hr before the experiment were used. After the neurons had been killed, animals were placed at the experimental temperature for 2–5 hr, after which time they were removed for confirmation of the kills. After confirmation, the animals were again placed at the experimental temperature. Mock controls were treated identically, but no cells were killed. Developing animals were observed at a minimum at 9 AM, 1 PM, 5 PM, and 11 PM daily until they had developed into either a dauer or an adult. The times at which the kills were performed and scored were chosen such that the dauer/L3 choice was made by each animal during the period from 9 AM to 11 PM. These precautions were taken to ensure that animals that formed a transient dauer would be appropriately scored (Bargmann and Horvitz, 1991; Malone et al., 1996). Tests at 20°C were treated identically to those at 25°C, except that parent animals were grown at 15°C and operated larvae were grown at 20°C. For tests of the role of ASJ in pheromone response in the wild type, larvae that had hatched from eggs less than 1 hr previous to the experiment were mounted for operation. After neurons were killed, the animals were placed immediately on plates containing dauer pheromone. Pheromone plates were made as described (Thomas et al., 1993). Plates were placed at 25°C, and dauer formation was assessed 20 hr, 28 hr, and 44 hr later.

To test pheromone response of *daf-7::GFP* strains JT8458 *lin-15(n765ts); saIs7* and JT8459 *lin-15(n765ts); saIs8*, five to ten adult animals were placed on pheromone assay plates with either no pheromone or the indicated amount of pheromone (assay and units were as described by Thomas et al., 1993) and were allowed to lay 40–60 eggs, and then the parents were removed. The plates were incubated at the experimental temperature until observation. A subset of the animals on each plate were mounted on a slide for observation of GFP fluorescence. All mounted animals on each slide were observed to ensure objectivity and were then discarded. Animals remaining on each plate after completing GFP observations were scored for dauer formation. An integrated *unc-119::GFP* fusion (*edIs1*, provided by D. Pilgrim) was used as a control for GFP expression and dauer formation. Response of experimental and control

strains to pheromone was similar and approximately that expected for the amount of pheromone and temperature used (Thomas et al., 1993). Tests on the effects of temperature were similar except that standard NG agar plates were used and an extrachromosomal array of *sra-6::GFP* was included as an additional control (*saEx163*, fusion DNA provided by C. Bargmann).

Construction and Analysis of the *daf-7::GFP* Fusion

A DNA fragment including 4.8 kb upstream and 1.3 kb downstream of the predicted *daf-7* ATG (Lim, 1993; Wilson et al., 1994; R. Waterston, A. Coulson, and J. Sulston, personal communication) was amplified by PCR with the following primers: 5′-CCGGCATGCGCCG CACACACCTGCAGCATACGG-3′ (upstream) and 5′-CGGCCCGGG CTTGACGAAGATACCTTGGATCGG-3′ (downstream). Amplification was performed with a mixture of Taq polymerase and a small amount of Pfu polymerase to achieve robust amplification with a low error rate (Barnes, 1994). Starting material was 20 ng of plasmid carrying the *daf-7* region, and the target was amplified only ~1000-fold, also to minimize errors. The fusions contain the first three introns of *daf-7* and the coding sequence up to amino acid 197, which is 37 amino acids upstream of the putative propeptide cleavage site that results in mature TGFβ ligands (Lim, 1993). The primers introduced unique SphI and XmaI sites, which were used to clone the PCR product into those sites in pPD95.70 and pPD95.79 (Chalfie et al., 1994; A. Fire, S. Xu, J. Aynn, and G. Seydoux, personal communication). These vectors carry the S65C mutation in GFP. Fusion junctions were sequenced to confirm the identity and frame of each fusion (Fire et al., 1990). Two independent clones were coinjected with a *lin-15(+)* carrying plasmid (Huang et al., 1994) into a *lin-15* mutant to generate heritable transgenic arrays (Mello et al., 1991). Two independent arrays from one of the clones were integrated into the chromosome as described (Mello et al., 1991). Expression was analyzed in detail from two integrated transgenic strains with nuclear localization sequences (JT8458 *lin-15(n765ts); saIs7* and JT8459 *lin-15(n765ts); saIs8*), and in less detail from an unintegrated transgenic strain without a nuclear localization signal (JT8454 *lin-15(n765ts); saEx128*). Expression was also assessed in other unintegrated arrays. In all cases, the pattern of GFP was qualitatively similar to the integrated arrays, except that expression was often absent in one or both ASI neurons as expected from the instability of extrachromosomal arrays. In the integrated lines, GFP expression was found predominantly in the perinuclear region of the cell body and was often punctate. Since the fusion proteins retain the signal sequence of *daf-7*, we suspect that the fusion protein was trapped in the endoplasmic reticulum or Golgi. Another GFP fusion carrying the same promoter region and the entire *daf-7* coding region was generated, but this fusion showed no GFP expression in transgenic strains, for reasons we do not know.

