Suppressors of Transforming Growth Factor-β Pathway Mutants in the *Caenorhabditis elegans* Dauer Formation Pathway

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ABSTRACT

The dauer is a developmentally arrested alternative third larval stage of *Caenorhabditis elegans*. Entry into this state is regulated by environmental cues, including temperature, food, and the concentration of constitutively secreted dauer pheromone. Genetically, three parallel pathways have been found that regulate this process. Of these, the group 2 pathway, which includes the genes *daf-1*, *daf-3*, *daf-4*, *daf-5*, *daf-7*, *daf-8*, and *daf-14*, mediates the transduction of environmental signals through the ASI chemosensory neuron and encodes a TGF-β-related signaling pathway. To identify additional genes that function in this pathway, we carried out a screen for suppressors of mutations in *daf-1*, *daf-8*, and *daf-14*. From the total of 36 mutations, seven complementation groups were identified. Three complementation groups correspond to the previously described genes *daf-3*, *daf-5*, and *daf-12*. Three correspond to novel genes *scd-1*, *scd-2*, and *scd-3*. Genetic analysis of these *scd* genes is presented here. A fourth complementation group was represented by a single mutation *sa315*, which affects the *daf-2/age-1* insulin-related signaling pathway.

THE dauer is a developmentally arrested third stage larva of the nematode *Caenorhabditis elegans* (Cassada and Russell 1975). Entry into dauer arrest is promoted by lack of food, high temperature, and high concentration of dauer pheromone, a constitutively secreted substance serving as an indicator of population density (Golden and Riddle 1982, 1984a,b). In the L1 and L2 larval stages, these environmental cues are sensed in part by chemosensory neurons in the amphid sensory organs in the head (Bargmann and Horvitz 1991; Schackwitz *et al.* 1996). These neurons in turn relay the environmental information to a complex regulatory system that makes the critical decision to proceed to the dauer or the L3 stage.

Genetic analysis of dauer formation in *C. elegans* has focused on two classes of mutations (Riddle *et al.* 1981). The first class, exhibiting the Daf-c (*da*uer *f*ormation *c*onstitutive) phenotype, forms dauers inappropriately under dauer-noninducing conditions. Most mutations of this class are temperature sensitive. This conditionality is a consequence of the inherent temperature sensitivity of the dauer-formation process and does not result from temperature-sensitive gene products (Malone and Thomas 1994). The second class, exhibiting the Daf-d (*da*uer *f*ormation *d*efective) phenotype, fails to form dauers under dauer-inducing conditions. Double mutant analyses placed these genes into a genetic pathway with three parallel branches (Thomas *et al.* 1993; Gottlieb and Ruvkun 1994). Although the cellular sites of action of these genetic pathways are not known in detail, they probably correspond to parallel streams of sensory information that feed into the decision to make a dauer.

The first branch, called the group 1 pathway, includes the Daf-c genes *daf-11* and *daf-21* (Thomas *et al.* 1993; Birnby *et al.* 2000). *daf-11* encodes a receptor guanylyl cyclase. *daf-21* encodes an HSP-90 heat-shock protein and is defined by a Daf-c gain-of-function mutation. Laser ablation studies have suggested that the function of these genes is associated with the chemosensory neuron ASJ (Schackwitz *et al.* 1996).

The second branch, called the group 2 pathway or the DAF-7/transforming growth factor (TGF)-β pathway, includes the Daf-c genes *daf-1*, *daf-4*, *daf-7*, *daf-8*, and *daf-14* and the Daf-d genes *daf-3* and *daf-5* (Thomas *et al.* 1993). Together, these genes encode a TGF-β-related signaling cascade. Specific components are DAF-7 (TGF-β/BMP related ligand; Ren *et al.* 1996), DAF-1 (type 1 receptor serine/threonine kinase; Georgi *et al.* 1990), DAF-4 (type 2 receptor serine/threonine kinase; Estevez *et al.* 1993), DAF-8, DAF-14, and DAF-3 (Smads; Patterson *et al.* 1997; Riddle and Albert 1997; Inoue and Thomas 2000). The molecular identity of the *daf-5* gene product has not yet been determined. By analogy with vertebrate and Drosophila TGF-β-related signaling (Massague 1998), ligand binding probably induces multimerization of receptors and the activation of the type 1 kinase through phosphorylation. Activated type 1 kinase in turn probably phosphorylates downstream Smad proteins, which transduce the signal to the nu-

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cleus and regulate transcription. In the group 2 pathway, the DAF-3 Smad has been demonstrated to exhibit a sequence-specific DNA-binding activity and probably functions as a downstream transcription factor (Thatcher *et al.* 1999).

Genetically, mutations in the Daf-d genes *daf-3* and *daf-5* suppress mutations in all five Daf-c genes (Vowels and Thomas 1992). Furthermore, four of five group 2 Daf-c genes, *daf-1*, *daf-7*, *daf-8*, and *daf-14*, display essentially identical pleiotropies, which include Egl (egg-laying defective), Din (dark intestine), and Cpy (clumpy; the tendency of animals to aggregate and form clumps on plates) phenotypes (Trent *et al.* 1983; Thomas *et al.* 1993). Although *daf-3* and *daf-5* mutants display no obvious pleiotropies, they suppress all pleiotropies in double mutant combinations with *daf-1*, *daf-7*, *daf-8*, or *daf-14*.

Laser ablation and expression studies indicate that this pathway directly mediates transduction of environmental signals, regulating dauer formation through the chemosensory neuron ASI (Bargmann and Horvitz 1991; Schackwitz *et al.* 1996). That this pathway is at least partly in parallel to the group 1 pathway is supported by the genetic evidence that *daf-3* and *daf-5* mutants do not strongly suppress *daf-11* and *daf-21*. Conversely, mutations that suppress the Daf-c phenotype of *daf-11* and *daf-21* do not suppress group 2 Daf-c mutants (Thomas *et al.* 1993).

The third pathway, here referred to as the *daf-2/age-1* pathway, includes *daf-2* (encoding an insulin/IGF1 receptor homolog; Kimura *et al.* 1997), *daf-16* (forkhead transcription factor; Lin *et al.* 1997; Ogg *et al.* 1997), *daf-18* (PTEN phosphatase; Ogg and Ruvkun 1998; Gil *et al.* 1999; Mihaylova *et al.* 1999; Rouault *et al.* 1999), *age-1* (PI3 kinase; Morris *et al.* 1996), *akt-1* and *akt-2* (two isoforms of AKT protein kinase; Paradis and Ruvkun 1998), and *pdk-1* (PDK protein kinase; Paradis *et al.* 1999). Loss-of-function mutations in *daf-2*, *age-1*, and *pdk-1* cause a Daf-c phenotype. RNAi (double stranded RNA interference) of *akt-1* and *akt-2* together also produces a Daf-c phenotype. Loss-of-function mutations in *daf-16* and *daf-18* are Daf-d and suppress mutations in *age-1* and/or *daf-2*. Dominant gain-of-function alleles of *akt-1* and *pdk-1* also exist and suppress mutations in *age-1*. Genetic interactions among *daf-2/age-1* pathway genes are complex and do not always follow the pattern expected from a linear pathway. One interesting feature of *daf-2/ age-1* pathway mutations is their tendency to cause formation of partial dauers, animals that exhibit only some characteristics of a dauer. This type of animal has not been observed in the wild type or in group 1 and group 2 pathway mutants.

