The *age-1* and *daf-2* Genes Function in a Common Pathway to Control the Lifespan of *Caenorhabditis elegans*

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ABSTRACT

Recessive mutations in two genes, *daf-2* and *age-1*, extend the lifespan of *Caenorhabditis elegans* significantly. The *daf-2* gene also regulates formation of an alternative developmental state called the dauer. Here we asked whether these two genes function in the same or different lifespan pathways. We found that the longevity of both *age-1* and *daf-2* mutants requires the activities of the same two genes, *daf-16* and *daf-18*. In addition, the *daf-2(e1370); age-1(hx546)* double mutant did not live significantly longer than the *daf-2* single mutant. We also found that, like *daf-2* mutations, the *age-1(hx546)* mutation affects certain aspects of dauer formation. These findings suggest that *age-1* and *daf-2* mutations do act in the same lifespan pathway and extend lifespan by triggering similar if not identical processes.

COMPARED to the detailed information we have about many biological processes, we know surprisingly little about what determines the lifespan of an animal. One attractive model is that some type of clock controls the rate of aging, including the time at which age-related processes such as human puberty and menopause take place. Such a clock would presumably run at vastly different rates in different organisms, since, for example, mice live 2 years, finches live 10, and bats live 30 years or more (FINCH 1990). Many processes have been proposed to influence lifespan, including a failure to replicate telomeres, to withstand oxidative damage, or to combat infectious agents effectively (FINCH 1990). However which, if any, of these processes actually determines the rate of aging is unknown.

In many instances, an effective way to dissect a regulatory process has been to identify mutations that alter it. If there are proteins that determine the lifespan of an animal, then it should be possible to mutate the genes encoding these proteins in such a way that the rate of aging is changed. The nematode *C. elegans* is especially well suited to a study of lifespan: it has a very short lifespan (approximately 19 days at 20°), and is amenable to genetic analysis.

Mutations in two genes, *age-1* and *daf-2*, are known to extend the lifespan of *C. elegans* adults. The *age-1(hx546)* mutation was initially identified in a screen for *C. elegans* mutants that live longer than wild type (KLASS 1983). The growth rate and behavior of the mutant is similar to wild type (FRIEDMAN and JOHNSON 1988) and it has approximately normal fertility (T. JOHNSON, personal communication). However, the mean lifespan is approximately 65% longer than wild type (FRIEDMAN and JOHNSON 1988).

Mutations in the second lifespan gene, *daf-2*, were originally isolated on the basis of their effects on dauer formation (RIDDLE *et al.* 1981). The dauer larva, somewhat analogous to a bacterial or yeast spore, is an alternative third larval (L3) form that is normally induced by crowding and food deprivation. The dauer is long-lived, reproductively immature, and resistant to starvation and desiccation (RIDDLE 1988). A number of genes that regulate dauer formation have been identified and assembled into a regulatory pathway (Figure 1; RIDDLE *et al.* 1981; THOMAS 1993; GOTTLIEB and RUVKUN 1994). Reduction or loss-of-function mutations in some of these genes prevent dauer formation (Daf-d mutations), whereas reduction or loss-of-function mutations in other genes cause constitutive dauer formation (Daf-c mutations). Previously we found that temperature-sensitive Daf-c mutations in the gene *daf-2* have a dramatic effect on the lifespan of nondauers (KENYON *et al.* 1993). Whereas *daf-2* mutants raised at a nonpermissive temperature become dauers, the same mutants raised at a permissive temperature, which does not induce dauer formation, become adults that live twice as long as normal. In contrast, Daf-c mutations in genes located at more upstream positions in the pathway did not affect the lifespan of nondauers (KENYON *et al.* 1993; LARSEN *et al.* 1995).

daf-2 adults appear to be healthy and active when grown at 15°, 20° or 25°. *daf-2(e1370)* young adults exhibit a pharyngeal pumping rate similar to that of wild-type young adults. At 20°, they exhibit a twofold extension in lifespan and have nearly normal numbers of self-progeny relative to the wild type (KENYON *et al.* 1993). Like *age-1* animals, *daf-2* adults appear healthier and more active than wild-type animals of the same