Tests for the Function of Group 2 Daf-c Genes

We unsuccessfully attempted two tests of the function of group 2 Daf-c genes. The first was whether killing ADF and ASI enhanced the group 2 Daf-c phenotype. The second was whether killing ASJ could eliminate pheromone response in group 2 mutants. Both tests were made impractical for the same reason: under our assay conditions, strong group 2 Daf-c mutations were too strongly Daf-c, even at low temperatures (Thomas et al., 1993; our unpublished data). Generating the very large number of animals that would be needed to test deviations from this condition was not practical. Weak group 2 Daf-c mutations (e.g., Figure 4B) reduce dauer formation but would make these experiments uninterpretable, since they presumably do not fully block the pathway.

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References

Ache, B.W. (1991). Phylogeny of taste and smell. In Smell and Taste in Health and Disease, T.V. Getchell, R.L. Doty, L.M. Bartoshuk, and J.B. Snow, Jr., eds. (New York: Raven Press), pp. 3–18.

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

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

Bargmann, C.I., and Horvitz, H.R. (1991). Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science *251*, 1243–1246.

Barnes, W.M. (1994). PCR amplification of up to 35-kb with high fidelity and high yield from lambda bacteriophage templates. Proc. Nat. Acad. Sci. USA *91*, 2216–2220.

Brenner, S. (1974). The genetics of *Caenorhabditis elegans*. Genetics *77*, 71–94.

Cassada, R., and Russell, R.L. (1975). The dauer larva, a post-embryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. *46*, 326–342.

Chalfie, M., Tu., Y., Euskirchen, G., Ward, W.W., and Prasher, D.C. (1994). Green fluorescent protein as a marker for gene expression. Science *263*, 802–805.

Estevez, M., Attisano, L., Wrana, J.L., Alberts, P.S., Massagué, J., and Riddle, D.L. (1993). The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature *365*, 644–649.

Fire, A., Harrison, S.W., and Dixon, D. (1990). A modular set of *lacZ* fusion vectors for studying gene expression in *Caenorhabditis elegans*. Gene *93*, 189–198.

Georgi, L.L., Albert, P.S., and Riddle, D.L. (1990). *daf-1*, a C. elegans gene controlling dauer larva development, encodes a novel receptor protein kinase. Cell *61*, 635–645.

Golden, J.W., and Riddle, D.L. (1982). A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science *218*, 578–580.

Golden, J.W., and Riddle, D.L. (1984a). A pheromone-induced developmental switch in *Caenorhabditis elegans*: temperature-sensitive mutants reveal a wild-type temperature dependent process. Proc. Natl. Acad. Sci. USA *81*, 819–823.

Golden, J.W., and Riddle, D.L. (1984b). The *Caenorhabditis elegans* dauer larva: developmental effects of pheromone, food, and temperature. Dev. Biol. *102*, 368–378.