Finally, *daf-12* encodes a nuclear hormone receptor that functions at a unique downstream position in the dauer pathway (Yeh 1991). Loss-of-function *daf-12* alleles are Daf-d and suppress Daf-c mutations in all three pathways, suggesting that this gene functions downstream of the convergence of the three parallel pathways.

Here, we describe the result of screens for suppressors of the group 2 Daf-c mutations *daf-1(sa184)*, *daf-8(sa234)*, and *daf-14(m77)*. Three new genes were identified, each represented by multiple alleles. In addition, a single mutation affecting the *daf-2/age-1* pathway was isolated and analyzed.

MATERIALS AND METHODS

Manipulation of *C. elegans* and nomenclature: Maintenance and manipulation of *C. elegans* were as described (Brenner 1974). All strains described here are in the N2 Bristol strain background, with the exception of a *scd-2(sa935)* strain used for some complementation tests. For clarity, genotypes of all double mutant strains are listed in the order *daf-c; sup* (rather than in the order of chromosomal locations as suggested by the standard *C. elegans* nomenclature system; Horvitz *et al.* 1979).

Isolation of suppressor mutations: *daf-14(m77)* and *daf-8(sa234)* were chosen as representative alleles because they were the strongest *daf-14* and *daf-8* alleles available at the time. *daf-1(sa184)* was chosen because of high penetrance and because this mutation does not exhibit strong maternal rescue like *daf-1(m40)* (Malone *et al.* 1996). Molecular data and phenotype analysis suggest that *daf-14(m77)* and *daf-8(sa234)* alleles may be null (Inoue and Thomas 2000); however, these alleles are less penetrant than *daf-1(sa184)* and presumably do not completely block DAF-7/TGF-β signaling. At restrictive temperatures (~25°), all of these alleles caused ~99–100% dauer formation, allowing easy isolation of suppressors. At permissive temperatures (15° and 20°), a significant fraction of each mutant grew as nondauers.

The screens for suppressors of Daf-c mutations were carried out essentially as described (Riddle *et al.* 1981; Thomas *et al.* 1993). EMS (ethyl methanesulfonate) mutagenesis was done as described (Brenner 1974). X-rays were used at the dose of 2000–4000 rads. Mutagenized worms were placed on seeded 10-cm NG agar plates and allowed to lay eggs overnight. The plates were placed at the permissive temperature to allow the F_1 progeny to grow to nondauer adults. Once the F_1 progeny were past the dauer stage (L3, L4, or adults), the plates were shifted to the restrictive temperature. Three days after the shift, the plates were visually screened for nondauers (L4 or young adult), using a dissecting microscope. These nondauers were picked to the restrictive temperature to confirm that they carried heritable suppressor mutations.

The restrictive temperature of 25° was used for *daf-14(m77)* and *daf-1(sa184)*. Although *daf-14* is not completely penetrant at this temperature (typically ~99% dauers), picked nondauers were significantly enriched for revertants. For *daf-8(sa234)*, 26° was used as the restrictive temperature, since at 25° the penetrance was too low to efficiently isolate suppressors.

The mutations we isolated varied significantly for their strength of suppression. Because of difficulties associated with analyzing low penetrance alleles, and because these were more likely to be nonspecific suppressors (see below), only strong suppressors were kept and analyzed. The cutoff was arbitrarily set at 90% suppression (<10% dauers). However, this is not a well-defined limit, because of the assay-to-assay variability in the frequency of dauer formation. The difference in strengths of the Daf-c mutations used [*daf-1(sa184)* > *daf-14(m77)* > *daf-8(sa234)*] probably also made this cutoff effectively different for each screen. In practice, however, types of mutations obtained from each screen were not obviously different.

sa756 was found as a spontaneous non-Daf-c revertant in the *unc-24 daf-14(m77)* background by M. Ailion. This mutation was analyzed in parallel with the suppressors generated by mutagenesis. Two additional alleles of *scd-1*, *mg94* and *mg99*, were provided by G. Patterson and G. Ruvkun. The *scd-2 (sa935)* allele was provided by M. Ailion.

Nonspecific suppression of group 2 Daf-c genes: Previous empirical observations suggested that most mutations used as genetic markers partially suppress the Daf-c phenotype of many mutants. To quantitate this effect, several *daf-14(m77); marker* double mutants were assayed. Mutations in *dpy-5*, *rol-6*, *unc-32*, *dpy-11*, *unc-22*, and *unc-44* significantly suppressed the Daf-c phenotype. With the exception of *unc-44*, the nonspecific suppression ranged from ~17 to 45%. Thus, the strong suppression (often >90% suppressed) observed for *scd* mutations analyzed here is likely to be caused by a more specific interaction. It is not clear why marker mutations, some in genes encoding cuticular collagens, suppress the Daf-c phenotype. The *unc-44(e362)* mutation we assayed strongly suppressed group 2 Daf-c mutants, perhaps indicating a specific interaction.

Mapping and complementation tests: Suppressors were mapped and complementation tested using the suppression phenotype. For *scd-2* and *scd-3*, complementation tests using single mutant phenotypes (dauer pheromone insensitivity and abnormal male tail morphology, respectively) were also done and gave consistent results. To assign mutations to previously described genes, complementation tests against *daf-3(e1376)*, *daf-5(sa205)*, *daf-5(e1385)*, and *daf-12(m20)* were done. Complementation tests against *scd-2(sa935)* (M. Ailion, personal communication) were also used to assign alleles to that complementation group. For all *scd* alleles presented here, the mutation was mapped to a chromosome before complementation tests were done. Thus at least two independent pieces of experimental data confirm the assignment of each allele to a *scd* gene.

The following type of complementation test was used most frequently to assign a mutation to a *scd* gene. *daf-c; scd-A* males were mated to marked *daf-c; scd-B* hermaphrodites and the unmarked progeny assayed at 25° for frequencies of dauers and nondauers. For *scd-3* alleles, *daf-c; scd/+* males were used, since *scd-3* males do not mate (data not shown).

To test for linkage to the X chromosome and for dominance, *daf-c* males were crossed to *daf-c; scd* hermaphrodites, and the progeny were assayed at 25°. X-linkage was indicated by the presence of *daf-c; scd/0* nondauer males and *daf-c; scd/+* dauer hermaphrodites. Dominance was indicated by the presence of *daf-c; scd/+* nondauer males and nondauer hermaphrodites (and by the absence of dauers, since hermaphrodite self-progeny are also nondauers). Autosomal linkage was indicated by the presence of *daf-c; scd/+* dauers (and the absence of male nondauers).

