Genetic Analysis of the Roles of *daf-28* and *age-1* in Regulating *Caenorhabditis elegans* Dauer Formation

Elizabeth A. Malone, Takao Inoue and James H. Thomas

Department of Genetics, University of Washington, Seattle, Washington 98195

Manuscript received November 13, 1995
Accepted for publication April 6, 1996

ABSTRACT

Based on environmental cues, the nervous system of *Caenorhabditis elegans* regulates formation of the dauer larva, an alternative larval form specialized for long-term survival under harsh conditions. Mutations that cause constitutive or defective dauer formation (Daf-c or Daf-d) have been identified and the genes ordered in a branched pathway. Most Daf-c mutations also affect recovery from the dauer stage. The semi-dominant mutation *daf-28(sa191)* is Daf-c but has no apparent effect on dauer recovery. We use this unique aspect of *daf-28(sa191)* to characterize the effects of several Daf-d and synthetic Daf-c mutations on dauer recovery. We present double mutant analysis that indicates that *daf-28(sa191)* acts at a novel point downstream in the genetic pathway for dauer formation. We also show that *daf-28(sa191)* causes a modest increase (12–13%) in life span. The phenotypes and genetic interactions of *daf-28(sa191)* are most similar to those of *daf-2* and *daf-23* mutations, which also cause a dramatic increase in life span. We present mapping and complementation data that suggest that *daf-23* is the same gene as *age-1*, identified previously by mutations that extend life span. We find that *age-1* alleles are also Daf-c at 27°.

LARVAL development in *Caenorhabditis elegans* is regulated in response to local environmental conditions. When conditions are favorable for growth, worm development proceeds from the embryo through four larval stages (L1–L4) to the adult. However, when conditions are inhospitable, worms adopt an alternative third-larval form called a dauer larva (CASSADA and RUSSELL 1975; see RIDDLE 1988 and THOMAS 1993 for reviews). Dauers are specialized for enduring harsh conditions: they are resistant to desiccation, detergents, and anesthetics, and are considered nonaging since a dauer can live for several months and still have a normal post-dauer life span (CASSADA and RUSSELL 1975; KLASS and HIRSH 1976). They are easily recognized as thin non-feeding larvae with dark bodies. If environmental conditions improve, dauers go through a process of recovery: they begin feeding, become thicker and paler, and finally molt to normal L4 larvae (CASSADA and RUSSELL 1975).

Three environmental factors are known to regulate dauer formation. The most critical is the concentration of a dauer-inducing pheromone, which is secreted constitutively by the worms and probably indicates population density (GOLDEN and RIDDLE 1982, 1984a). The pheromone is not well characterized chemically, but it is very stable and seems to be composed of several related compounds similar to hydroxylated fatty acids and bile acids (GOLDEN and RIDDLE 1984c). Crude laboratory preparations of dauer pheromone can be used to induce dauer formation (GOLDEN and RIDDLE 1984b). While dauer pheromone is necessary and sufficient to induce dauer formation (GOLDEN and RIDDLE 1984a; 1985), its effectiveness is enhanced by higher temperatures and smaller amounts of food (GOLDEN and RIDDLE 1984a,c). The same three factors regulate dauer recovery, with low pheromone levels, low temperature, and abundant food promoting recovery (GOLDEN and RIDDLE 1982, 1984a,c). In the recovery process, the relative amounts of pheromone and food, rather than their absolute amounts, are most important (GOLDEN and RIDDLE 1982).

Neurons of the amphid sensilla in the worm's head regulate dauer formation (ALBERT *et al.* 1981; PERKINS *et al.* 1986; SHAKIR *et al.* 1993a; VOWELS and THOMAS 1994). Experiments in which neurons were killed with a laser microbeam have identified amphid sensory neurons that repress dauer formation (ADF, ASI, and ASG) (BARGMANN and HORVITZ 1991) and others that promote dauer formation (ASJ, ASK, and ADL) (W. S. SCHACKWITZ and J. H. THOMAS, unpublished results). In addition, ASJ promotes dauer recovery (BARGMANN and HORVITZ 1991). It is thought that these sensory neurons assess the level of pheromone and possibly the level of food and the temperature. This information is then integrated to control larval development.

Analysis of two kinds of mutations has led to the formal genetic model shown in Figure 1 (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; THOMAS *et al.*, 1993; GOTTLIEB and RUVKUN 1994). One class of mutations prevents dauer formation (*da*uer *f*ormation *d*efective or Daf-d) and the other inappropriately activates dauer formation (*da*uer *f*ormation *c*onstitutive or Daf-c). All

Corresponding author: James H. Thomas, Department of Genetics, Box 357360, University of Washington, Seattle, WA 98195-7360. E-mail: jht@genetics.washington.edu

of the Daf-c mutants shown here are also defective in dauer recovery (Dar) (RIDDLE *et al.* 1981; THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1994; MALONE and THOMAS 1994; VOWELS and THOMAS 1994; LARSEN *et al.* 1995). In most cases, the Daf-c and Dar phenotypes are stronger at higher temperature. A mutation in the gene most upstream in the pathway, *daf-22,* prevents the production or secretion of dauer pheromone (GOLDEN and RIDDLE 1985). The two parallel branches represented by the group 1 and group 2 Daf-c genes are thought to be required for the function of the dauer-promoting and -repressing sensory neurons, respectively (BARGMANN and HORVITZ 1991; W. S. SCHACKWITZ and J. H. THOMAS, unpublished results). Three of the group 2 Daf-c genes have been cloned and are predicted to encode components of a TGF-β signaling system: *daf-7* encodes a putative ligand homologous to TGF-β (LIM 1993), and *daf-1* and *daf-4* encode type 1 and type 2 receptor protein kinases of the TGF-β family (GEORGI *et al.* 1990; ESTEVEZ *et al.* 1993). Mutations in many genes (*che-2, daf-10, etc.,* see Figure 1) cause defects in the ciliated sensory endings of amphid neurons and result in a Daf-d phenotype (ALBERT *et al.* 1981; PERKINS *et al.* 1986; SHAKIR *et al.* 1993a; STARICH *et al.* 1995). One of these genes, *osm-3,* encodes a probable kinesin that is presumed necessary for proper transport of components of the cilia (SHAKIR *et al.* 1993b). The most downstream Daf-d gene, *daf-12,* is predicted to encode a nuclear hormone receptor, which may act in particular tissues to signal the developmental changes that occur during dauer formation (YEH 1991).

The nonaging quality of dauer larvae (KLASS and HIRSH 1976) suggested a link between the regulation of dauer formation and life span (reviewed in KENYON 1996). Recently it was found that Daf-c mutations in two genes, *daf-2* and *daf-23,* also extend life span (Age) (KENYON *et al.* 1993; LARSEN *et al.* 1995). It has been proposed that a specific life span extension program is activated in dauer larvae and that this program is inappropriately activated in certain Daf-c mutants (KENYON *et al.* 1993). *age-1* mutants were isolated based on their increased life span (KLASS 1983; FRIEDMAN and JOHNSON 1988a,b). Mutations that suppress the *daf-2* and *daf-23* Daf-c phenotype also suppress the Age phenotype of *daf-2, daf-23* and *age-1* mutations (KENYON *et al.* 1993; DORMAN *et al.* 1995; LARSEN *et al.* 1995). An analysis of these genes and their possible targets and regulators will lead to a better understanding of the aging process and how it is regulated.

We previously reported the identification of a new Daf-c mutation, *daf-28(sa191),* that has unique properties (MALONE and THOMAS 1994). First, *sa191* is a semi-dominant, gain-of-function mutation. Second, *daf-28(sa191)* mutants have apparently normal dauer recovery, unlike other Daf-c mutants. Here we present further characterization of *daf-28(sa191).* We analyze double mutants that indicate that *daf-28(sa191)* acts at a novel step late in the dauer formation pathway. We also use the *daf-28* mutant background as an expedient way to analyze the effect of many Daf mutations on dauer recovery. In addition, we identify a modestly longer life span in *daf-28(sa191)* mutants (12–13% longer than wild type). We provide an additional link between dauer formation and aging by demonstrating that an *age-1* mutant is Daf-c. Mapping and complementation results strongly suggest that the *daf-23* gene is the same as *age-1.*

MATERIALS AND METHODS

General genetic methods: Worms were grown on standard NG agar plates seeded with *Escherichia coli* strain OP50 (BRENNER 1974). Our OP50 was obtained from S. BRENNER via H. R. HORVITZ. The standard growth temperature was 20° unless otherwise noted. The *Caenorhabditis* Genetics Center (supported by the National Institutes of Health National Center for Research Resources) supplied wild-type (N2) and mutant strains. Standard *C. elegans* nomenclature is followed throughout (HORVITZ *et al.* 1979).

Mutations used: Linkage group I: *dpy-5(e61), unc-13(e376), che-3(e1124), daf-8(e1393ts), unc-29(e1072am), daf-16(m26), daf-16(m27), egl-32(n155), che-13(e1805), unc-75(e950).* Linkage group II: *dpy-10(e128), let-23(n1045cs), unc-4(e120), bli-1(e769), sqt-1(sc1), age-1(hx546), daf-23(mg44mat), daf-23(mg108mat), daf-23(mg109mat), lin-29(n333), rol-1(e91), daf-22(m130), daf-5(e1385), daf-5(sa205), unc-52(e444), mnC1.* Linkage group III: *unc-45(m94ts), daf-7(e1372ts), daf-2(e1370ts), daf-4(e1364ts), unc-36(e251), dpy-19(e1259ts, mat), unc-64(e246), qC1.* Linkage group IV: *daf-18(e1375), daf-1(sa184ts), dpy-9(e12), egl-4(n478), osm-3(e1806), unc-24(e138am), daf-10(e1387), daf-14(m77ts), unc-43(e408), him-8(e1489), unc-31(e169).* Linkage group V: *unc-42(e270), daf-11(sa195ts), sma-1(e30), osm-6(p811), che-11(e1810), sqt-3(sc63ts), egl-1(n986dm), daf-21(p673ts, mat), unc-76(e911), daf-28(sa191sd, ts).* Linkage group X: *egl-17(e1313), aex-3(sa5), che-2(e1033), unc-1(e719), daf-3(e1376), daf-3(sa206), osm-5(p803), lon-2(n1442spo), unc-58(e757), daf-12(m20), unc-3(e151), daf-6(e1377), osm-1(p808).*

Analysis of dauer formation and dauer recovery: Starvation assays were used to test for a Daf-d phenotype. One to four animals were picked to plates and grown at either 20° or 25°. Five days after the worms depleted the food, the plates were flooded with 1% SDS and screened for resistant dauer larvae. Pilot experiments showed that this is the optimal time in the wild type. The semi-quantitative analysis of dauers formed on each plate included the categories thousands, hundreds, a few, and none.