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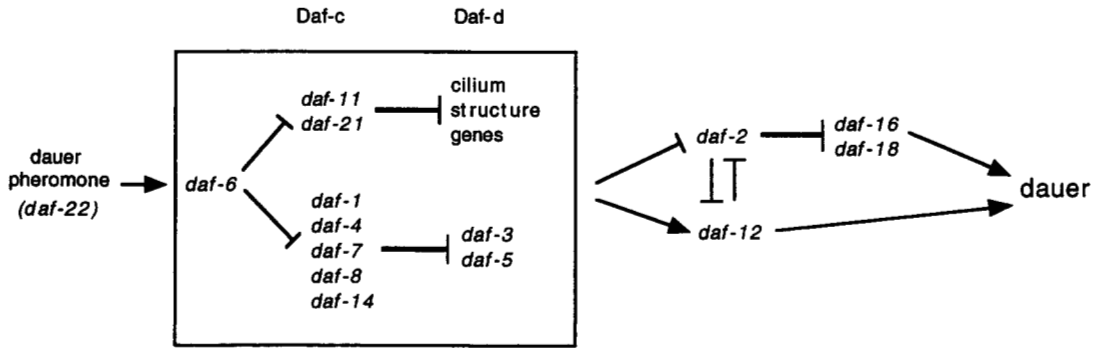

FIGURE 1.—A genetic model for the control of dauer formation. In this model, abstracted from THOMAS (1993) and GOTTLIEB and RUVKUN (1994), dauer pheromone triggers the activity of genes in two parallel pathways, each containing a group of Daf-d genes acting downstream of a group of Daf-c genes. Downstream of *daf-11* and *daf-21*, for example, is a group of nine cilium structure genes (including *che-2, che-3, che-11,* and *osm-3*) which, when mutated, disrupt cilium structure and give a Daf-d phenotype (VOWELS and THOMAS 1992). This is a formal pathway derived from genetic epistasis; in addition, the cilium structure genes may act upstream of the Daf-c genes in both branches of the pathway (THOMAS 1993). In response to dauer pheromone, the gene activities in these parallel pathways negatively regulate *daf-2*, which, in turn, is a negative regulator of *daf-16* and *daf-18*. In addition, they also activate the gene *daf-12*, which is required to proceed from the L2d state to dauer. Based on its single and double mutant phenotypes, *daf-20(m25)* would be positioned with *daf-16* and *daf-18*. However, daf-*20(m25)* has recently been shown to be an allele of *daf-12* (LARSEN *et al.* 1995).

age. As they age, the tissues of both wild-type and *daf-2* mutants become progressively more mottled and disorganized; however, this physical decline occurs at a much slower rate in the mutant.

The identification of lifespan mutations in these two genes immediately raises the question of whether they extend lifespan in the same way. Do *age-1* and *daf-2* affect the same process or different processes? One way to address this question is to ask whether the lifespan extensions caused by *age-1* and *daf-2* mutations depend upon the same downstream gene activities. *daf-2* mutants are dependent on the gene *daf-16* for both their dauer-constitutive (VOWELS and THOMAS 1992; GOTTLIEB and RUVKUN 1994) and extended lifespan phenotypes (KENYON *et al.* 1993). Here we show that *daf-16* is also required for the lifespan extension of *age-1* mutant animals. We also find that both *age-1* and *daf-2* mutants require the activity of a second gene, *daf-18*, for lifespan extension. Finally, we find that the *age-1* mutation enhances some aspects of the *daf-2* phenotype that appear to be related to dauer formation. Together these findings argue that *age-1* and *daf-2* act in a common genetic pathway that regulates *C. elegans* lifespan.

MATERIALS AND METHODS

Methods and strains: Strains were handled as described in BRENNER (1974). The mutations used in this study were as follows:

LGI: *unc-29(e1072), daf-16(m26), lin-11(n566)*
LGII: *age-1(hx546), fer-15(b26ts)*
LGIII: *daf-2(e1370ts)*
LGIV: *daf-18(e1375), dpy-9(e12)*
LGV: *him-5(e1490)*
LGX: *daf-12(m20), daf-12(m25) [formerly daf-20]*