Suppressor mutations were mapped to chromosomes as follows. At 25°, nondauer progeny of *daf-c; scd/mk* (or *scd/+ mk/+*) were picked (*mk*, marker). These were placed individually onto separate plates at 25° and allowed to self. The progeny were first scored to confirm that the suppressor was homozygous and then scored for segregation of the *mk* mutation. Confirmation of the suppressor genotype was necessary because of the incomplete penetrance of the Daf-c mutations. If the suppressor mutation was not linked to the marker, two-thirds of the nondauers segregated the marker mutation. If the suppressor mutation was linked, very few sup *scd* homozygotes segregated the marker mutation. Various methods were used to map the suppressor mutations to specific intervals on chromosomes. Methods, as well as results, are summarized in Table 3.

The following data argue that *scd-1*, *scd-2*, and *scd-3* are new genes. *scd-1(sa248)* maps to the *mes-1 unc-9* interval on chromosome X and complements *daf-3* and *daf-12*. A similar map position (within the *egl-15 unc-9* interval) was obtained independently for *scd-1(sa318)* (data not shown). *scd-2(sa249)* was mapped to the *dpy-11 rol-3* interval of chromosome V. Furthermore, an independently isolated allele *scd-2(sa935)* was mapped to the *dpy-11 unc-70* interval (M. Ailion, personal communication). *scd-3(sa253)* and *scd-3(sa246)* were mapped to the *unc-11 dpy-5* interval on chromosome I. No previously described genes affecting dauer formation map to these intervals. For *scd-3*, complementation tests were carried out against Mab genes *lin-44* and *egl-34* in the same interval. *scd-3* complemented mutations in both of these genes.

Assays for Daf-c and Daf-d phenotypes: Starvation assays were done to test for the ability to form dauers under strong dauer-inducing conditions (Vowels and Thomas 1992). One to four animals of each genotype were placed on seeded 5-cm NG agar plates and allowed to starve at 25°. Four to 6 days after the food was exhausted, the plates were flooded with 1% sodium dodecyl sulfate (SDS) solution to select for dauers. (Dauers are resistant to SDS; Cassada and Russell 1975.) After 10–15 min, the plates were scored visually for dauers under the dissecting microscope. For *scd-3* alleles, two to eight parents per plate were used because of low brood size.

Assays of dauer formation efficiency under high concentration of dauer pheromone (pheromone plate assay) were done essentially as described (Golden and Riddle 1984b). Briefly, synchronous populations of worms were grown on plates supplemented with crude dauer pheromone preparation. Food was provided as a thick suspension of *Escherichia coli* placed on the agar media, which contained streptomycin and lacked peptone to inhibit bacterial growth.

Frequencies of dauers formed by *daf-c; scd* double mutants under normal growth conditions were measured essentially as described (Vowels and Thomas 1992). Typically, 10 adult animals were allowed to lay eggs on seeded 5-cm NG plates for ~6 hr at room temperature (~23°). The parents were then removed and the plates were placed at 25°. The numbers of dauers and nondauers were scored ~50 hr later. For some slow growing strains, the plates were scored at ~72 hr (3 days). Formation of *pdk-1(lf)* type partial dauers (Table 9) was also scored at 72 hr, since this made it easier to distinguish them from true dauers. Although some fluctuations in the incubator temperature were inevitable, most of the assays were done between 25.0° and 25.5°.

Other phenotypes: Egl (egg-laying defect and retention of eggs) and Din (dark intestine) phenotypes were scored visually. To score the Cpy (clumping behavior) phenotype, three parent worms were placed on seeded 5-cm NG agar plates and left undisturbed for 3 days at 20°. The distribution of the progeny on the plate was observed using a dissecting microscope. Typically, two to four replicate plates of each strain were scored. The Mab phenotype and the gonad position defect of *scd-3* were assayed using Nomarski optics. Brood sizes were counted by transferring the parent animal from one plate to another until animals stopped laying eggs or died. The partial dauer phenotype was scored under Nomarski optics. Life spans of mutants and the wild type were measured as described. Life spans of *scd-1(sa248)*, *scd-2(sa249)*, and *sa315* were wild type (data not shown). The life span of *daf-2(e1370); sa315* was not distinguishable from *daf-2(e1370)*.

***sa315* dosage analysis and sequencing:** The following results suggest that *sa315* is recessive. First, in a mapping cross, nondauer progeny of *daf-1; sa315/+* were picked and the genotype confirmed in the next generation. All nondauers picked were *sa315/sa315*, suggesting that most *sa315/+* animals were dauers. Second, *sa315* homozygous males were crossed to *unc-4* hermaphrodites and progeny grown on pheromone

TABLE 1

Screens for suppressors of *daf-1(sa184)*, *daf-8(sa234)*, and *daf-14(m77)*

Daf-c mutation	Mutagen	Genomes screened	No. of alleles found	Allele nos.
daf-14(m77)	EMS	5000	10	*sa244–sa246*, *sa248–sa253*, *sa303*
daf-8(sa234)	EMS	4200	13	*sa317–sa329*
daf-1(sa184)	EMS	3500	7	*sa309–sa315*
daf-14(m77)	X ray	6000	5	*sa737*, *sa793–sa796*
daf-14(m77)	Spontaneous	NA	1	*sa756*

NA, not applicable.

plates. Many non-Unc dauers were observed, indicating that *sa315/+* animals are capable of forming dauers, unlike *sa315/sa315* homozygotes, which are Daf-d.

To test the phenotype of *sa315/Df*, progeny of *sa315/mnDf89* parents were grown on pheromone plates. From one set of assays, 19 were dauers or partial dauers and 116 were nondauers. Since *sa315* homozygotes assayed in parallel did not form any dauers or partial dauers ($n = 140$), this suggests that *sa315/Df* is less Daf-d than is *sa315/sa315*, which would indicate that *sa315* is a gain-of-function mutation. Curiously, most of the dauers appeared to be partial dauers.

Genomic fragments containing *age-1* exons were amplified by PCR, using purified total genomic DNA of an *sa315*-bearing strain as the template. The bulk PCR product was sequenced and the result was compared with the reported *age-1* mRNA sequence (Morris *et al.* 1996) as well as sequences from the genome project and an EST database.

RESULTS AND DISCUSSION

Screens for suppressors of *daf-14*, *daf-8*, and *daf-1*: To identify genes that interact with group 2 Daf-c genes *daf-1*, *daf-8*, and *daf-14*, we isolated mutations that suppressed the Daf-c phenotype of *daf-1(sa184)*, *daf-8(sa234)*, and *daf-14(m77)* (Table 1; materials and methods). Both X-ray and EMS mutagenesis were used. In addition, a single suppressor allele (*sa756*) was found as a spontaneous mutation in *daf-14(m77)* background (M. Ailion, personal communication).

Each screen produced suppressor mutations with a wide range of suppression strength. For example, in the case of the *daf-14(m77)* EMS screen, the level of suppression varied from 20 to 100% depending upon the suppressor mutation (data not shown). Because of the potential difficulty in analyzing weak suppressor mutations and also to avoid nonspecific suppressors, weak mutations were not kept for analysis (materials and methods). In total, the 36 strongest suppressor mutations were kept for analysis. Although the screens were capable of isolating both recessive and dominant suppressors, all alleles were recessive (data not shown).