For assays of dauer formation under noninducing conditions, hermaphrodites were allowed to lay eggs on well-seeded plates for 4–6 hr at the assay temperature. To measure dauer formation, dauers and non-dauers on the agar and sides of dish were counted after ~55 hr at 25° or 5 days at 15°. This timing was important because *daf-28* mutants (MALONE and THOMAS 1994), as well as many double mutants, recover quickly. For each assay, *daf-28* single mutants were used in parallel to confirm that dauers had formed but had not recovered at the time of scoring.

To assay dauer recovery defects, dauers were induced in two ways. For one method, the *daf-28* mutation caused dauer formation at 25°, and non-dauers were removed from the plate. Alternatively, partially purified dauer pheromone was used to induce dauer formation at 25° as described (GOLDEN and RIDDLE 1984a). In this procedure, parents were picked to plates containing 5 units (THOMAS *et al.* 1993) of dauer pheromone and allowed to lay eggs over a period of ~4–6

hr. In most cases, dauers were picked after 3 days to well-seeded NG plates with no pheromone. For *unc-58* mutants, which develop more slowly, dauers were sometimes picked after 4 days. Following either method of dauer induction, dauers were incubated at 25°. Dauers and recovered worms on the agar were counted 24 hr later to measure dauer recovery. Because worms dried on the plastic sides of the dish did not have an opportunity to recover, they were not included in the dauer recovery data. Worms were scored as recovered if they had resumed pharyngeal pumping. To assay dauer recovery in the presence of pheromone, parents were allowed to lay eggs at 25° on plates containing 10 units of dauer pheromone. Animals that did not form dauers after 55 hr were removed, and dauer recovery was measured 24 hr later.

Construction of *daf-28; daf-d* double mutants: To construct *daf-22; daf-28, daf-28/+* males were crossed to *daf-22* hermaphrodites. F_2 dauer progeny were picked singly from cross progeny that segregated *daf-28*. After recovery, to verify that the dauers were *daf-28* homozygotes, we allowed individuals to lay eggs for 1 day and confirmed that nearly all of these F_3 progeny formed dauers at 25°. The F_3 dauers recovered at 20° and were screened carefully for those with tiny intestinal blobs, a phenotype associated with *daf-22* (J. H. THOMAS, unpublished results). To prove that *daf-22* was present in these putative double mutants, we reisolated *daf-22* as a single mutant and tested this strain for a Daf-d phenotype as described (VOWELS and THOMAS 1992).

We constructed *daf-28* double mutants with *daf-6* or cilium-structure mutations in a similar way. After identifying F_2 *daf-28* homozygotes, we screened several to identify those that segregated osmotic-avoidance defective (Osm) progeny. These Osm animals were saved as putative *daf-28;* cilium-structure double mutants, and several progeny were retested for the Osm phenotype to verify the presence of the cilium-structure mutation.

To construct *daf-3, daf-5,* and *daf-12* double mutants, we employed closely linked visible mutations in *trans* as balancing markers. For example, *daf-28/+* males were crossed to *daf-3* hermaphrodites, and male progeny were crossed to *unc-1* hermaphrodites. F_1 cross progeny that segregated dauers were of the genotype *daf-28/+; daf-3/unc-1.* (*daf-3* is *X*-linked.) Non-Unc F_2 dauers were picked singly, and *daf-28* homozygotes were identified as above. Those that segregated no Unc progeny were saved as putative *daf-28; daf-3* double mutants. For *daf-5,* the *trans* marker was *unc-52;* for *daf-12,* it was *unc-58.* In the *daf-3* and *daf-5* double mutants, the Daf-c phenotype of *daf-28* was epistatic. Therefore, we verified the presence of *daf-3* or *daf-5* by taking advantage of the fact that mutations in these genes suppress the Daf-c and *egg-l*aying defective (Egl) phenotypes of *daf-14* mutations. Wild-type males were crossed to the candidate double mutant, and male progeny were crossed to *unc-24 daf-14* hermaphrodites. F_1 cross progeny were picked singly and their broods were screened for non-Daf non-Egl Uncs (*daf-3* or *-5; unc-24 daf-14*). Several of these F_2 animals were picked singly to verify that *daf-14* was suppressed. In the *daf-28; daf-12* double mutant the Daf-d phenotype of *daf-12* was epistatic. Therefore, we verified the presence of *daf-28* by crossing wild-type males to the double mutant and demonstrating the segregation of F_2 dauers.

Construction of double mutants containing *daf-28* and *daf-16* or *daf-18*: As in constructing *daf-d* double mutants, we obtained *daf-16/dpy-5 unc-75; daf-28/+* and *daf-18/dpy-9; daf-28/+* heterozygotes. Wild-type F_2 dauers were picked to 25° and allowed to recover. F_3 progeny were screened to show that *daf-28* was homozygous. It was possible to distinguish the partial dauers characteristic of *daf-16* and *daf-18* at this stage, and candidate double mutants were saved. F_4 progeny were screened to verify absence of the balancer. Because the phenotypes of both mutations were visible in the double mutants, further confirmation of these strains was unnecessary.

Construction of *daf-28; daf-c* double mutants: To construct these strains, we took advantage of the fact that *daf-28* dauers recover well at 25°, whereas dauers induced by other Daf-c mutations recover poorly. Balancing markers for the Daf-c mutations were used as follows: *dpy-9* for *daf-1, qC1 dpy-19* for *daf-2, unc-36* for *daf-4, unc-45* for *daf-7, unc-29* for *daf-8, unc-42 sma-1* for *daf-11, unc-43* for *daf-14, unc-76* for *daf-21,* and *mnC1 dpy-10 unc-52* for *age-1* (*daf-23*). In general, males heterozygous for the balancing marker were crossed to *daf-28* hermaphrodites. The resulting male progeny were crossed to the appropriate *daf-c* mutant, and F_1 cross progeny were grown singly at 25°. We picked many F_2 dauers from several F_1s that segregated the balancer (*daf-c/bal*). These dauers were kept at 25°, and those that recovered after only 1 day were presumed to be *daf-28; daf-c/bal.* F_3 progeny of these candidates were grown at 25° to verify that *daf-28* was homozygous. F_4 progeny that failed to recover from the dauer state were potential *daf-c* homozygotes. These dauers were transferred to 15°, where they recovered, though inefficiently. Those that recovered were picked individually to plates at 15° to test for absence of the balancing marker. For *daf-1, daf-21,* and *age-1* (*daf-23*), the Daf-c phenotypes are maternally rescued and it was not possible to pick nonrecovering F_3 dauers. Instead, several F_3s were picked to 15°, and those that did not segregate the balancer were saved. We tested all candidates for the presence of *daf-28* and the second Daf-c mutation by complementation.

Construction of double mutants containing *daf-28* and synthetic Daf-c mutations: Synthetic Daf-c mutations cause a Daf-c phenotype at 25° in certain double mutant combinations but not as single mutants (AVERY 1993; J. J. VOWELS, K. IWASAKI, M. AILION and J. H. THOMAS, unpublished results; I. KATSURA, M. URASAKI, N. SUZUKI and T. ISHIHARA, personal communication). For these constructions, we used the additional phenotypes of the synthetic Daf-c mutations to follow them in crosses. We tracked *egl-4* and *egl-32* based on their Egl phenotypes. We followed *unc-3, unc-13, unc-31, unc-58,* and *unc-64* based on their *unc*oordinated (Unc) phenotypes. We identified *aex-3* mutants first by their scrawny slow-growing phenotype and later confirmed they were defective in contraction of the *a*nterior body muscles and *ex*pulsion muscles during defecation. In general, males heterozygous for a given synthetic Daf-c mutation were crossed to *daf-28* hermaphrodites. For some mutations on the *X* chromosome, this cross was reversed. F_1 cross progeny were picked singly to 25° and screened for those that segregated dauers and the synthetic Daf-c mutation. F_2 dauers were allowed to recover, and their progeny were grown at 25° to verify that *daf-28* was homozygous. Synthetic Daf-c mutants (Egl, Unc, or Aex) were identified among these F_3 animals. In the case of the dominant *unc-58* mutation, we verified that all F_4 progeny were Unc.

Construction of *daf-7; daf-28; daf-3*: In this construction, *daf-7* was homozygous at all times. *daf-7; him-5/+; daf-3/0* males were crossed to *daf-7; daf-28* hermaphrodites. F_1 cross progeny hermaphrodites were picked singly to 25°, and F_2 dauer progeny were picked to 15° to recover. After dauer recovery, non-Egl animals were used to establish lines. We inferred that *daf-3* was homozygous from the suppression of the *daf-7* Egl phenotype. *daf-28* was present based on its Daf-c phenotype. Two lines had indistinguishable Daf-c and Dar phenotypes. One that produced no males was saved for quantitative analysis.