Construction of the *daf-16; age-1 fer-15* and *age-1 fer-15; daf-18* triple mutants: The *fer-15(b26)* mutation has a temperature-sensitive defect in sperm production (WARD *et al.* 1981) and a lifespan similar to wild type (JOHNSON 1990). This mutation was reported to be closely linked to *age-1* (T. JOHNSON, personal communication) and was present in all the *age-1* mutants analyzed. To construct the *daf-16; age-1 fer-15* triple mutant, we first constructed an *unc-29 lin-11; age-1 fer-15* multiple mutant by crossing *+/unc-29 lin-11* males with *age-1 fer-15* hermaphrodites and looking for Unc Lin Fer F_2 segregants. These animals were then crossed with wild-type males, and the male progeny, in turn, were crossed with *daf-16(m26)* hermaphrodites. Cross-progeny that segregated Unc Lin animals were picked, and from the descendants of these, we obtained Fer mutants that did not segregate Unc Lin animals. These animals were candidates for *daf-16; age-1 fer-15* mutants. To verify that the strain contained *daf-16*, we asked whether it contained a suppressor of the dauer-constitutive phenotype of *daf-2(e1370)*. We crossed the putative *daf-16; age-1 fer-15* mutant with *daf-2(e1370); him-5(e1490)* males (*him-5* increases the frequency of male self-progeny). F_2 progeny were incubated at 25°, where *daf-2/daf-2* homozygotes would become dauers. Individual dauers were picked, and allowed to exit the dauer stage at 15°. The presence of *daf-16* was ascertained by shifting the former dauers back to 25° and observing non-dauer self-progeny. To verify the presence of *age-1*, we displaced the *daf-16* chromosome by crossing the putative *daf-16; age-1 fer-15* mutant with *+/unc-29* males (*unc-29* is closely linked to *daf-16)* and identifying Unc Fer animals among the descendants of the cross-progeny. To determine whether *age-1* was present, we examined the lifespans of these animals, to see whether they exhibited extended lifespans relative to control *unc-29* and N2 animals (data not shown).

This verification, though laborious, proved to be essential. We found that the *age-1* mutation segregated with *fer-15* in one of these strains, but was lost in a second strain constructed in parallel. The *age-1* mutation was also lost in two of six additional strain constructions we performed using *fer-15* as a linked marker.

To construct *age-1 fer-15; daf-18* triple mutants, we first crossed *+/dpy-9* males with *age-1 fer-15* hermaphrodites. The males issuing from this cross were mated individually to *daf-18(e1375)* hermaphrodites. (*dpy-9* is linked to *daf-18*.) Cross-progeny that produced Dpy progeny were analyzed further. From these, we isolated progeny that were Fer, and that did

not produce Dpy progeny. These were candidates for *age-1 fer-15; daf-18* animals. The presence of *daf-18* was verified by its morphological phenotypes (see RESULTS). The presence of *age-1* was verified as described above for the *daf-16; age-1 fer-15* mutant, using *dpy-9* to displace *daf-18.*

Construction of the *age-1 fer-15; daf-2* triple mutant: *+/daf-2(e1370)* males were crossed with *age-1 fer-15* hermaphrodites, and F_2 animals were picked onto individual plates and grown at 25°. *age-1 fer-15; daf-2/+* animals were recognized by the presence of 3/4 Fer and 1/4 dauer animals among their progeny. Dauers were picked to establish strains of the *age-1 fer-15; daf-2* mutant. Two such triple mutant strains were constructed and analyzed in parallel; these had similar novel phenotypes (see RESULTS). A *fer-15; daf-2* control strain was made the same way.

Dauer formation assay: The frequency of dauer formation was assayed under non-inducing conditions as described in VOWELS and THOMAS (1992). Animals raised at 15° were transferred to 20° and allowed to lay eggs. These progeny were then examined for dauer formation.

Lifespan determination: Worms were cultured on petri dishes containing NG media and seeded with *Escherichia coli* strain OP50 (SULSTON and HODGKIN 1988). Animals were allowed to lay eggs overnight and then were removed from the plates. The day the adults were removed was counted as the day of hatching, the $t = 0$ for lifespan measurement of their progeny. When these staged animals reached the L4 or young adult stage, groups of five animals were placed on each of a number of plates (see figure legends for details of each experiment). When lifespan curves were obtained at 25°, animals were cultured at 15° until the L4 or young adult stage and then shifted to the higher temperature. While producing progeny, adults were transferred to new plates daily. Once reproduction ceased, the animals were transferred to new plates approximately once a week. Animals were judged dead when they did not move after repeated proddings with a pick, or after being tugged gently on the tail by a pick covered with bacteria. Animals that crawled off the plates were not included in the analysis.