Complementation groups: Mapping and complementation tests were carried out to place suppressor mutations into complementation groups (materials and methods, Table 2, Table 3, and Figure 1). As expected, some suppressors were alleles of the previously described Daf-d genes, *daf-3*, *daf-5*, and *daf-12* (Riddle *et al.* 1981; Thomas 1993; Thomas *et al.* 1993). In addition, four other complementation groups were identified. Three complementation groups correspond to previously undescribed genes, which we named *scd-1*, *scd-2*, and *scd-3* (*scd*, *s*uppressor of *c*onstitutive *d*auer). The fourth complementation group is represented by a single allele, *sa315*, which maps to the same genetic interval as the *age-1* gene. As we have not determined whether *sa315* defines a new gene or is an unusual allele of *age-1*, we have not given it a gene name.

With the exception of *sa315*, multiple alleles of each complementation group were found. This result suggests that most genes that mutate to cause strong suppression of the group 2 Daf-c mutations were identified. The spectrum of mutations obtained in each screen appears similar, despite the use of three different Daf-c alleles. *daf-5*, *daf-12*, and *scd-1* alleles were obtained as suppressors of all three Daf-c mutations, and *scd-3* and *daf-3* alleles were isolated in two out of three. Similarly, subsequent tests showed that mutations in all three *scd* genes suppressed all group 2 Daf-c mutations (Table 5). However, as comparatively weaker suppressor mutations [*e.g.*, *scd-2(sa303)*] did not strongly suppress the stronger Daf-c mutations [*e.g.*, *daf-1(sa184)*], there was likely a bias toward stronger suppressors in screens carried out using *daf-1(sa184)*.

***scd* single mutant phenotype:** The effect of *scd* mutations on dauer formation was tested using two assays, the starvation assay and the pheromone plate assay (Table 4 and materials and methods; Golden and Riddle 1984b; Vowels and Thomas 1992). In the starvation assay, dauer formation is induced by allowing plates of worms to starve naturally. This causes a very strong dauer induction as a result of overcrowding and starvation. Strains carrying strong alleles of previously described Daf-d genes, *daf-3*, *daf-5*, and *daf-12*, formed very few dauers under this condition. In contrast, dauer formation by the *scd-1* single mutant was indistinguishable from the wild type. *scd-2* and *scd-3* single mutant strains

TABLE 2

Complementation groups

Gene	Alleles	Comments
daf-5 II	*sa244*	
	sa250	Weak
	sa309	Weak
	sa310	Weak
	sa312	
	sa323	
	sa326	Weak
	sa327	
	sa328	
	sa756	
	sa793	
	sa794	Weak
daf-3 X	*sa319*	
	sa324	
	sa325	
daf-12 X	*sa251*	
	sa314	
	sa322	
	sa737	
	sa796	
scd-1 X	*sa248*	
	sa252	
	sa313	
	sa317	
	sa318	
scd-2 V	*sa249*	
	sa303	Weaker than *sa249*
scd-3 I	*sa246*	Unc, short, Egl
	sa253	Mab, short, Egl, gonad position defect, low brood size
	sa320	Mab, short, Egl, gonad position defect, low brood size
	sa795	Mab, short, Egl, gonad position defect, low brood size
scd(sa315) II	*sa315*	May be an *age-1* allele

Reference alleles of *scd-1*, *scd-2*, and *scd-3* are underlined. Mab, abnormal male tail morphology. Weak, alleles of *daf-5* that exhibit a weak phenotype similar to *scd* alleles. Strains bearing *scd-3(sa246)* exhibit a weak coiler Unc (uncoordinated) phenotype, which also maps to IC. However, this phenotype is not present in other *scd-3* alleles and may be caused by a linked mutation. The following alleles were not assigned unambiguously to complementation groups: chromosomal locations of *sa245* and *sa311* were not determined; *sa329* is located on the X chromosome; and *sa321* is located on chromosome V. *sa321*-bearing strains were weakly Daf-d, Egl, and Mab; however, it was not determined whether the pleiotropies are caused by the same mutation as *sa321*. The possibility that *sa321* is allelic with *scd-2* has not been ruled out.

also formed dauers under this condition, although less efficiently than the wild type. The other assay used is a pheromone plate assay (Table 4), in which dauers are induced by the addition of crudely purified dauer pheromone to the media. With this assay, quantitative measurement of dauer frequency is possible, allowing detection of subtler differences in sensitivity to dauer-inducing stimuli. *scd-2* and *scd-3* single mutant strains formed very few dauers in this assay. In contrast, all five alleles of *scd-1* tested (*sa248*, *sa317*, *sa318*, *mg94*, and *mg99*) had dauer pheromone responses indistinguishable from the wild type.

***daf-c; scd* double mutants:** To test the generality of suppression of group 2 Daf-c mutations, *scd* mutations were constructed in combination with *daf-1*, *daf-7*, *daf-8*, and *daf-14*. In general, the *scd* mutations suppressed all group 2 Daf-c mutations (Table 5). The suppression was incomplete in all cases, and the level of suppression varied according to the strengths of the alleles. For example, *daf-1(sa184)* and *daf-7(e1372)* produce a stronger Daf-c phenotype than do *daf-8(sa234)* and *daf-14(m77)*. Accordingly, *daf-1; sa249* and *daf-7; sa249* formed significantly more dauers than *daf-8; sa249* and *daf-14; sa249*. This incompleteness of suppression is not due to the choice of *scd* alleles we tested. From our screens, all isolated alleles that completely suppressed were alleles of *daf-3*, *daf-5*, or *daf-12* (*sa244*, *sa312*, *sa323*, *sa327*, *sa328*, *sa756*, *sa793*, *sa319*, *sa324*, *sa325*, *sa251*, *sa314*, *sa322*, *sa737*, and *sa796*; data not shown). The weak phenotype of the *scd* alleles probably also accounts for the lack of *scd* alleles from earlier group 2 Daf-c suppressor screens, which were carried out using the strong Daf-c mutations *daf-7(e1372)* and *daf-4(e1364)* (Riddle *et al.* 1981; Thomas *et al.* 1993).

In addition to dauer formation, the suppression of the pleiotropic phenotypes caused by the group 2 Daf-c mutation *daf-14(m77)* was scored (Table 6; Trent *et al.* 1983; Thomas *et al.* 1993). *scd-1* alleles suppressed all of the pleiotropies, although the suppression was partial for some phenotypes. *scd-2* alleles did not exhibit any effect on the pleiotropies. However, given the weaker Daf-c suppression phenotype of the *scd-2* alleles in comparison to the *scd-1* alleles (Table 5), the possibility that *scd-2* alleles weakly affect the pleiotropies cannot be ruled out. *scd-3* alleles suppressed the Cpy phenotype but not the Din phenotype. Although this may indicate a phenotype-specific interaction, suppression of the Cpy phenotype may also be a result of an unrelated pleiotropic sensory or motor defect of *scd-3*.