Phenotypic analysis: We tested *daf-28* mutants for several phenotypes associated with other Daf-c mutations. Mutations in the group 1 Daf-c genes *daf-11* and *daf-21* cause defects in the response to nonvolatile and specific volatile attractants

(Che, Odr) (VOWELS and THOMAS 1994). Group 2 Daf-c mutants are Egl, have *d*arker *in*testines (Din) and associate in clumps (Cpy) (TRENT *et al.* 1983; THOMAS *et al.* 1993). *daf-2* and *daf-23* mutants are Din and long-lived (Age) (KENYON *et al.* 1993; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995; our unpublished observations). All of the synthetic Daf-c single mutants have pleiotropic behavioral phenotypes. They cause either *unc*oordinated movement (Unc), *e*gg-*l*aying defects (Egl), *con*stipation (Con), a short *de*fecation *c*ycle period (Dec), resistance to fluoride toxicity (Flr), pleiotropic chemosensory deficits, or some combination of the above. (BRENNER 1974; TRENT *et al.* 1983; THOMAS 1990; AVERY 1993; KATSURA *et al.* 1994; IWASAKI *et al.* 1995; J. J. VOWELS, K. IWASAKI, M. AILION and J. H. THOMAS, unpublished results; I. KATSURA, M. URASAKI, N. SUZUKI and T. ISHIHARA, personal communication).

Based on dissecting microscope observation, *daf-28* mutants moved normally (non-Unc), did not retain excess eggs (non-Egl), and dispersed normally (non-Cpy). Although *daf-28* worms sometimes appeared to have a slightly darker intestine, the difference from wild type could not be distinguished reliably in blind experiments (non-Din or weak Din). To assay defecation, four *daf-28* young adults raised at 20° were observed for 10 min each at 20° (CROLL and SMITH 1978; THOMAS 1990; LIU and THOMAS 1994). The three defecation muscle contractions were executed correctly (non-Con), and the defecation cycle period was normal (non-Dec). Forty *daf-28* animals had a normal mechanosensory response to light touch (non-Mec) when stroked with an eyelash as described (SULSTON *et al.* 1975; CHALFIE and SULSTON 1981). Sensitivity to fluoride was tested on NG plates containing 150 and 400 μg/ml NaF as described (KATSURA *et al.* 1994). The *daf-28* mutants were wild type (non-Flr). We previously reported that sensory neurons of the amphid and phasmid sensilla are structurally normal based on their ability to take up a fluorescent dye (non-Dyf) (MALONE and THOMAS 1994). We assayed avoidance of high osmotic strength as a further test of sensory function (CULOTTI and RUSSELL 1978; modified by VOWELS and THOMAS 1994). More than 50 *daf-28* animals were observed and responded normally (non-Osm). *daf-28* mutants also display normal thermotaxis (non-Ttx, I. MORI, personal communication).

Life span assays: Parents were allowed to lay eggs for 6–8 hr at 20° (day 0), a temperature at which *daf-28* mutants form dauers rarely. Two days later, progeny at the same developmental stage (L4) were transferred singly to plates. Therefore, the *daf-28* mutants analyzed did not spend longer in early larval stages (*e.g.*, L2d or dauer) than the wild-type controls. They were transferred to fresh plates every 2–3 days as their progeny developed. Every 1–4 days, worms were scored as alive, dead, missing or bagged (progeny hatched inside the mother, creating a bag of worms and killing the mother). Dead worms were those that did not move spontaneously, did not pump, and did not respond to tapping the plate or prodding. For each time point, the percentage alive was calculated as number alive/(number alive + number dead) × 100. In one assay, we started with 60 worms per strain, of which one to seven bagged or were lost. In a second assay, we started with 50 worms, of which two to four bagged or were lost. The life spans of individuals of a given strain were used to calculate the mean life span. Significance of differences between strains was determined with the Mann-Whitney U test using Instat 2.01 software.

Mapping the Daf-c phenotype of *age-1*: Progeny of *age-1(hx546)/let-23 unc-4* heterozygotes were picked singly to plates at 15° and six independent lines not segregating *let-23 unc-4* (non-Vul non-Unc) were kept and tested for the Daf-c phenotype at 27°. All were homozygous for the Daf-c mutation, demonstrating that the Daf-c phenotype associated with the *age-1* strain is on linkage group II, as is *age-1* (FRIEDMAN and JOHNSON 1988a). Additional three-factor crosses were used to map *age-1(hx546)* more precisely. These crosses were similar except that we picked progeny in which recombination had occurred between the two markers. For example, *unc-4 bli-1/age-1* heterozygotes yielded Unc non-Bli (*unc-4* +/ *unc-4 bli-1*) and Bli non-Unc (+ *bli-1/unc-4 bli-1*) recombinants. We analyzed the Daf-c phenotype at 27° in animals in which the recombinant chromosome was homozygous.

Complementation tests with *daf-23* and *age-1*: Because of the maternal rescue of *age-1* and *daf-23*, phenotypic analysis was carried out on progeny of *age-1/daf-23* heterozygotes. If the mutations complement, we expect very few mutant progeny, since both *age-1* and *daf-23* are maternally rescued. If the mutations fail to complement, we expect nearly all the progeny to show the mutant phenotype. We tested complementation for both the Daf-c and Age phenotypes. We analyzed the Daf-c phenotype using three *daf-23* alleles. For *daf-23(mg44)*, wild-type males were crossed to *daf-23(mg44)/sqt-1* hermaphrodites. *sqt-1*/+ animals *rol*l when they swim (Rol). Non-Rol male progeny (*daf-23*/+) were mated to *let-23 age-1(hx546)* or *unc-4 age-1(hx546)* hermaphrodites, and cross progeny were picked singly to plates at 27° or 25°. Of those at 27°, approximately half (17/36) segregated nearly 100% dauers, whereas the rest segregated none or very few. The animals that segregated none or very few indicate that *age-1(hx546)* is recessive. Of those at 25° (where *hx546* normally forms no dauers), about half (16/24) segregated partially arrested dauers characteristic of *daf-23* (GOTTLIEB and RUVKUN 1994). For *daf-23(mg108)* and *daf-23(mg109)*, N2 males were crossed to non-Dpy non-Unc progeny of *daf-23/mnC1 dpy-10 unc-52*. Male progeny were mated to *unc-4 age-1(hx546)*, and non-Unc progeny were picked to 27°. One in four is expected to be *daf-23/unc-4 age-1*. At least one non-Unc animal segregated over 50% dauers for both *mg108* (1/35) and *mg109* (7/30), indicating noncomplementation.

We analyzed the Age phenotype using *daf-23(mg44)*. Wild-type males were crossed to *daf-23(mg44)/sqt-1* hermaphrodites. Non-Rol hermaphrodite progeny (*daf-23*/+) were analyzed in life span assays at 25°. Non-Rol male progeny (*daf-23*/+) were mated to *unc-4 age-1(hx546)*, and non-Unc hermaphrodite progeny were analyzed in life span assays. In addition, to determine whether these animals were *daf-23/age-1* or +/*age-1*, their progeny were grown at 25° and scored for partially arrested dauers characteristic of *daf-23*.

RESULTS

Epistasis analysis of *daf-28(sa191)* and Daf-d mutations: The opposite phenotypes of Daf-c and Daf-d mutations have facilitated the ordering of gene function through epistasis analysis (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992). For this pathway, the phenotype of a *daf-c; daf-d* double mutant corresponds to the gene that acts downstream (AVERY and WASSERMAN 1992). As a first step in determining where the *daf-28(sa191)* gain-of-function mutation acts in the dauer formation pathway, we made double mutants containing *daf-28(sa191)* and one of several Daf-d mutations and measured dauer formation at 25°, the restrictive temperature for *daf-28* (Table 1). In most cases the double mutants displayed a strong Daf-c phenotype. We conclude that *daf-28(sa191)* acts downstream or independently of *daf-22* [required for pheromone production (GOLDEN and RIDDLE

TABLE 1

Dauer formation and dauer recovery at 25° of double mutants containing *daf-28(sa191)* and Daf-d mutations

Daf-d mutation	Dauer formation (%)	Dauer recovery (%)
+	98 (1768)	98 (1105)
daf-22(m130)	99 (451)	99 (472)
daf-6(e1377)	99 (698)	4 (376)
che-2(e1033)	98 (272)	1 (252)
che-3(e1124)	100 (542)[a]	1 (318)
che-11(e1810)	100 (688)	0 (327)
che-13(e1805)	100 (363)	1 (325)
daf-10(e1387)	99 (495)	14 (323)
osm-1(p808)	99 (241)	6 (109)
osm-3(e1806)	100 (452)	9 (165)
osm-5(p803)	100 (551)	5 (392)
osm-6(p811)	99 (603)	16 (358)
daf-3(e1376)	98 (772)	96 (756)
daf-5(e1385)	89 (308)	91 (282)
daf-5(sa205)	98 (536)	100 (133)
daf-12(m20)	0 (758)	NA

For all tables values in parentheses are the numbers of animals counted. NA, not applicable.

[a] Approximately 20% of the progeny did not hatch or were L1-L2 larvae and are not included in the numbers presented here.

1985)], *daf-6* [required for proper morphology of the amphid sheath cell (ALBERT *et al.* 1981; HERMAN 1984)], several genes required for correct structure of the amphid chemosensory cilia (*che-2, che-13, daf-10, osm-1, osm-3, osm-5,* and *osm-6*) (ALBERT *et al.* 1981; PERKINS *et al.* 1986), and *daf-3* and *daf-5*. In contrast, the *daf-28; daf-12* double mutant was not Daf-c and failed to form dauers in starvation assays (Daf-d), indicating that *daf-28(sa191)* acts upstream of *daf-12,* which encodes a protein similar to nuclear hormone receptors (YEH 1991).