RESULTS

The *daf-16 (m26)* mutation suppresses the lifespan extension of *age-1(hx546)* mutants: The *age-1(hx546)* mutation could extend lifespan in one of several ways. It could act in a pathway that is different from that affected by *daf-2* mutants. Alternatively, it could act in the same pathway as *daf-2,* either upstream, in parallel, or downstream of *daf-2.* Some of these possibilities can be distinguished by asking whether the lifespan extensions of both mutants require the same downstream gene activities. Previously we showed that the lifespan extension of *daf-2* mutants requires the *daf-16* gene, which is also required for *daf-2* mutants to form normal dauers. Therefore, we asked whether *daf-16* mutations would also suppress the longevity of *age-1* mutants. To address this question, we constructed the *daf-16(m26); age-1(hx546) fer-15(b26)* triple mutant (see MATERIALS AND METHODS). The *fer-15* mutation is linked to *age-1,* and does not affect lifespan on its own (JOHNSON 1990). The *age-1 fer-15* double mutant is well characterized, unlike the single *age-1(hx546)* mutant; and its presence facilitates strain construction. For these reasons, we analyzed the *age-1 fer-15* double mutant, rather than the single *age-1* mutant, in all our experiments. As shown in Figure 2, we found that the *daf-16* mutation suppressed the lifespan extension of *age-1 (hx546).* Whereas the *age-1 fer-15* mutant had a mean lifespan of 24 days when grown at 25°, the *daf-16; age-1 fer-15* mutant had a mean lifespan of 13 days, similar to *daf-16* and wild-type animals. We verified that the strain actually contained *age-1* by crossing out the *daf-16* mutation and testing the lifespans of the Fer segregants (see MATERIALS AND METHODS). This verification proved to be essential, because in a number of strains the *age-1* mutation had been lost (see MATERIALS AND METHODS). Because the lifespan of *daf-16(m26); age-1(hx546) fer-15(b26)* triple mutants was similar to wild type, we conclude that in order for *age-1 fer-15* mutants to live longer than wild type, they require *daf-16* function.

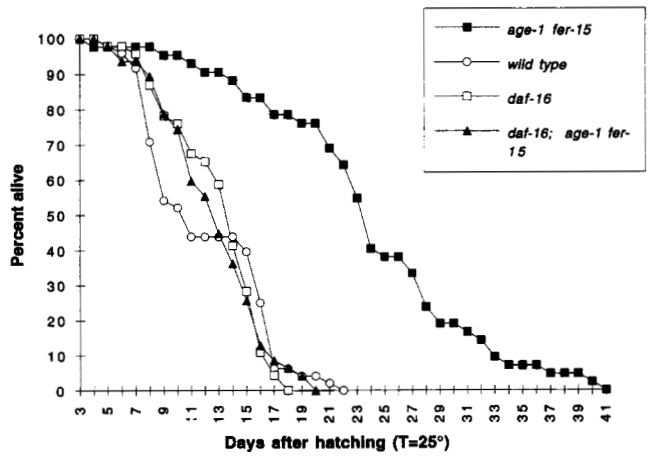

FIGURE 2.—The *daf-16* gene is required for the longevity of *age-1(hx546)* mutants. Lifespan curves were obtained as described in MATERIALS AND METHODS. Animals were cultured at 15° until the L4 stage, when they were transferred to 25°, at which temperature the *age-1(hx546)* mutation is reported to have a strong effect on lifespan (FRIEDMAN and JOHNSON 1988). A fraction of the mutants died as "bags of worms" (HORVITZ 1988) in early adulthood. In all experiments, the "bags" were excluded from our lifespan curves and statistical analyses. Mean lifespans were 12 days for wild type (n = 48, none died as bags), 13 days for *daf-16(m26)* (n = 46, one additional animal died as a bag), 24 days for *age-1(hx546) fer-15(b26)* (n = 42, one additional animal died as a bag), and 13 days for *daf-16(m26); age-1(hx546) fer-15(b26)* (n = 47, none died as bags). Comparisons of the *age-1(hx546) fer-15(b26)* and *daf-16(m26); age-1(hx546) fer-15(b26)* populations using the Log-Rank test (WOOLSON 1987) yielded a chi-square value of 59.3, ($P \ll 0.0001$); thus *age-1(hx546) fer-15(b26)* animals lived significantly longer than *daf-16(m26); age-1(hx546) fer-15(b26)* animals. The lifespan of the *daf-16(m26); age-1(hx546) fer-15(b26)* mutants was not significantly different from that of *daf-16* single mutants ($\chi^2 = 0.006$, $P = 0.94$) or wild-type animals ($\chi^2 = 0.11$, $P = 0.74$).