Placement of *scd* genes in the dauer pathway: To further place the *scd* genes in the dauer pathway, double mutants were also built between *scd* mutations and *daf-c* mutations from the group 1 pathway (*daf-11*) and the *daf-2/age-1* (*daf-2*) pathways (Table 5). Mutations in *scd-1* and *scd-2* suppressed group 2 Daf-c mutations but not *daf-11(sa195)* or *daf-2(e1370)*, tentatively placing *scd-1* and *scd-2* in the group 2 (DAF-7/TGF-β) pathway. For *scd-1*, this placement is further supported by the suppression of group 2 pleiotropies (Egl, Din, and Cpy). Like *daf-3* (Smad) mutations, in which these pleiotropies are also suppressed (Thomas *et al.* 1993; Patterson *et al.* 1997), *scd-1* is likely to encode a component of the DAF-7/TGF-β signaling pathway. *scd-2* mutations do not suppress the pleiotropies, but have a stronger

TABLE 3

Map data

Parent genotype	Progeny picked	*n*	Progeny genotype	Comments
daf-14; lon-2 lin-2/scd-1	Non-Daf-c (25°)	18/47	*lon-2 scd-1/scd-1*	*scd-1* is to the right of *lon-2*
		29/47	*scd-1/scd-1*	
daf-14; lon-2 scd-1/unc-3 lin-15	Non-Daf-c	12/23	*lon-2 scd-1/lon-2 scd-1*	Between *lon-2* and *unc-3*
		5/23	*lon-2 scd-1/scd-1*	
		2/23	*lon-2 scd-1 unc-3 lin-15/scd-1*	
		2/23	*lon-2 scd-1 unc-3 lin-15/lon-2 scd-1*	
		1/23	*lon-2 scd-1 lin-15/scd-1*	
		1/23	*lon-2 scd-1 lin-15/lon-2 scd-1 unc-3 lin-15*	
daf-14; scd-1/egl-15 unc-3	Non-Daf-c	3/33	*egl-15 scd-1/scd-1*	Between *egl-15* and *unc-3*
		6/33	*scd-1 unc-3/scd-1*	
		24/33	*scd-1/scd-1*	
daf-14; lon-2 scd-1 lin-15/lin-2 flr-1	Non-Daf-c non-Muv	1/8	*lon-2 scd-1 flr-1/lon-2 scd-1 lin-15*	*scd-1* is to the left of *flr-1*
		1/8	*lon-2 scd-1/scd-1 lin-15*	
		6/8	*lon-2 scd-1/lon-2 scd-1 lin-15*	
daf-14; lon-2 scd-1 flr-1/unc-9	Non-Daf-c non-Flr-1	4/4	*lon-2 scd-1 flr-1/lon-2 scd-1 unc-9*	*scd-1* is to the left of *unc-9*
daf-14; lin-2 unc-9/scd-1	Lin non-Unc	9/9	*lin-2 unc-9/lin-2*	
daf-14; nDf19/scd-1				*nDf19* complements *scd*-1
daf-14; scd-2/dpy-11	Non-Daf-c	7/7	*scd-2/scd-2*	*scd-2* is linked to *dpy-11* V
daf-14; scd-2/dpy-11 unc-76	Non-Daf-c	1/29	*dpy-11 scd-2/scd-2*	*scd-2* is between *dpy-11* and *unc-76*
		5/29	*scd-2 unc-76/scd-2*	
		23/29	*scd-2/scd-2*	
daf-14; scd-2/rol-3 unc-42	Non-Daf-c	3/155	*scd-2 rol-3 unc-42/scd-2*	*scd-2* is to the left of *rol-3*
		3/155	*scd-2 unc-42/scd-2*	
		149/155	*scd-2/scd-2*	
daf-14; unc-14/scd-3(sa253)	Non-Daf-c	9/9	*scd-3/scd-3*	*scd-3* is linked to *unc-14* I
daf-14; unc-14/scd-3(sa795)	Non-Daf-c	2/2	*scd-3/scd-3*	
daf-14; dpy-5/scd-3(sa795)	Non-Daf-c	3/3	*scd-3/scd-3*	
daf-14; unc-11 dpy-5/scd-3 (sa253)	Non-Daf-c	18/19	*scd-3/scd-3*	Right of *unc-11*
		1/19	*unc-11 scd-3/scd-3*	
daf-14; unc-11 dpy-5/scd-3 (sa253)	Dpy non-Unc	1/7	*unc-11 dpy-5/dpy-5*	*scd-3* is between *unc-11* and *dpy-5*
		6/7	*unc-11 dpy-5/scd-3 dpy-5*	
scd-3(sa320) daf-8/dpy-5 unc-75	Daf-c	7/9	*dpy-5 daf-8/scd-3 daf-8*	*scd-3* is to the left of *dpy-5*
		2/9	*daf-8/scd-3 daf-8*	
scd-3(sa320) daf-8/unc-11 dpy-5	Dpy non-Unc	3/7	*scd-3 dpy-5/unc-11 dpy-5*	*scd-3* is between *unc-11* and *dpy-5*
		4/7	*dpy-5/unc-11 dpy-5*	
scd-3(sa253)/unc-11 dpy-5	Unc non-Dpy	1/7	*unc-11 scd-3/unc-11 dpy-5*	*scd-3* is between *unc-11* and *dpy-5*
		6/7	*unc-11/unc-11 dpy-5*	
	Dpy non-Unc	2/2	*dpy-5/unc-11 dpy-5*	
daf-1; sa315/rol-6	Non-Daf-c	6/6	*sa315/sa315*	*sa315* is linked to *rol-6* II

(*continued*)

TABLE 3

(Continued)

Parent genotype	Progeny picked	*n*	Progeny genotype	Comments
daf-1; sa315/dpy-10 unc-4	Non-Daf-c	22/28	*sa315/sa315*	*sa315* is to the right of *unc-4*
		3/28	*dpy-10 sa315/sa315*	
		3/28	*dpy-10 unc-4 sa315/sa315*	
daf-1; dpy-10 sa315/unc-4 bli-1 rol-1	Non-Daf-c non-Dpy	8/16	*dpy-10 sa315/sa315*	*sa315* is to the right of *bli-1*
		4/16	*dpy-10 sa315/unc-4 sa315*	
		4/16	*dpy-10 sa315/unc-4 bli-1 sa315*	
daf-1; sa315/unc-4 bli-1 rol-1	Non-Daf-c	51/52	*sa315/sa315*	
		1/52	*unc-4 bli-1 sa315/sa315*	
	Bli non-Rol	1/1	*unc-4 bli-1 sa315/unc-4 bli-1 rol-1*	
daf-1/+; sa315/unc-4 bli-1 rol-1	Wild type	1	*sa315/sa315 rol-1; daf-1/+*	*sa315* is to the left of *rol-1*
sa315/sqt-1 lin-29	Rol non-Pvl	1/4	*sqt-1 sa315/sqt-1 lin-29*	Between *sqt-1* and *lin-29*
		3/4	*sqt-1/sqt-1 lin-29*	

daf-c alleles are omitted from progeny genotypes.