Effects of *daf-16* and *daf-18* on *daf-28(sa191)*: Mutations in *daf-16* and *daf-18* cause defects in the response to dauer pheromone in two ways: (1) the dauers formed are incomplete or partial, based on the presence or absence of individual characteristics that differentiate a dauer from an L3; and (2) the efficiency of formation of even the partial dauers is much lower than in the wild type (VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994). With group 1 and group 2 Daf-c mutations, *daf-16* or *daf-18* double mutants form partial dauers, although in some cases the frequency is reduced from that of the Daf-c single mutant (VOWELS and THOMAS 1992). In contrast, *daf-16* mutations fully suppress the Daf-c phenotype of *daf-2* and *daf-23* mutations. The only known *daf-18* mutation suppresses some but not all alleles of *daf-2* and *daf-23,* and in several cases the suppression is incomplete (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995). Therefore, we have not included *daf-18* in the pathway in Figure 1. We constructed *daf-28(sa191)* double mutants with *daf-16* and *daf-18* and analyzed dauer formation (Table 2). In each case, any dauers that formed were partial and recovered well. Mutations in both *daf-16* and *daf-18* reduced the frequency of dauer formation but did not fully suppress *daf-28(sa191).* We examined the *daf-16(m26); daf-28* partial dauers by Nomarski microscopy and found that they have all the previously described features of partial dauers (VOWELS and THOMAS 1992).

Synergistic interactions between *daf-28(sa191)* and other Daf-c mutations: Previously, strong synergy between two groups of Daf-c mutations was identified (THOMAS *et al.* 1993). These interactions suggested that the two groups of Daf-c mutations (group 1 and group 2) define partially redundant sensory pathways that control dauer formation. Group 1 consists of *daf-11* and *daf-21,* and group 2 consists of *daf-1, daf-4, daf-7, daf-8,* and *daf-14.* We tested for synergy in double mutants containing *daf-28(sa191)* and other Daf-c mutations by measuring dauer formation at 15°, where all of the single mutants have relatively weak phenotypes (RIDDLE *et al.* 1981; THOMAS *et al.* 1993; Table 3). Because *daf-23* alleles are highly penetrant, we used *age-1(hx546)* given its weaker Daf-c phenotype and evidence that it is allelic to *daf-23* (see below). Strong synergy was observed between *daf-28(sa191)* and mutations in all group 1 and group 2 genes. These double mutants could recover with low efficiency (data not shown), in contrast to double mutants with one group 1 and one group 2 Daf-c mutation, which are completely dauer recovery defective (THOMAS *et al.* 1993). There was no synergy with mutations *daf-2* or *daf-23,* genes believed to function downstream of, or in parallel to, both the group 1 and group 2 Daf-c genes (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995).

Synergistic interactions between *daf-28(sa191)* and synthetic Daf-c mutations: Many double mutant combinations result in a strong Daf-c phenotype at 25° even though the single mutations cause no obvious Daf-c phenotype under the same conditions (synthetic Daf-c). There are perhaps more than 40 genes that have this property (AVERY 1993; J. J. VOWELS, K. IWASAKI, M. AILION and J. H. THOMAS, unpublished results; I. KATSURA, M. URASAKI, N. SUZUKI and T. ISHIHARA, personal communication). Some of these genes are predicted to encode components of the synapse (D. LIVINGSTONE, R. MARTIN, M. NONET, A. POTTER and J. RAND, personal communication) or signal transduction proteins (MARUYAMA and BRENNER 1991; KATSURA *et al.* 1994). We tested whether *daf-28(sa191)* has synergistic interactions with selected synthetic Daf-c mutations by constructing double mutants and assaying dauer formation at 15°. The degree of enhancement varied with the mutation tested (Table 4). For example, *unc-13, unc-31, unc-58,* and *unc-64* strongly enhanced the *daf-28* Daf-c phenotype, whereas an *egl-32* mutation did not. The results with *aex-3, egl-4* and *unc-3* were intermediate.

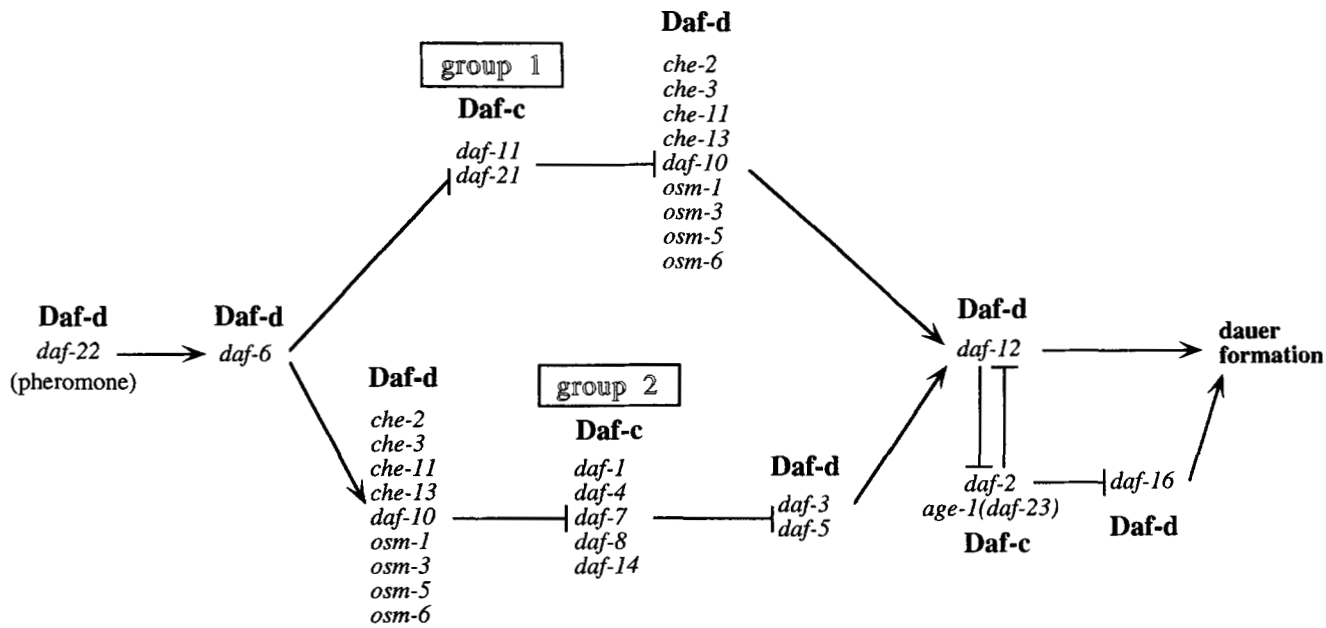

FIGURE 1.—Genetic model for the control of dauer formation. Modified from THOMAS *et al.* (1993) and GOTTLIEB and RUVKUN (1994). The order of gene action is based mainly on gene interactions. The cilium-structure mutations (*e.g., che-2*) are shown as affecting both branches because these mutations cause morphological defects in all of the amphid sensory neurons known to be involved in regulating dauer formation (PERKINS *et al.* 1986; BARGMANN and HORVITZ 1991; VOWELS and THOMAS 1992, 1994; W. SCHACKWITZ and J. H. THOMAS, unpublished results).

The effects of *daf-28(sa191)* on dauer recovery: The effect of Daf-c mutations on dauer recovery has been previously described. With the exception of *daf-28(sa191)*, all Daf-c mutants are dauer recovery defective (Dar). In general, Daf-c mutants at 25° form dauers that recover poorly at 25°. Upon transfer to 15°, most group 1 Daf-c mutants recover slowly (a week or more), while group 2 Daf-c mutants generally recover quickly (1–2 days) (RIDDLE *et al.* 1981; THOMAS *et al.* 1993; VOWELS and THOMAS 1994; our unpublished observations). Some alleles of *daf-2* entirely prohibit dauer recovery at any temperature (MALONE and THOMAS 1994; LARSEN *et al.* 1995) and *daf-23* mutants recover poorly (GOTTLIEB and RUVKUN 1994). Double mutants containing one group 1 Daf-c mutation and one group 2 Daf-c mutation fail to recover (THOMAS *et al.* 1993). In addition, mutations in the synthetic Daf-c gene *unc-31* impair dauer recovery (AVERY *et al.* 1993).

TABLE 2

Dauer formation at 25° of double mutants containing *daf-28(sa191)* and partial Daf-d mutations

Partial Daf-d mutation	*daf-28(+)* (%)	*daf-28(sa191)* (%)
+	0 (860)	99 (868)
daf-16(m26)	0 (373)	47 (950)[a]
daf-16(m27)	0 (572)	56 (1029)[a]
daf-18(e1375)	0 (453)	20 (893)[a]

[a] Percentage partial dauers (VOWELS and THOMAS 1992). The rest of the population formed neither complete nor partial dauers.

We previously reported that *daf-28(sa191)* does not cause a dauer recovery defect; mutants at 25° form dauers that begin to recover within 2 hr (MALONE and THOMAS 1994). We tested whether this mutation activates dauer recovery constitutively by growing *daf-28(sa191)* with a high concentration of dauer pheromone (10 units/plate) and limiting food (dauer-recovery-repressing conditions) (GOLDEN and RIDDLE 1984a). This amount of pheromone fully inhibited dauer recovery of both the wild-type and *daf-28* mutants (Table 5). Therefore, *daf-28* has no apparent effect on dauer recovery, although it remains possible that these mutants have subtle, undetected defects.

The effects of Daf-d mutations on dauer recovery: The effects of Daf-d mutations on dauer recovery

TABLE 3

Dauer formation at 15° of double mutants containing *daf-28(sa191)* and Daf-c mutations

Daf-c mutation	*daf-28(+)* (%)	*daf-28(sa191)* (%)
+	0 (600)	4 (536)
daf-11(sa195)	9 (266)	100 (372)
daf-21(p673)	43 (100)	100 (372)
daf-1(sa184)	77 (161)	100 (200)
daf-4(e1364)	37 (215)	100 (118)
daf-7(e1372)	14 (129)	100 (198)
daf-8(e1393)	1 (502)	100 (592)
daf-14(m77)	61 (157)	99 (314)
daf-2(e1370)	0 (402)	0.7 (556)
age-1(hx546)	0 (530)	0 (288)

TABLE 4

Dauer formation at 15° of double mutants containing *daf-28(sa191)* and syn-Daf mutations

Synthetic Daf-c mutation	*daf-28(+)* (%)	*daf-28(sa191)* (%)
+	0 (600)	5 (1259)
aex-3(sa5)	0 (394)	24 (309)
egl-4(n478)	0 (538)	23 (634)
egl-32(n155)	0 (234)	0 (214)
unc-3(e151)	0 (573)	24 (974)
unc-13(e376)	0 (511)	99 (403)
unc-31(e169)	0 (389)	97 (510)
unc-58(e757dm)	0 (226)[a]	100 (111)[a]
unc-64(e246)	0 (364)	99 (551)

[a] For these strains, the synchronous egg-lay was not sufficient to give synchronous development of all progeny. At the time of assaying, 2–20% of the progeny had not yet become L3s or dauers and are not included in the numbers presented here.