The *daf-18(e1375)* mutation suppresses the lifespan extension of *daf-2(e1370)* mutants: Next, we asked whether any additional genes known to function downstream of *daf-2* in the dauer pathway might also be required for the longevity of *daf-2* adults. *daf-16* had been positioned downstream of *daf-2* in the dauer path-

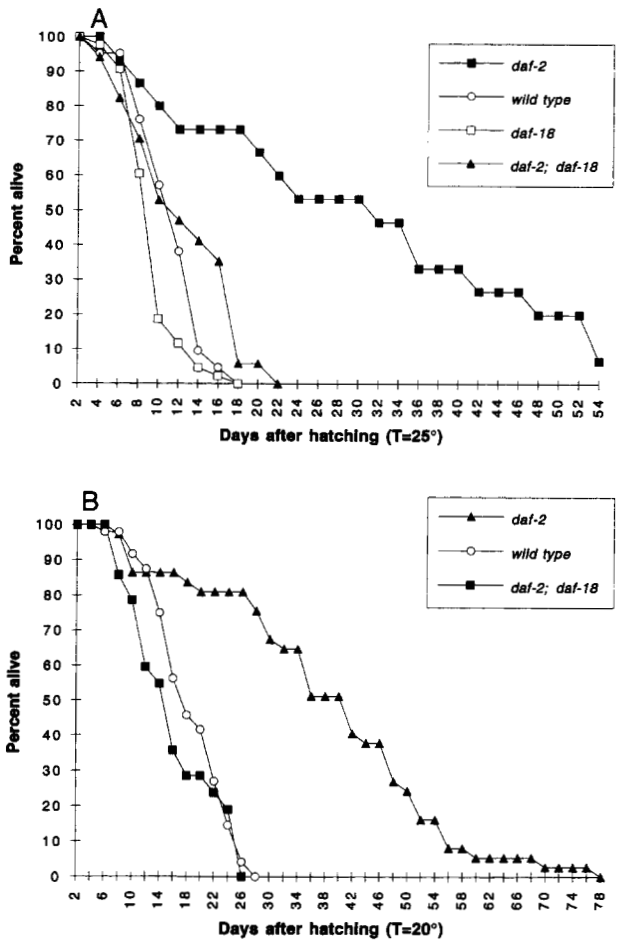

FIGURE 3.—The *daf-18(e1375)* mutation suppresses the lifespan extension of *daf-2* mutants. (A) Animals were shifted to 25° as L4 larvae or young adults, as described in MATERIALS AND METHODS. In this experiment, all animals containing the *daf-18 (e1375)* mutation were monitored individually (1 per plate). A fraction of animals containing the *daf-18* mutation displayed gross morphological abnormalities in the midbody region (see RESULTS). These animals died soon (within 1 day, on average) after the abnormality was first detected, and, like bags, have been excluded from our lifespan curves and statistical analyses. Of the 21 *daf-2* animals we monitored, seven died as bags and one died of morphological abnormalities; the remaining 14 animals had a mean lifespan of 29 days. Mean lifespans were 13 days for *daf-2;daf-18* (n = 17, four additional animals died as bags, 27 additional animals died of morphological abnormalities); 12 days for N2 (n = 21, 2 additional animals died as bags); and 10 days for *daf-18* (n = 43, 5 additional animals died as bags; 17 additional animals died of morphological abnormalities). *daf-2* animals lived significantly longer than N2 (χ^2 = 14.31, P = 0.0001). The lifespan of the *daf-2; daf-18* population was not significantly different from wild-type (χ^2 = 1.60, P = 0.21); *daf-2; daf-18* animals lived significantly less long than the *daf-2* population (χ^2 = 7.14, P = 0.007). *daf-18* mutants lived slightly less long than wild type (χ^2 = 4.22, P = 0.04). (B) The lifespan curve was obtained as described in MATERIALS AND METHODS at 20°, at which temperature *daf-2* mutants do not enter dauer but become long-lived adults. Of the 43 *daf-2* animals we monitored, 6 died as bags; the remaining 37 animals had a mean lifespan of 39 days. Mean lifespans were 16 days for *daf-2;daf-18* (n = 42, 3 additional animals died as bags) and 19 days for N2 (n = 48, none died as bags). *daf-2* lived significantly longer than N2 (χ^2 = 47.31, $P \ll 0.0001$). The lifespan of the *daf-2; daf-18* population, however, was not significantly different from wild-type (χ^2 = 1.08, P = 0.29). The *daf-2; daf-18* population lived significantly less long than the *daf-2* population (χ^2 = 38.35, $P \ll 0.0001$).