Daf-d phenotype than do *scd-1* alleles. Thus, *scd-2* probably functions at a different point in the dauer pathway from *scd-1*.

scd-3 mutants suppressed group 2 Daf-c mutants and *daf-11(sa195)* but not *daf-2(e1370)*. Strong *daf-3* and *daf-5* mutations partially suppress *daf-11* mutants. Unlike those genes, the level of suppression by *scd-3* appears comparable for both *daf-11* and group 2 Daf-c mutations, suggesting that *scd-3* functions downstream of both.

Pleiotropic phenotypes of *scd-3* mutants: Uniquely among genes identified here, *scd-3* alleles exhibited multiple pleiotropic phenotypes not obviously related to their dauer phenotype. The same pleiotropies were observed for three independently isolated alleles, *sa253*, *sa320*, and *sa795*, and are thus caused by mutations in the *scd-3* gene. The fourth *scd-3* allele, *sa246*, fails to complement the other *scd-3* alleles for the suppressor phenotype, but did not exhibit some of the pleiotropies (Table 2). The pleiotropies include the following:

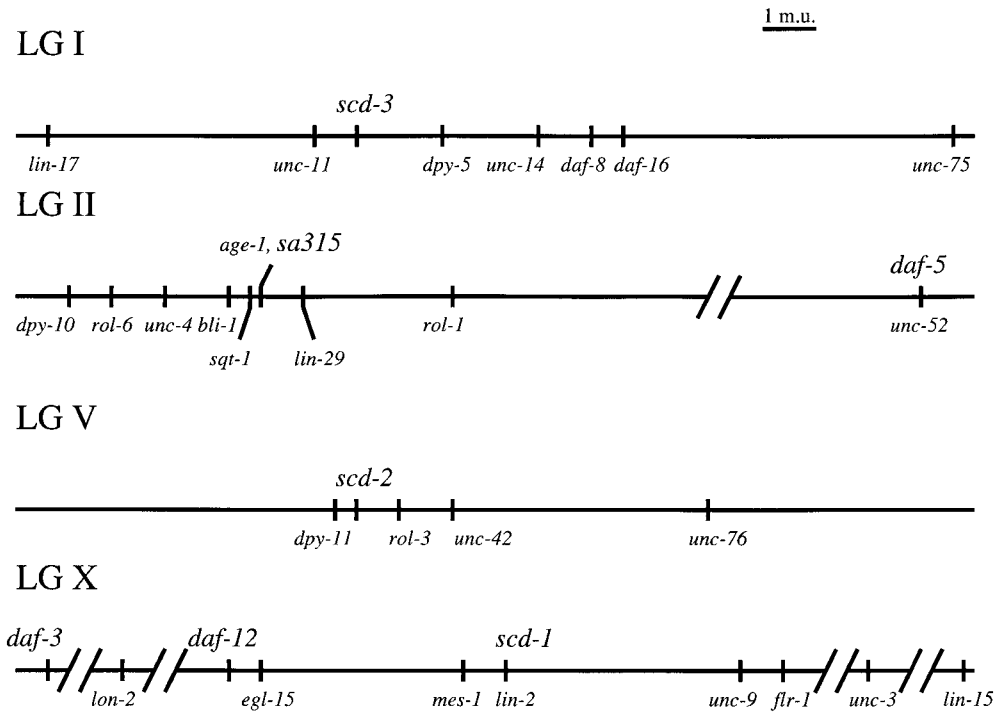

FIGURE 1.—Chromosomal locations of *scd* genes.

TABLE 4

Single mutant phenotypes of *scd* genes

Genotype	Pheromone plate assay		Starvation assay (no. of dauers on starved plates at 25°)
	Dauers at 25° (%)	*N*	
Wild type	89	175	Many (>100)
scd-1(sa248)	87	193	Many (>100)
scd-1(sa318)	5[a]	55	Many (>100)
scd-2(sa249)	0	154	Few (<100)
scd-2(sa303)	0	157	Many (>100)
scd-3(sa253)	0	168	Few (<100)
scd-3(sa795)	3	38	Few (<100)
sa315	0	146	Very few (<10)
daf-3(e1376)	—	—	Very few (<10)
daf-12(m20)	—	—	None (0)

—, dauer formation by these strains was not measured in parallel with other strains shown here. Previous experiments indicate that these strains form no dauers under this condition.

[a] Although *scd-1(sa318)* formed relatively few dauers in this particular assay, other experiments with this allele and with other *scd-1* alleles failed to detect any difference between mutants and the wild type. The low frequency of dauers in this assay may be attributable to the low population density on the assay plate. In another set of pheromone plate assays, dauer frequencies were as follows: wild type, 15%; *sa248*, 23%; and *sa318*, 11%.

1. Low brood size: *sa253*, *sa320*, and *sa795* reduced the hermaphrodite brood size (Table 7). As indicated by the large standard deviation, the phenotype was quite variable and individual brood sizes ranged from 0 to >100.
2. Egg-laying defect (Egl): Strains carrying any of the four *scd-3* alleles retained eggs in their gonad and became bloated. Often, eggs hatched inside the parent and formed a "bag of worms," killing the parent. This probably contributes to the low brood size, although it is not the sole cause, since some animals that did not bag also had reduced brood size.
3. Gonadal L/R reversal: In the bilobed gonad of the wild-type *C. elegans*, the anterior half of the gonad is located on the right side of the intestine whereas the posterior half is located on the left side. In strains carrying the *scd-3* alleles *sa253*, *sa320*, or *sa795*, the left/right positions of the gonad halves were frequently reversed (Table 8). The gonad position of the *sa246* mutant was also affected, although less frequently. Anterior/posterior and dorsal/ventral organizations of each gonad arm were normal in most individuals. However, occasionally a severely misplaced or abnormally shaped gonad was observed ("other" in Table 8). The reversal of the posterior half of the gonad was the most frequent, occurring in 30 out of 75 animals (data for three strong alleles combined).

TABLE 5

Percentage dauer formation by *daf-c; scd* double mutants at 25°

	Group 2 Daf-c genes					
	daf-1(sa184)	*daf-7(e1372)*	*daf-8(sa234)*	*daf-14(m77)*	*daf-11(sa195)*	*daf-2(e1370)*
+	100	100	99	97	100	100
scd-1(sa248)	24	41	10	1	100	100
scd-1(sa318)	13	7	0	3	96	100
scd-2(sa249)	51	62	6	3	100	100
scd-2(sa303)	99	99	37	21	ND[a]	100
scd-3(sa253)	3	35	ND[a]	0	35	100
scd-3(sa795)	0	96	ND[a]	0	13	100
sa315	19[b]	6[b]	0	0	27[b]	—[c]

[a] ND, no data. These linked double mutants were not built. An independently isolated *scd-2* allele, *sa935*, does not suppress *daf-11(sa195)* (M. Ailion, personal communication). *scd-3(sa320)* suppresses *daf-8(e234)*.

[b] Percentage partial dauers.

[c] See Table 9.