TABLE 5

Dauer recovery in the presence of dauer pheromone at 25°

Genotype	Dauer recovery (%)
+	0 (132)
daf-28(sa191)	0 (93)
daf-28(sa191); daf-3(e1376)	1 (97)
daf-5(e1385); daf-28(sa191)	0 (101)

are difficult to study because Daf-d mutants do not form dauers. Because *daf-28(sa191)* is epistatic to nearly all Daf-d mutations and does not itself affect recovery, it is a useful tool for analyzing dauer recovery in Daf-d mutants. We tested the effect of Daf-d mutations on *daf-28*-induced dauers at 25°. We found that mutations in all of the cilium-structure genes tested (*che-2, che-3, che-11, che-13, daf-10, osm-1, osm-3, osm-5,* and *osm-6*) drastically reduced the efficiency of dauer recovery at 25° (Table 1). A *daf-6* mutation, which affects the amphid sheath cell, also impaired recovery (Table 1). Since these sensory-defective mutants were unable to recover, it seems likely that dauer recovery is activated by a favorable signal rather than repressed by an unfavorable signal. In contrast, *daf-22, daf-3,* and *daf-5* did not inhibit dauer recovery (Table 1).

Mutations in *daf-3* and *daf-5* suppress group 2 Daf-c mutations in *daf-1, daf-7, daf-8,* and *daf-14*. These group 2 mutants are Daf-c, Egl, have darker intestines (Din) and associate in clumps (Cpy), and each of these phenotypes is suppressed by *daf-3* and *daf-5* mutations (TRENT *et al.* 1983; THOMAS *et al.* 1993). We tested whether a *daf-3* mutation would also suppress a group 2 dauer recovery defect by constructing a *daf-7; daf-28; daf-3* triple mutant and assaying dauer recovery after growth at 25° (Table 6). In contrast to the *daf-7; daf-28* double mutant, the triple mutant recovered well, indicating that *daf-3* suppresses the *daf-7* recovery defect. We infer that the relationship between *daf-7* and *daf-3* is the same in controlling dauer formation and dauer recovery.

We also tested whether *daf-3* or *daf-5* mutations inappropriately activate dauer recovery. We reasoned that, since many mutations cause constitutive dauer formation and inhibit recovery, mutations that inhibit dauer formation might cause constitutive recovery. Therefore, we grew *daf-28; daf-3* and *daf-5; daf-28* worms on plates containing a high level of dauer pheromone (10 units/ plate) and limiting food. The *daf-28* mutation induced dauer formation in spite of the Daf-d mutations. For both *daf-3* and *daf-5*, the dauers did not recover in the presence of dauer pheromone (Table 5), indicating that *daf-3* and *daf-5* mutations do not cause constitutive dauer recovery.

The effects of synthetic Daf-c mutations on dauer recovery: We examined the effect of individual synthetic Daf-c mutations on dauer recovery in two ways. First, we grew *daf-28* double mutants at 25° and determined whether the synthetic Daf-c mutations blocked recovery (Figure 2, stippled bars). *unc-31* mutants were previously described as Dar (AVERY *et al.* 1993), and an *unc-31* mutation caused a profound dauer recovery defect in the *daf-28(sa191)* background, as expected. In addition, *egl-4, unc-3, unc-13, unc-58* and *unc-64* mutations had very strong Dar phenotypes. In contrast mutations in *aex-3* and *egl-32* had little or no effect.

Although *daf-28* dauers recover like wild type in all of our assays, they might in principle have a subliminal dauer recovery defect. Therefore we used a second assay that allowed us to analyze the synthetic Daf-c mutants in the absence of the *daf-28* mutation. We used dauer pheromone to induce dauer formation, transferred the dauers to plates with no pheromone at 25°, and determined the percentage that recovered. Generally, the results of this assay were similar in *daf-28(+)* and *daf-28(sa191)* backgrounds, although the *unc-31; daf-28* dauers recovered somewhat better than the *unc-31* dauers (Figure 2, black and gray bars, respectively). For most synthetic Daf-c mutants, the results after pheromone induction were similar to those obtained in the first experiment without pheromone induction. However, *egl-4, unc-58,* and *unc-64* mutants were strongly recovery defective in the first assay and nearly wild type in the second. Possible explanations for these discrepancies are explored in the DISCUSSION.

Extended life span in *daf-28(sa191)* mutants: In addition to defects in dauer formation and dauer recovery, all Daf-c and synthetic Daf-c mutations confer other phenotypes (BRENNER 1974; TRENT *et al.* 1983; THOMAS 1990; AVERY 1993; KENYON *et al.* 1993; THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1994; KATSURA *et al.* 1994; VOWELS and THOMAS 1994; IWASAKI *et al.* 1995; LARSEN *et al.* 1995; J. J. VOWELS, K. IWASAKI, M. AILION and J. H. THOMAS, unpublished results; I. KATSURA, M. URASAKI, N. SUZUKI and T. ISHIHARA, personal communication).

TABLE 6

Dauer formation and recovery of *daf-3* double and triple mutants at 25°

Daf-c mutation	*daf-3(+)*		*daf-3(e1376)*	
	Dauer formation (%)	Dauer recovery (%)	Dauer formation (%)	Dauer recovery (%)
daf-28(sa191)	99 (626)	100 (681)	98 (772)	96 (756)
daf-7(e1372)	100 (324)	0 (324)	0 (602)	NA
daf-7(e1372); daf-28(sa191)	100 (516)	1 (511)	98 (1074)	97 (1077)

NA, not applicable.

As described in MATERIALS AND METHODS, we tested *daf-28(sa191)* mutants and found that they were wild type in every test except for life span assays. At 20°, *daf-28(sa191)* increased the mean life span from 20.7 days to 23.4 days (Table 7, Figure 3). This modest increase of 13% is statistically significant ($P = 0.0048$). It was reproduced in a second assay (12% increase, $P = 0.0003$) as well as in assays by J. REITER and C. KENYON (personal communication). *daf-12* suppressed the *daf-28* Age phenotype ($P = 0.0011$) (Table 7, Figure 3). We examined a *daf-16; daf-28* double mutant, but the result was difficult to interpret since the life span of the *daf-16* single mutant was shorter than wild type in this experiment (Table 7, reported previously in KENYON *et al.* 1993 and LARSEN *et al.* 1995). It has been shown previously that *daf-2* and *daf-23* mutations dramatically increase the life span of *C. elegans*, whereas other Daf-c mutations do not (KENYON *et al.* 1993; LARSEN *et al.* 1995). Although the *daf-28(sa191)* mutation does not extend life span as dramatically as other Age mutations, we tentatively classify *daf-28* among a small group of genes that affect both dauer formation and aging.

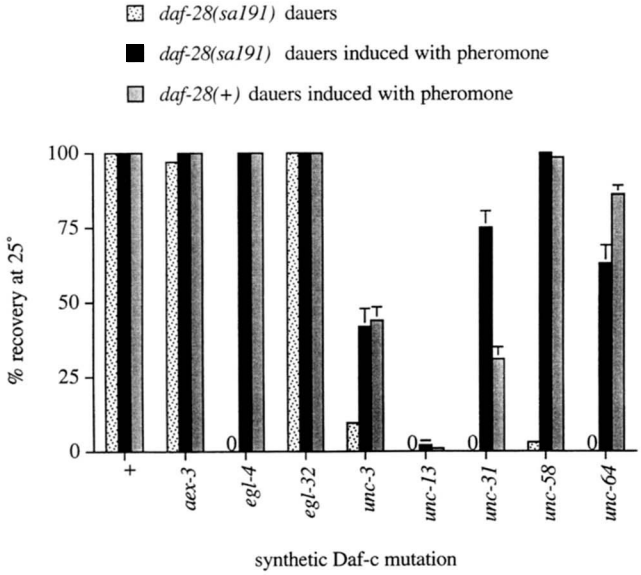

FIGURE 2.—Dauer recovery of syn-Daf mutants. Induction of dauers is described in MATERIALS AND METHODS. The percentage of dauers that recovered on bacterial lawns after 24 hr at 25° is shown. Each synthetic Daf-c mutation was tested under all three conditions. A zero marks each instance of 0% recovery. When dauers were induced at 25° with no pheromone (stippled bars), 230–994 dauers were assayed. When dauers were induced with dauer pheromone (black and gray bars), 48–120 dauers were assayed. Standard errors are shown as vertical lines.

***age-1(hx546)* is allelic to *daf-23*:** *daf-2, daf-23,* and *daf-28* mutations were identified by their effects on dauer formation and later found to affect life span. This lead us to consider whether other mutations known to affect aging also affect dauer formation. *age-1(hx546)* was originally isolated and characterized as a mutation that increases life span (KLASS 1983; FRIEDMAN and JOHNSON 1988a,b). The Age phenotypes of *daf-2, daf-23,* and *age-1* mutations are all suppressed by *daf-16* mutations, indicating a similar underlying mechanism (KENYON *et al.* 1993; DORMAN *et al.* 1995; LARSEN *et al.* 1995). We therefore tested the *age-1* mutant for a Daf-c phenotype. At 25°, the standard restrictive condition for dauer formation, the *age-1* mutant did not form dauers. However, at 27°, 100% of *age-1(hx546)* animals formed dauers while the wild type did not ($n = 430$). This phenotype was extremely sensitive to temperature, with variations of 0.5° having dramatic effects on penetrance.