way because *daf-16* mutations suppress the Daf-c phenotype of *daf-2* mutants. Mutations in two other genes, *daf-18* and *daf-20*, were also known to prevent *daf-2* mutants from forming normal dauers (VOWELS and THOMAS 1992). The *daf-18* gene is defined by a single mutation, *e1375*. At the nonpermissive temperature, *daf-2; daf-18* double mutants initially form dauers but then quickly exit the dauer state (VOWELS and THOMAS 1992; LARSEN *et al.* 1995). Unlike *daf-16* mutants, a fraction of *daf-18(e1375)* animals appear morphologically abnormal; they have swollen midbody regions. For this reason, we wanted to assay lifespan in a way that allowed us monitor the appearance and lifespan of each animal individually. To do this, we carried out a lifespan determination experiment in which all animals containing the *daf-18* mutation were cultured singly on individual plates (Figure 3A). We found that all the animals that had swollen midbody regions (the fraction differed between strains and is given in the figure legend) exploded and died as young adults within 1 or 2 days after the abnormality became apparent. Because these animals clearly did not die of old age, they were excluded from the lifespan curves. The remaining *daf-18* animals looked normal as young adults and appeared to undergo a normal process of senescence. We found that the *daf-18* mutation suppressed the lifespan extension of *daf-2(e1370)*. At 25°, the mean lifespan of *daf-2* animals was 29 days, whereas the mean lifespans of N2, *daf-18*, and *daf-2; daf-18* animals were 12, 10, and 13 days, respectively. We also determined the lifespans of *daf-2* and *daf-2; daf-18* animals at 20° (Figure 3B). At this temperature, the mean lifespan of *daf-2* animals was 39 days, and, again, the mean lifespans of wild type and *daf-2; daf-18* mutants were much shorter (19 and 16 days, respectively). LARSEN *et al.* (1995) have independently found that *daf-18(e1370)* suppresses the lifespan extension of *daf-2* mutants.

The *daf-18(e1375)* mutation also suppresses the lifespan extension of *age-1(hx546)*: Does the *daf-18(e1375)* mutation also suppress the lifespan extension of *age-1*? We addressed this question by constructing the *age-1 fer-15; daf-18* triple mutant and determining its lifespan at 25°. As with *daf-2; daf-18* animals, we cultured these animals singly on individual plates, and excluded from the lifespan determination those that had swollen midbodies and exploded as young adults. The abnormal animals accounted for about 24% of the *daf-18* population and 27% of the *age-1 fer-15; daf-18* triple mutants.

We found that whereas the *age-1 fer-15* mutant had an average lifespan of 27 days, the mean lifespan of the triple mutant was similar to wild type (both 12 days;