TABLE 6

Suppression of group 2 pleiotropies by *scd* mutant alleles

Genotype	Daf-c	Egl	Din	Cpy
+	+	+	+	+
daf-14(m77)	−	−	−	−
daf-14(m77); scd-1(sa248)	±	±	±	+
daf-14(m77); scd-1(sa318)	±	±	±	+
daf-14(m77); scd-2(sa249)	±	−	−	−
daf-14(m77); scd-2(sa303)	±	−	−	−
daf-14(m77); scd-3(sa253)	±	−[a]	−	+
daf-14(m77); scd-3(sa795)	±	−[a]	−	+
daf-14(m77); sa315	±[b]	−	−	−
daf-14(m77); daf-16(m27)	−[b]	−	−	−
daf-14(m77); daf-18(e1375)	−[b]	−	−	−
daf-14(m77); akt-1(mg144)	−	−	−	−
daf-14(m77); pdk-1(mg142)	−	−	−	−

For double mutant strains: −, a phenotype indistinguishable from the *daf-14* single mutant; +, a phenotype indistinguishable from the wild type; ±, an intermediate phenotype.

[a] Since *scd-3* single mutants are Egl, this result is not interpretable.

[b] Formed partial dauers; see Table 9.

4. Weak Dpy (dumpy): All four *scd-3* mutant alleles caused a variable and weak short body shape phenotype similar to weak Dpy mutants.
5. Male abnormal (Mab) phenotype: *sa253*, *sa320*, and *sa795* mutant males exhibited abnormal tail morphology (Figure 2). Defects included deformation of the spicule, absence of the hook, and abnormal sensory rays. Defects in the sensory rays included missing rays, fused rays, and abnormally shaped rays, and were variable from animal to animal. All rays (one to nine) were affected to some extent. The combination of short body shape and male abnormality is also caused by mutations in the Sma/Mab pathway, a separate TGF-β-related signaling pathway (Savage *et al.* 1996). However, most Sma/Mab pathway mutants specifically affect rays four, five, six, and seven, and the defects are milder than those observed for *scd-3*. Therefore, *scd-3* probably does not affect the Sma/Mab pathway.

TABLE 7

***scd-3* brood size counts**

Genotype	Brood size ± SD		
	15°	20°	25°
Wild type	264 ± 79	236 ± 76	177 ± 87
scd-3(sa253)	49 ± 50	42 ± 51	25 ± 19
scd-3(sa320)	25 ± 22	53 ± 80	12 ± 26
scd-3(sa795)	14 ± 29	74 ± 79	5 ± 10

Brood size per hermaphrodite animal is shown. Ten animals were scored for each temperature and genotype.

TABLE 8

***scd-3* gonad position defect**

Genotype	Classes				
	RL	RR	LR	LL	Other
Wild type	34	1	0	0	1
dpy-20(e1282)	19	0	0	1	0
scd-1(sa248)	18	0	0	0	0
scd-2(sa249)	18	1	0	0	2
sa315	9	1	0	0	2
scd-3(sa246)	33	8	0	1	2
scd-3(sa253)	17	12	2	1	6
scd-3(sa320)	9	4	0	1	3
scd-3(sa795)	15	11	1	2	6

The animals were classified into different classes based on positions of the anterior and posterior halves of the gonad. RL, right anterior gonad and left posterior gonad (the wild-type condition); RR, right anterior gonad and right posterior gonad; LR, left anterior gonad and right posterior gonad; LL, left anterior gonad and left posterior gonad; other, animals in which a gonad arm appeared to be missing or misshapen, or in which the gonad was not confined to one side of the animal. *dpy-20(e1282)* was tested to control for possible effects of the short body shape on gonad positioning.

***sa315* affects the *age-1/daf-2* pathway:** The last complementation group is represented by a single allele *sa315*, originally isolated as a suppressor of *daf-1(sa184)*. *sa315* affected dauer formation of group 1 and group 2 Daf-c mutants in two ways (Table 9). First, the frequency of dauer formation was reduced. Second, any dauers that formed were partial dauers (defined as animals mosaic for dauer and nondauer phenotypes; Figure 3 and MATERIALS AND METHODS). Formation of partial dauers is a hallmark of mutations in the *age-1/daf-2* pathway, as partial dauers are not made by the wildtype or by group 1 and group 2 mutants under any condition (Vowels and Thomas 1992). Partial dauers formed by group 2 *daf-c; sa315* double mutants had dauer alae but the pharynx was not remodeled to a dauer-like state. This combination of phenotypes and the overall appearance of the partial dauers (thin but pale) were similar to those of group 2 *daf-c; daf-16* and group 2 *daf-c; daf-18* double mutants (Vowels and Thomas 1992), suggesting that the *sa315* mutation caused a defect similar to *daf-16(lf)* and *daf-18(lf)*. In general, *daf-c; sa315* double mutants formed fewer partial dauers than *daf-c; daf-16* and *daf-c; daf-18* (Table 9); however, this difference may be merely quantitative.

sa315 also interacted with Daf-c mutations in the *age-1/daf-2* pathway (Table 9). First, *sa315* suppressed the Daf-c phenotype of *pdk-1(sa680)*, as do *daf-16(lf)* and *akt-1(d)*. Second, *sa315* interacted with *daf-2*. The *daf-2(e1370)* single mutant formed normal dauers only, whereas the *daf-2(e1370); sa315* double mutant formed partial dauers that were dark and thick (different from group 2 *daf-c; sa315* partial dauers). These partial dauers

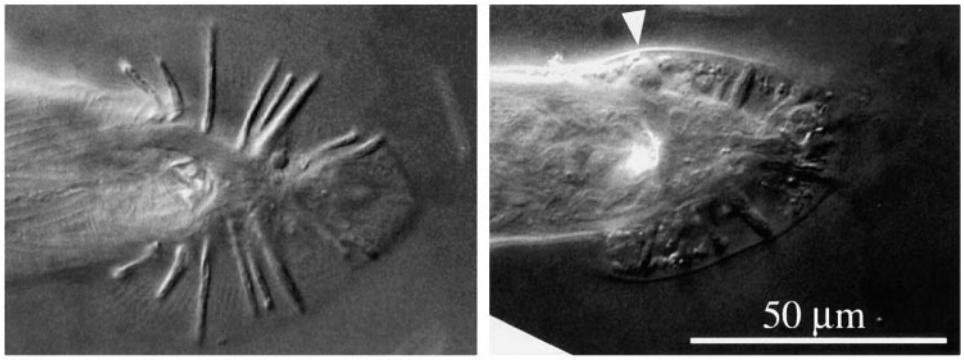

FIGURE 2.—The Mab phenotype of *scd-3*. Left, the tail of a phenotypically wild-type *C. elegans* male [*scd-3(sa795)/+*] showing the sensory rays. Eight rays are visible on either side. Right, the tail of a typical *scd-3(sa253)* male. The defect caused by the *scd-3* mutation is variable and includes missing rays, fused rays (not shown), and deformed rays (open arrowhead). Other structures in the male tail, including the hook and spicules, are also affected (not shown).

resembled partial dauers made by the *daf-2; akt-1 (mg144dm)* double mutant. In contrast, *daf-2; daf-16(lf)* and *daf-2; daf-18(lf)* double mutants formed partial dauers like group 2 *daf-c; sa315* mutants. Therefore, *sa315* differs from *daf-16(lf)* and *daf-18(lf)* in its interaction with *daf-2*. Although the pattern of interactions does not exactly match that of any previously identified mutants, *sa315* does appear to affect the *daf-2/age-1* pathway.