To confirm that the Daf-c phenotype of the *age-1* mutant is not caused by a background mutation, we mapped the Daf-c phenotype in the *age-1* strain (see MATERIALS AND METHODS). *age-1* was previously assigned to linkage group II based on its Age phenotype (FRIEDMAN and JOHNSON 1988a). Our results indicate that the Daf-c mutation is also on linkage group II, between *sqt-1* and *lin-29* (Table 8), a map position indistinguishable from that of *daf-23* (GOTTLIEB and RUVKUN 1994). We also noted during these crosses that it was very difficult to find dauer progeny of *age-1*/+ parents at 27°, indicat-

TABLE 7

Life span at 20° of *daf-28* and *daf-28; daf-d* double mutants

Daf-d mutation	*daf-28(+)* (days)	*daf-28(sa191)* (days)
+	20.7 ± 5.0	23.4 ± 4.9
daf-16(m26)	18.0 ± 4.3	14.0 ± 2.5
daf-12(m20)	21.7 ± 4.7	20.1 ± 5.2

Values are means ± SD.

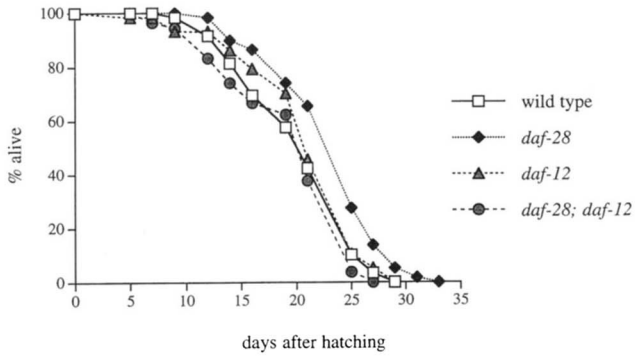

FIGURE 3.—The effect of *daf-28(sa191)* on life span. Worms were grown continuously at 20°. These data are for animals grown in parallel. Day 1 is the day of hatching. The mean life spans are shown in Table 7. The life span for *daf-28(sa191)* is significantly different from the wild type ($P = 0.0048$) and *daf-28; daf-12* ($P = 0.0011$). The life spans for wild type and *daf-12* are not significantly different (P = 0.2963). The wild-type strain is the direct parent of the *daf-28(sa191)* mutant. A second experiment gave similar results.

ing that the *age-1* Daf-c phenotype is maternally rescued like that of *daf-23*. Because of these similarities between *age-1* and *daf-23*, we performed complementation tests between *age-1(hx546)* and three *daf-23* alleles, and analyzed the Daf-c phenotype at 27°. All alleles were fully recessive and failed to complement (see MATERIALS AND METHODS). In addition, we tested complementation for the Age phenotype (see MATERIALS AND METHODS). The mean life spans ± SD were as follows: 13.0 ± 2.9 ($N = 29$) for *daf-23/+*, 14.1 ± 3.4 ($N = 56$) for *age-1/+*, and 28.3 ± 8.7 ($N = 27$) for *daf-23/age-1*. These data indicate that both mutations are recessive and that they fail to complement. These results strongly suggest that *age-1(hx546)* causes both the Age and the Daf-c phenotypes and that it is allelic with *daf-23*.

DISCUSSION

***daf-28* and the dauer pathway:** We analyzed 35 double mutants containing *daf-28(sa191)* to understand its relationship to other genes known to regulate dauer formation. Although this *daf-28* allele is a gain-of-function mutation, deficiency analysis indicates that the wild-type *daf-28* gene product interferes with the dominance of *sa191* (MALONE and THOMAS 1994), suggesting that *daf-28(+)* is normally involved in regulating dauer formation. The epistasis analysis presented here reveals at what point the mutant gene product acts, with a downstream Daf-d mutation blocking the Daf-c phenotype. Our results indicate that *daf-28(sa191)* occupies a novel position in the dauer formation pathway.

daf-28(sa191) is clearly distinct from group 1 and group 2 Daf-c mutations. Daf-d mutations that disrupt the chemosensory cilia suppress group 1 Daf-c mutations in *daf-11* and *daf-21* (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992), but not *daf-28(sa191)*. Daf-d mutations in *daf-3* and *daf-5* suppress group 2 Daf-c mutations in *daf-1, daf-4, daf-7, daf-8,* and *daf-14* (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; THOMAS *et al.* 1993; LARSEN *et al.* 1995), but not *daf-28(sa191)*. Group 1 Daf-c mutations are strongly synergistic with group 2 Daf-c mutations but not with other group 1 mutations; the reciprocal is true for group 2 mutations (THOMAS *et al.* 1993). In contrast, *daf-28(sa191)* is synergistic with mutations in both group 1 and group 2 Daf-c genes.

We found some intriguing similarities between *daf-28(sa191)* and Daf-c mutations in *daf-2* and *daf-23*. Like *daf-28(sa191), daf-2* and *daf-23* mutations are not suppressed by Daf-d mutations that disrupt sensory cilia or by Daf-d mutations in *daf-3* and *daf-5* (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995). In addition, *daf-2(e1370)* has synergistic interactions with mutations in both group 1 genes (*daf-11* and *daf-21*) and at least one group 2 gene (*daf-14*) (our data, not shown), similar to *daf-28(sa191)*. Finally, mutations in all three genes increase life span, although the effect of *daf-28(sa191)* is comparatively small.

On the other hand, we also found certain ways in which *daf-28(sa191)* is different from *daf-2* and *daf-23* mutations. First, combining *daf-23* or some *daf-2* mutations with a *daf-12* mutation (Daf-d) causes embryonic or early larval lethality, suggesting that *daf-2* and *daf-23* act antagonistically or in parallel to *daf-12* (VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995, see Figure 1). As shown in Table 1, this is not

TABLE 8

Three factor map data for the Daf-c phenotypes of *age-1(hx546)*

Genotype of heterozygote	Recombinant phenotype	Recombinant genotype	*age-1* position
age-1/ let-23 unc-4	Vul non-Unc	1/1 *let-23* + *age-1*	
	Unc non-Vul	7/7 + *unc-4* +	Right of *let-23*
age-1/unc-4 bli-1	Unc non-Bli	9/9 *unc-4* + *age-1*	
	Bli non-Unc	2/2 + *bli-1* +	Right of *unc-4*
age-1/bli-1 rol-1	Bli non-Rol	7/18 *bli-1* + +	
		11/18 *bli-1 age-1* +	Between *bli-1* and *rol-1*
age-1/sqt-1 lin-29	Rol non-Lin	11/14 *sqt-1* + +	
		3/14 *sqt-1 age-1* +	Between *sqt-1* and *lin-29*

the case for *daf-28(sa191)*. In this respect, *daf-28* is similar to the *daf-2(m41)* allele. Second, all tested double mutant combinations of cilium-structure mutations with *daf-2* or *daf-23* result in early larval or embryonic arrest (VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994). For *daf-28*, we observed no lethality in most cilium-structure double mutants (Table 1). A lethal interaction was found in the *che-3; daf-28* double mutant, but additional analysis is required to rule out the possibility that this results from a background mutation. Third, the Daf-c phenotype of *daf-2* and *daf-23* mutations is fully suppressed by *daf-16* mutations; most *daf-23* alleles and weak *daf-2* alleles are suppressed by *daf-18* (RIDDLE *et al.* 1981; VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995). In contrast, suppression of *daf-28(sa191)* by either *daf-16* or *daf-18* is incomplete. Fourth, *daf-2* and *daf-23* mutants have unusually dark intestines (Din) (THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1994; our unpublished observations). This phenotype is weak and variable in *daf-28(sa191)* mutants. Fifth, although *daf-28(sa191)* increases life span (Age), the increase is fairly modest compared to that conferred by *daf-2* or *daf-23* mutations (KENYON *et al.* 1993; LARSEN *et al.* 1995). Some of these differences may be explained by hypothesizing that *daf-28(sa191)* is a relatively weak allele. Even so, we find the similarities between *daf-28* and *daf-2* or *daf-23* to be intriguing, but not compelling enough to conclude that these genes function at the same physiological step.

With regard to the genetic pathway in Figure 1, our results are consistent with several possibilities. *daf-28(sa191)* could act downstream in each of the two branches represented by the group 1 and group 2 Daf-c genes. Alternatively, *daf-28(sa191)* could function at a step downstream of the convergence point of these branches. *daf-28(sa191)* may act at the same step as *daf-2* and *daf-23*, but the poor suppression by *daf-16* and *daf-18* argues against this. Finally, *daf-28(sa191)* could activate a separate branch entirely. Isolation and analysis of *daf-28* loss-of-function mutations may help to clarify the function of this gene.

It has only recently been appreciated that the synthetic Daf-c mutations play a role in dauer formation, and their interactions with other Daf mutations are not well characterized. Thus it is not possible to use such interactions as a point of comparison for *daf-28(sa191)* and synthetic Daf-c mutations. The varied and pleiotropic effects of synthetic Daf-c mutations on nervous system function suggest that many of these genes are important for several behaviors (BRENNER 1974; TRENT *et al.* 1983; THOMAS 1990; AVERY 1993; KATSURA *et al.* 1994; IWASAKI *et al.* 1995; J. J. VOWELS, K. IWASAKI, M. AILION and J. H. THOMAS, unpublished results; I. KATSURA, M. URASAKI, N. SUZUKI and T. ISHIHARA, personal communication). One model is that they act in interneurons that integrate sensory information. The probable involvement of at least some of these gene products in signal transduction (KATSURA *et al.* 1994; MARUYAMA and BRENNER 1991) or synaptic function (D. LIVINGSTONE, R. MARTIN, M. NONET, A. POTTER and J. RAND, personal communication) is compatible with such a model. In contrast, *daf-28(sa191)* does not obviously disrupt neuronal processes other than dauer formation. A more complete description of the genetic interactions among synthetic Daf-c genes and their molecular functions will help with interpretation of the *daf-28* double mutant phenotypes presented here.