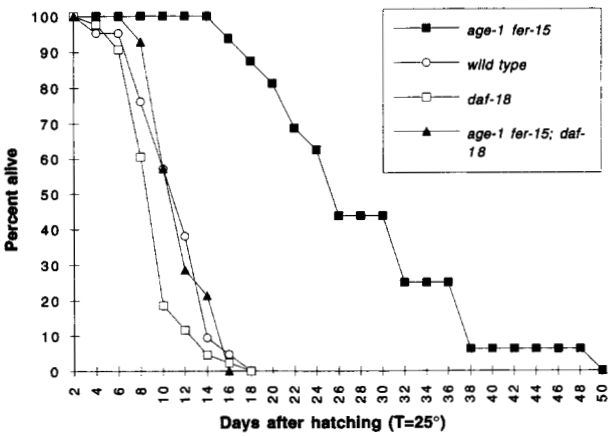

FIGURE 4.—The *daf-18(e1375)* mutation suppresses the lifespan extension of *age-1(hx546) fer-15(b26)* mutants. This experiment was carried out in parallel with the experiment described in Figure 3A. Animals were shifted to 25° as L4 larvae or young adults, as described in MATERIALS AND METHODS. All animals containing the *daf-18 (e1375)* mutation were monitored individually (1 per plate). The mean lifespan of the *age-1(hx546) fer-15(b26)* population was 27 days (n = 16), whereas *age-1(hx546) fer-15(b26); daf-18(e1375)* triple mutants lived significantly less long (mean lifespan = 12 days, n = 14; an additional two animals died as bags, while six others died soon after displaying gross morphological abnormalities, χ^2 = 234.2, $P \ll 0.0001$). The *age-1(hx546) fer-15(b26); daf-18(e1375)* lifespan does not differ significantly from the wild-type mean of 12 days (n = 21, $\chi^2 = 0.02$, $P = 0.87$). As mentioned above, *daf-18* single mutants lived a mean of 10 days (n = 43), slightly less long than wild type ($\chi^2 = 4.22$, $P = 0.04$). The same N2 and *daf-18* lifespan data is shown in both this figure and in Figure 3A, to facilitate comparison with the double or triple mutant strains.

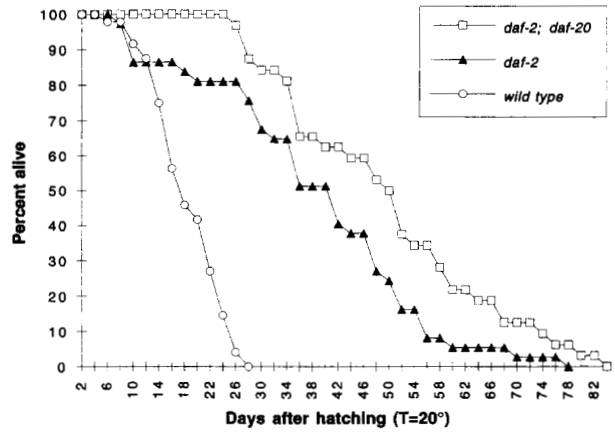

FIGURE 5.—The *daf-20(m25)* mutation did not suppress the lifespan extension of *daf-2(e1370)* mutants. In the same experiment described in Figure 3B, we also assayed the mean lifespan of *daf-2; daf-20* mutants, and found it to be 50 days (n = 32, two additional animals died as bags). The *daf-2; daf-20* population lived significantly longer than wild type (χ^2 = 72.45, $P \ll 0.0001$) and slightly longer than *daf-2* ($\chi^2 = 5.35$, $P = 0.02$).

Figure 4). Again, we verified that these triple mutants actually carried the *age-1* mutation (see MATERIALS AND METHODS). Thus, like *daf-16(m26)*, the *daf-18(e1375)* mutation also suppressed the lifespan extension of *age-1* mutants, suggesting that the wild-type function of *daf-18* is required for the longevity of both *age-1* and *daf-2* animals.

The *daf-20(m25)* mutation did not suppress the lifespan extension of *daf-2(e1370)* mutants: Like *daf-18(e1375)*, the mutation *daf-20(m25)* also suppresses dauer formation caused by Daf-c mutations in a number of genes, including *daf-2*. At the nonpermissive temperature, *daf-2; daf-20* double mutants quickly exit the dauer state (VOWELS and THOMAS 1992). We found that, unlike *daf-18* and *daf-16* mutations, the *daf-20* mutation did not suppress the lifespan extension of *daf-2*; in fact, it increased the lifespan extension slightly (Figure 5). While this study was in progress, we learned that the *m25* mutation fails to complement *daf-12* mutations, and thus is likely to be an allele of *daf-12* (LARSEN *et al.* 1995). LARSEN *et al.* have carried out an extensive analysis of the effect of *daf-12* mutations on lifespan (LARSEN *et al.* 1995).

The lifespan of *age-1 fer-15; daf-2* triple mutants: Since both *age-1 fer-15* and *daf-2* mutants have extended lifespans, we wondered whether or not the triple mutant would have an even longer lifespan. To determine this, we constructed the triple mutant (see MATERIALS AND METHODS) and determined its lifespan at two temperatures. In one experiment we shifted animals to 25° as young adults; in a second, we measured the lifespans of animals cultured continuously at 15°. At 25°, we found that the triple mutant had a lifespan that did not differ significantly from that of *daf-2* mutants, although it did live longer than *age-1 fer-15* mutants (Figure 6A). At 15°, the mean lifespan of *daf-2(e1370)* was 43 days and the mean lifespan of *age-1 fer-15* was 31 days. The mean lifespan of the triple mutant was 49 days, which was slightly longer than the *daf-2* single mutant (Figure 6B).