The similarity between *daf-2; sa315* and *daf-2; akt-1(d)* led us to test the interaction of *akt-1(d)* and *pdk-1(d)* with group 2 Daf-c alleles (Table 9). Unlike *sa315*, *daf-16(lf)*, and *daf-18(lf)*, the *akt-1* and *pdk-1* alleles did not show any obvious effect on dauer formation by *daf-7(e1372)* or *daf-14(m77)*. Closer examination of the dauers formed by the *daf-7; akt-1(d)* double mutant indicated that the dauers were not partial (Figure 3).

The nature of the *sa315* mutation: Genetic mapping placed the *sa315* mutation in the same region of chromosome II as *age-1*. Since *age-1(null)* is Daf-c, this suggested the possibility that *sa315* is a gain-of-function mutation in *age-1*. The fact that only one *sa315*-like allele was found among the 36 mutations analyzed in this study is also consistent with this idea, since gain-of-function mutations are typically rare. Although *sa315* is recessive, a preliminary gene dosage analysis of *sa315* suggested that *sa315* is a gain-of-function mutation (MATERIALS AND METHODS). However, sequencing of the entire *age-1*

TABLE 9

Interactions of *daf-2/age-1* pathway mutants

Genotype	Frequency at 25°			
	Dauer	Partial dauer *pdk-1(lf)* type[a]	Partial dauer *daf-16* type[b]	Nondauer
daf-7(e1372)	100	0	0	0
daf-7(e1372); sa315	0	0	6	94
daf-7(e1372); daf-16(m27)	0	0	99	1
daf-7(e1372); daf-18(e1375)	0	0	100	0
daf-7(e1372); akt-1(mg144)	100	0	0	0
daf-14(m77)	98	0	0	2
daf-14(m77); sa315	0	0	0	100
daf-14(m77); daf-16(m27)	0	0	77	23
daf-14(m77); daf-18(e1375)	0	0	91	9
daf-14(m77); akt-1(mg144)	100	0	0	0
daf-14(m77); pdk-1(mg142)	99	0	0	1
daf-2(e1370)	100	0	0	0
daf-2(e1370); sa315	81	19	0	0
daf-2(e1370); daf-16(m27)	0	0	1	99
daf-2(e1370); daf-18(e1375)	0	0	100	0
daf-2(e1370); akt-1(mg144)	95	5	0	0
pdk-1(sa680)	19	76	0	5
pdk-1(sa680); sa315	0	0	0	100
pdk-1(sa680); daf-16(m27)	0	0	0	100
pdk-1(sa680); akt-1(mg144)	0	0	0	100

[a] These partial dauers appear thick but dark.

[b] These partial dauers have dauer alae but the pharynx is incompletely remodeled (Figure 3, C–F). Under a dissecting microscope, these appear thin but pale.

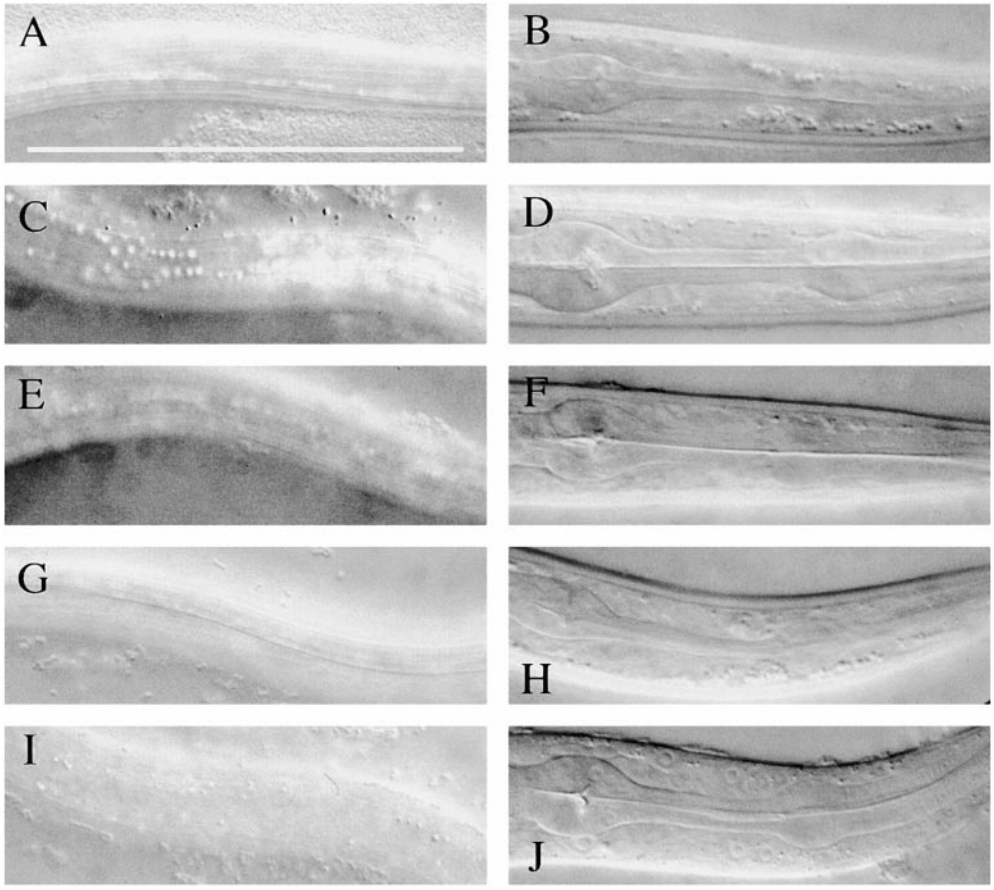

FIGURE 3.—*sa315* partial dauer phenotype. Shown are the dauer alae (or lack thereof; A, C, E, G, and I) and the pharynx (B, D, F, H, and J) from dauers, partial dauers, and an L3. The scale bar is 0.1 mm. (A and B) a *daf-7(e1372)* dauer. (C and D) a *daf-7; sa315* partial dauer. (E and F) a *daf-7; daf-16(m27)* partial dauer. (G and H) a *daf-7; akt-1(mg144)* dauer. (I and J) wild-type L3 larva. In a dauer, the dauer alae are present (A and G) and the pharynx is narrower and more tapered (B and H). In an L3 larva, the alae are absent (I) and the pharynx is wide (J). In partial dauers, the alae are present (C and E) but the pharynx shape is closer to that of the wild-type L3 (D and F).

coding region from the *sa315* mutant detected no mutations (MATERIALS AND METHODS). Therefore, *sa315* may be a mutation in the noncoding region of *age-1* or an allele of a novel gene in the region.

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