Dauer recovery: In dauer formation, the two partially redundant branches of the genetic pathway (THOMAS *et al.* 1993) are thought to depend on two different groups of sensory neurons (BARGMANN and HORVITZ 1991; W. S. SCHACKWITZ and J. H. THOMAS, unpublished results). This complexity may reflect the importance to the worm of choosing the most appropriate developmental pathway, the L3 or the dauer larva. Likewise, a worm must carefully choose when to recover from the dauer state. Detailed analyses of genes and cells important for recovery have not yet been undertaken. Dauer pheromone, food, and temperature are all factors that influence both decisions (GOLDEN and RIDDLE 1982, 1984a,c). ASJ, a ciliated amphid neuron, activates both dauer recovery and dauer formation (BARGMANN and HORVITZ 1991; W. S. SCHACKWITZ and J. H. THOMAS, unpublished results). Most mutations that constitutively activate dauer formation also inhibit dauer recovery (RIDDLE *et al.* 1981; THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1994; MALONE and THOMAS 1994; VOWELS and THOMAS 1994; LARSEN *et al.* 1995; our unpublished observations). It seems that common sensory cells and gene products mediate two different responses to the same environmental factors. Each process must also involve one or more unique downstream steps. We hypothesize that the *daf-28(sa191)* mutation acts exclusively in dauer formation at such a step because it has a Daf-c phenotype but no detected Dar phenotype and because it functions downstream in the dauer formation pathway.

Two simple models for the sensory regulation of dauer recovery can be imagined. Harsh conditions may repress dauer recovery or advantageous conditions may activate dauer recovery. Alternatively, both types of signals may be important. Using *daf-28(sa191)* double mutants, we showed that mutations that disrupt sensory input impaired dauer recovery, suggesting that dauers rely on favorable environmental signals to activate recovery. It is possible that the Dar phenotype results from effects of the cilium-structure mutations on neuronal activity rather than on sensory capabilities. However, a *daf-6* mutation, which causes the nonneuronal amphid sheath cell to occlude the amphid pore (ALBERT *et al.* 1981; HERMAN 1984), also causes a dauer recovery defect. We think it is unlikely that the dauer recovery defects are related to *daf-28(sa191)* because cilium-structure mutations seem to inhibit dauer recovery in other

Daf-c backgrounds (our unpublished observations), although this result is complicated by the fact that these Daf-c mutations affect dauer recovery themselves.

We found that a *daf-3* mutation suppresses the *daf-7* Dar phenotype. This suggests that *daf-3* acts downstream of *daf-7* in dauer recovery, as it does in dauer formation and other processes (TRENT *et al.* 1983; VOWELS and THOMAS 1992; THOMAS *et al.* 1993). However, we found that *daf-3* and *daf-5* mutations do not strongly inhibit or promote dauer recovery in a *daf-28* background. These results suggest the possibility that other genes are redundant with *daf-3* in dauer recovery. These may function in parallel to *daf-3* in the dauer formation pathway as well, perhaps in the branch defined by the group 1 Daf-c genes (see Figure 1).

Mutations in many of the synthetic Daf-c genes tested also confer a Dar phenotype. *unc-31* mutations were previously shown to block dauer recovery (AVERY *et al.* 1993). We show here that *unc-3* and *unc-13* mutants also have dauer recovery defects. They may be comparable to Daf-c mutations that affect both dauer formation and dauer recovery. *aex-3* and *egl-32,* on the other hand, have little or no effect on dauer recovery. Results with *egl-4, unc-58,* and *unc-64* mutants are more difficult to interpret. In these cases, *daf-28(sa191)* double-mutants that formed dauers with no added dauer pheromone recovered quite poorly. In contrast, dauers induced with dauer pheromone recovered well, regardless of the *daf-28* background. Similar but less dramatic differences between the two assays were found with *unc-3* and *unc-31* mutants. We think it is probable that differences in population density are responsible. In the experiments in which *daf-28(sa191)* was used for dauer induction, the recovering dauers were much more crowded, although they had plenty of food. We hypothesize that these synthetic Daf-c mutants are sensitized to the known inhibition of dauer recovery by crowding (GOLDEN and RIDDLE 1982). A second difference was that pheromone-induced dauers were picked to new plates, while *daf-28*-induced dauers were left undisturbed. It is possible that the picking process somehow promoted dauer recovery (G. RUVKUN, personal communication). In either case, it seems likely that dauer recovery can be influenced by relatively subtle differences in the environment. Among the synthetic Daf-c mutants, we found no absolute correlation between the Dar phenotype and enhancement of the *daf-28* Daf-c phenotype.

Although we have not performed a comprehensive analysis of dauer recovery, our results broaden our knowledge significantly. We have determined that mutations in several genes, including cilium-structure genes, the Daf-d gene *daf-3,* and some synthetic Daf-c genes influence dauer recovery. Our results suggest that the process of dauer recovery involves a large number of genes that presumably function in a pathway comparable in complexity to that regulating dauer formation.

Aging and dauer formation: Dauer larvae are described as nonaging because arrest at this stage for months has no effect on post-dauer life span (KLASS and HIRSH 1976). Previous studies showed that mutations in *daf-2* and *daf-23* affect both dauer formation and aging and that these effects are suppressed by *daf-16* mutations (RIDDLE *et al.* 1981; KENYON *et al.* 1993; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995). *daf-28(sa191)* might also be classified in this group, although the Age phenotype of *daf-28* mutants is much weaker and suppression of the Daf-c phenotype by *daf-16* is not complete. One model is that the nonaging property of dauers results from activation of a specific life span extension program dependent on *daf-16(+)* and that this program is inappropriately activated in adults by mutations in some Daf-c genes (KENYON *et al.* 1993).

age-1 mutants were first identified by their extended life spans (KLASS 1983). We report here that *age-1(hx546)* mutants are strongly Daf-c at 27°, a higher temperature than that normally used to assay this phenotype. This was the only *age-1* allele available to us, but others have confirmed that three other recently isolated *age-1* alleles are also Daf-c (S. A. DUHON and T. E. JOHNSON, personal communication). This strongly argues that the Daf-c phenotype results from an *age-1* mutation rather than a background mutation. The *age-1* Daf-c phenotype mapped to the same interval as *daf-23,* and complementation tests showed that *age-1* and *daf-23* mutations fail to complement. The simplest model is that these mutations all affect a single gene that controls both dauer formation and aging. It is also possible that *age-1* and *daf-23* are separate genes that fail to complement. Although examples of this phenomenon do exist, they are rare and are generally thought to reflect physical interactions of the protein products (*i.e.,* KUSCH and EDGAR 1986; REGAN and FULLER 1988; STEARNS and BOTSTEIN 1988; HAYES *et al.* 1989; GREEN *et al.* 1990; SWANSON and WINSTON 1992). Lacking convincing evidence for two genes, we favor the concept of a single gene. We suggest calling it *age-1,* the name published first.

We currently understand very little about the mechanisms of aging. In *C. elegans* there is some evidence that increased resistance to stress is correlated with increased life span. *age-1* mutants are resistant to oxidative stress (LARSEN 1993; VANFLETEREN 1993). Levels of superoxide dismutase (SOD) and catalase, two enzymes that protect against oxidative damage, are elevated in dauers and *age-1* mutants (LARSEN 1993). Both *age-1* and *daf-2* mutants have increased thermotolerance (LITHGOW *et al.* 1994, 1995). Possibly related to this, mild thermal stress induces thermotolerance and extends life span in the wild type (LITHGOW *et al.* 1995). Although the Age phenotype of *daf-28(sa191)* is relatively weak, it would be reasonable to examine stress resistance in this mutant.

In summary, by characterizing *daf-28(sa191)* mutants

and double mutants, we demonstrate that this dominant mutation acts at a novel point in the genetic pathway regulating dauer formation and that it has no obvious effect on dauer recovery. *daf-28(sa191)* provides a useful tool for analyzing dauer recovery, and we identified several genes that influence this process. Finally, we report two findings that further link the processes of dauer formation and aging. First, the *daf-28(sa191)* mutation leads to a slight increase in life span. Second, *age-1* mutations are Daf-c and fail to complement *daf-23*. Identification of *daf-28* loss-of-function mutations and supressors of *daf-28(sa191)*, as well as molecular analysis, will further define the function of this gene.

We thank JEREMY REITER, JENNIE DORMAN and CYNTHIA KENYON for initial testing of the life span of *daf-28* mutants and for communicating unpublished results that motivated us to study the Daf-c phenotype of *age-1*. We thank MICHAEL AILION, who first discovered that some mutant strains form dauers at 27°. We thank IKUE MORI, who tested thermotaxis in *daf-28* mutants. We thank GARY RUVKUN and members of his laboratory for helpful discussions and for kindly sharing unpublished *daf-23* alleles. Other mutant strains were provided by the *Caenorhabditis* Genetics Center [supported by the National Institutes of Health (NIH) National Center for Research Resources]. We thank MICHAEL AILION, DEBORAH BIRNBY, HELEN CHAMBERLIN, KOUICHI IWASAKI, DUNCAN JOHNSTONE, DAVID REINER, WENDY SCHACKWITZ, and PETER SWOBODA for many helpful comments on the manuscript. This work was supported by NIH grant R01GM-48700. E.A.M. was supported by a postdoctoral fellowship from the American Cancer Society and NIH Training grant AG-0057 (Genetic Approaches to Aging).

LITERATURE CITED

ALBERT, P. S., S. J. BROWN and D. L. RIDDLE, 1981 Sensory control of dauer larva formation in *Caenorhabditis elegans*. J. Comp. Neurol. **198:** 435–451.