Gottlieb, S., and Ruvkun, G. (1994). *daf-2*, *daf-16*, and *daf-23*: genetically interacting genes controlling dauer formation in *Caenorhabditis elegans*. Genetics *137*, 107–120.

Heim, R., Prasher, D., and Tsien, R. (1994). Wavelength mutations and posttranslational autooxidation of green fluorescent protein. Proc. Natl. Acad. Sci. USA *91*, 12501–12504.

Huang, L.S., Tzou, P., and Sternberg, P.W. (1994). The *lin-15* locus encodes two negative regulators of *Caenorhabditis elegans* vulval development. Mol. Biol. Cell *5*, 395–412.

Lim, C.S. (1993). The *Caenorhabditis elegans daf-7* gene encodes a novel member of the transforming growth factor-β superfamily. PhD thesis, University of Missouri, Columbia, Missouri.

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

Malone, E.A., Inoue, T., and Thomas, J.H. (1996). Genetic analysis of the roles of *daf-28* and *age-1* in regulating *Caenorhabditis elegans* dauer formation. Genetics *143*, 1193–1205.

Mello, C., Kramer, J.M., Stinchcomb, D., and Ambros, V. (1991). Efficient gene transfer in *C. elegans*: extrachromosomal maintenance and integration of transforming sequences. EMBO J. *10*, 3959–3970.

Mori, I., and Ohshima, Y. (1995). Neural regulation of thermotaxis in *Caenorhabditis elegans*. Nature *376*, 344–348.

Perkins, L.A., Hedgecock, E.M., Thomson, J.N., and Culotti, J.G. (1986). Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. *117*, 456–487.

Riddle, D.L., Swanson, M.M., and Albert, P.S. (1981). Interacting genes in nematode dauer larva formation. Nature *290*, 668–671.

Schiller, P.H. (1982). Central connections of the retinal ON and OFF pathways. Nature *297*, 581–583.

Schiller, P.H., Sandell, J.H., and Maunsell, J.H.R. (1986). Functions of the ON and OFF channels of the visual system. Nature *322*, 824–825.

Shakir, M.A., Miwa, A.J., and Saddiqui, S.S. (1993). A role of ADF chemosensory neurones in dauer formation behavior in *C. elegans*. Neuroreport *4*, 1151–1154.

Solomon, J.M., Magnuson, R., Srivastava, A., and Grossman, A.D. (1995). Convergent sensing pathways mediate response to two extracellular competence factors in *Bacillus subtilis*. Genes Dev. *9*, 547–558.

Sulston, J.E., Schierenberg, E., White, J.G., and Thomson, J.N. (1983). The embryonic cell lineage of the nematode *C. elegans*. Dev. Biol. *100*, 64–119.

Swanson, M.M., and Riddle, D.L. (1981). Critical periods in the development of the *Caenorhabditis elegans* dauer larva. Dev. Biol. *84*, 27–40.

Tessier-Lavigne, M. (1991). Phototransduction and information processing in the retina. In Principles of Neural Science, E.R. Kandel, J.H. Schwartz, and T.M. Jessell, eds. (Norwalk, Connecticut: Appleton and Lange).

Thomas, J.H. (1993). Chemosensory regulation of development in *C. elegans*. Bioessays *15*, 791–797.

Thomas, J.H., Birnby, D.A., and Vowels, J.J. (1993). Evidence for parallel processing of sensory information controlling dauer formation in *C. elegans*. Genetics *134*, 1105–1117.

Vowels, J.J., and Thomas, J.H. (1992). Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics *130*, 105–123.

Vowels, J.J., and Thomas, J.H. (1994). Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. Genetics *138*, 303–316.

Wilson, R., Ainscough, R., Anderson, K., Baynes, C., Barks, M., and Bonfie, J. (1994). 2.2 Mb of contiguous nucleotide sequence from chromosome III of *C. elegans*. Nature *368*, 32–38.