AVERY, L., 1993 The genetics of feeding in *Caenorhabditis elegans*. Genetics **133:** 897–917.

AVERY, L., and S. WASSERMAN, 1992 Ordering gene function: the interpretation of epistasis in regulatory hierarchies. Trends Genet. **8:** 312–316.

AVERY, L., C. I. BARGMANN and H. R. HORVITZ, 1993 The *Caenorhabditis elegans unc-31* gene affects multiple nervous system-controlled function. Genetics **134:** 455–464.

BARGMANN, C. I., and H. R. HORVITZ, 1991 Control of larval development by chemosensory neurons in *Caenorhabditis elegans*. Science **251:** 1243–1246.

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

CASSADA, R. C., and R. L. RUSSELL, 1975 The dauerlarva, a postembryonic developmental variant of the nematode *Caenorhabditis elegans*. Dev. Biol. **46:** 326–342.

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

CROLL, N. A., and J. M. SMITH, 1978 Integrated behavior in the feeding phase of *Caenorhabditis elegans* (Nematoda). J. Zool. **184:** 507–517.

CULLOTI, J. G., and R. L. RUSSELL, 1978 Osmotic avoidance defective mutants of the nematode *Caenorhabditis elegans*. Genetics **90:** 243–256.

DORMAN, J. B., B. ALBINDER, T. SHROYER and C. KENYON, 1995 The *age-1* and *daf-2* genes function in a common pathway to control the lifespan of *Caenorhabditis elegans*. Genetics **141:** 1399–1406.

ESTEVEZ, M., L. ATTISANO, J. L. WRANA, P. S. ALBERT, J. MASSAGUE *et al.*, 1993 The *daf-4* gene encodes a bone morphogenetic protein receptor controlling *C. elegans* dauer larva development. Nature **365:** 644–649.

FRIEDMAN, D. B., and T. E. JOHNSON, 1988a A mutation in the *age-1* gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. Genetics **118:** 75–86.

FRIEDMAN, D. B., and T. E. JOHNSON, 1988b Three mutants that extend both mean and maximum life span of the nematode *Caenorhabditis elegans* define the *age-1* gene. J. Gerontol. **43:** 102–109.

GEORGI, L. L., P. S. ALBERT and D. L. RIDDLE, 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 D. L. RIDDLE, 1982 A pheromone influences larval development in the nematode *Caenorhabditis elegans*. Science **218:** 578–580.

GOLDEN, J. W., and D. L. RIDDLE, 1984a The *Caenorhabditis elegans* dauer larva: developmental effects of food, pheromone and temperature. Dev. Biol. **102:** 368–378.

GOLDEN, J. W., and D. L. RIDDLE, 1984b 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 D. L. RIDDLE, 1984c A *Caenorhabditis elegans* dauer-inducing pheromone and an antagonistic component of the food supply. J. Chem. Ecol. **10:** 1265–1280.

GOLDEN, J. W., and D. L. RIDDLE, 1985 A gene affecting production of the *Caenorhabditis elegans* dauer-inducing pheromone. Mol. Gen. Genet. **198:** 534–536.

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

GREEN, L. L., N. WOLF, K. L. MCDONALD and M. T. FULLER, 1990 Two types of genetic interaction implicate the *whirligig* gene of *Drosophila melanogaster* in microtubule organization in the flagellar axoneme. Genetics **126:** 961–973.

HAYES, T. S., R. DEURING, B. ROBERTSON, M. PROUT and M. T. FULLER, 1989 Interacting proteins identified by genetic interactions: a missense mutation in α-tubulin fails to complement alleles of the testis-specific β-tubulin gene of *Drosophila*. Mol. Cell. Biol. **9:** 875–884.

HERMAN, R. K., 1984 Analysis of genetic mosaics of the nematode *Caenorhabditis elegans*. Genetics **108:** 165–180.

HORVITZ, H. R., S. BRENNER, J. HODGKIN and R. K. HERMAN, 1979 A uniform genetic nomenclature for the nematode *Caenorhabditis elegans*. Mol. Gen. Genet. **175:** 129–133.

IWASAKI, K., D. LIU and J. H. THOMAS, 1995 Genes that control a temperature compensated clock in *C. elegans*. Proc. Natl. Acad. Sci. USA **92:** 10317–10321.

KATSURA, I., K. KONDO, T. AMANO, T. ISHIHARA and M. KAWAKAMI, 1994 Isolation characterization and epistasis of fluoride-resistant mutants of *Caenorhabditis elegans*. Genetics **136:** 145–154.

KENYON, C., 1996 Ponce d'elegans: genetic quest for the fountain of youth. Cell **84:** 501–504.

KENYON, C., J. CHANG, E. GENSCH, A. RUDNER and R. TABTIANG, 1993 A *C. elegans* mutant that lives twice as long as wild type. Nature **366:** 461–464.

KLASS, M. R., 1983 A method for the isolation of longevity mutants in the nematode *Caenorhabditis elegans* and initial results. Mech. Aging and Dev. **22:** 279–286.

KLASS, M. R., and D. HIRSH, 1976 Non-ageing developmental variant of *Caenorhabditis elegans*. Nature **260:** 523–525.

KUSCH, M., and R. S. EDGAR, 1986 Genetic studies of unusual loci that affect body shape of the nematode *Caenorhabditis elegans* and may code for cuticle structural proteins. Genetics **113:** 621–639.

LARSEN, P. L., 1993 Aging and resistance to oxidative damage in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **90:** 8905–8909.

LARSEN, P. L., P. S. ALBERT and D. L. RIDDLE, 1995 Genes that regulate both development and longevity in *Caenorhabditis elegans*. Genetics **139:** 1567–1583.

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

LITHGOW, G. J., T. M. WHITE, D. A. HINERFELD and T. E. JOHNSON, 1994 Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. J. Gerontol. Biol. Sci. **48:** B270–276.

LITHGOW, G. J., T. M. WHITE, S. MELOV and T. E. JOHNSON, 1995 Thermotolerance and extended life span conferred by single

gene mutations and induced by thermal stress. Proc. Natl. Acad. Sci. USA **92:** 7540–7544.

LIU, D. W., and J. H. THOMAS, 1994 Regulation of a periodic motor program in *C. elegans*. J. Neurosci. **14:** 1953–1962.

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

MARUYAMA, I. N., and S. BRENNER, 1991 A phorbol ester/diacylglycerol-binding protein encoded by the *unc-13* gene of *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **88:** 5729–5733.

PERKINS, L. A., E. M. HEDGECOCK, J. N. THOMPSON and J. G. CULOTTI, 1986 Mutant sensory cilia in the nematode *Caenorhabditis elegans*. Dev. Biol. **117:** 456–487.

REGAN, C. L., and M. T. FULLER, 1988 Interacting genes that affect microtubule function: the *nc2* allele of the *haywire* locus fails to complement mutations in the testis-specific β-tubulin gene of *Drosophila*. Genes Dev. **2:** 82–92.

RIDDLE, D. L., 1988 The dauer larva, pp. 393–412 in *The Nematode Caenorhabditis elegans*, edited by W. WOOD. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

RIDDLE, D. L., M. M. SWANSON and P. S. ALBERT, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 668–671.

SHAKIR, M. A., J. MIWA and S. S. SIDDIQUI, 1993a A role of ADF chemosensory neurones in dauer formation behaviour in *C. elegans*. Dev. Neurosci. **4:** 1151–1154.

SHAKIR, M. A., T. FUKUSHIGE, H. YASUDA, J. MIWA and S. S. SIDDIQUI, 1993b *C. elegans osm-3* gene mediating osmotic avoidance behavior encodes a kinesin-like protein. Mol. Neurosci. **4:** 891–894.

STARICH, T. A., R. K. HERMAN, C. K. KARI, W.-H. YEH, W. S. SCHACKWITZ *et al.*, 1995 Mutations affecting the chemosensory neurons of *Caenorhabditis elegans*. Genetics **139:** 171–188.

STEARNS, T., and D. BOTSTEIN, 1988 Unlinked noncomplementation: isolation of new conditional-lethal mutations in each of the tubulin genes of *Saccharomyces cerevisiae*. Genetics **119:** 249–260.

SULSTON, J. E., M. DEW and S. BRENNER, 1975 Dopaminergic neurons in the nematode *Caenorhabditis elegans*. J. Comp. Neurol. **163:** 215–226.

SWANSON, M. S., and F. WINSTON, 1992 *SPT4, SPT5,* and *SPT6* interactions: effects on transcription and viability in *Saccharomyces cerevisiae*. Genetics **132:** 325–336.

THOMAS, J. H., 1990 Genetic analysis of defecation in *Caenorhabditis elegans*. Genetics **124:** 855–872.

THOMAS, J. H., 1993 Chemosensory regulation of development in *C. elegans*. BioEssays **15:** 791–797.

THOMAS, J. H., D. A. BIRNBY and J. J. VOWELS, 1993 Evidence for parallel processing of sensory information controlling dauer formation in *Caenorhabditis elegans*. Genetics **134:** 1105–1117.

TRENT, C., N. TSUNG and H. R. HORVITZ, 1983 Egg-laying defective mutants of the nematode *Caenorhabditis elegans*. Genetics **104:** 619–647.

VANFLETEREN, J. R., 1993 Oxidative stress and ageing in *Caenorhabditis elegans*. Biochem. J. **292:** 605–608.

VOWELS, J. J., and J. H. THOMAS, 1992 Genetic analysis of chemosensory control of dauer formation in *Caenorhabditis elegans*. Genetics **130:** 105–123.

VOWELS, J. J., and J. H. THOMAS, 1994 Multiple chemosensory defects in *daf-11* and *daf-21* mutants of *Caenorhabditis elegans*. Genetics **138:** 303–316.

YEH, W.-Y., 1991 Genes acting late in the signalling pathway for *Caenorhabditis elegans* dauer larval development. Ph.D. Thesis, University of Missouri, Columbia.

Communicating editor: R. K. HERMAN