The *age-1(hx546)* mutation and dauer formation: Unlike *daf-2* mutants, the *age-1(hx546)* mutant has not been reported to form dauers spontaneously, and we also did not observe dauers among populations of animals growing in the presence of food. However, because *age-1* was similar to *daf-2* in its ability to extend lifespan in a *daf-16* and *daf-18*-dependent fashion, we wondered whether *age-1* might play a role in dauer formation. Therefore, we asked whether the *age-1* mutation could enhance the dauer phenotype of *daf-2* mutants in any way. It seemed possible that the double mutant might form dauers at a lower temperature, such as 20°. We found that it did not. However, surprisingly, at 20°, the *age-1 fer-15; daf-2* triple mutant grew very slowly to adulthood and never became fertile. In addition, the animals had some dauer-like characteristics: they had dark intestines and they tended to lie motionless in a posture typical of dauers. They did exhibit pumping behavior, but they pumped much more slowly than normal adults. A *fer-15; daf-2* control strain did not exhibit this arrest phenotype at 20°. We have seen a similar phenotype in

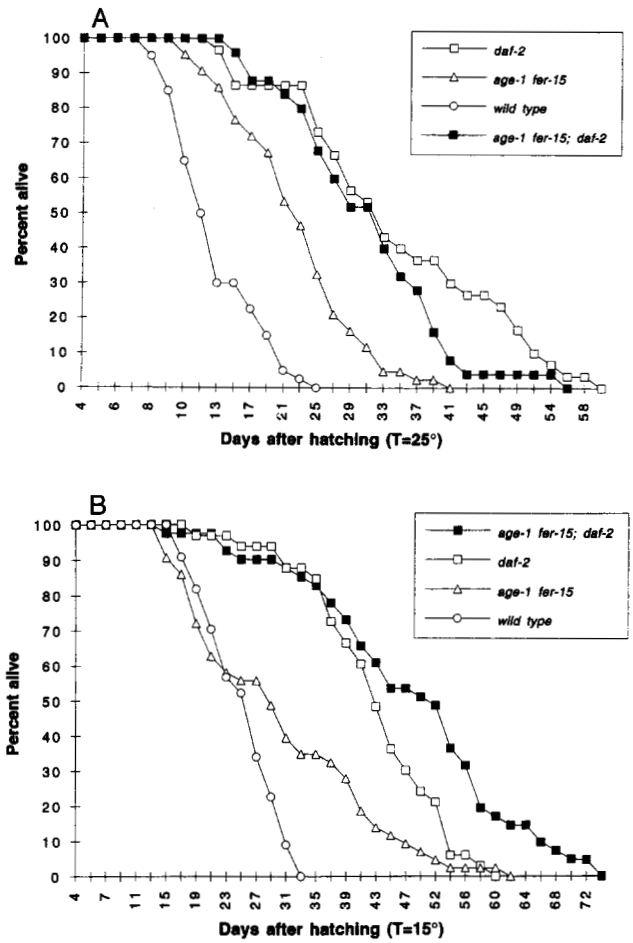

FIGURE 6.—Lifespan of *age-1 fer-15; daf-2* mutants at 25° and 15°. (A) All strains were transferred to 25° as L4s or young adults (see MATERIALS AND METHODS). Both *daf-2* (mean lifespan = 35 days, n = 30, an additional 17 animals died as bags) and *age-1 fer-15* (mean lifespan = 23 days, n = 43, one additional animal died as a bag) strains display extended lifespans relative to wild type (mean lifespan = 14 days, n = 40, five additional animals died as bags). The *age-1 fer-15; daf-2* triple mutant (mean lifespan = 29 days, n = 25, 24 additional animals died as bags), however, did not show an enhanced lifespan extension relative to *daf-2*, although it did live longer than *age-1 fer-15* ($\chi^2 = 10.34$, $P = 0.001$). (B) All strains were cultured continuously at 15°. At this temperature, the *age-1 fer-15; daf-2* triple mutant strain (mean lifespan = 49 days, n = 41, two additional animals died as bags) lived slightly longer than either *daf-2* (mean lifespan = 43 days, n = 33, nine additional animals died as bags, $\chi^2 = 6.75$, $P = 0.009$) and significantly longer than *age-1 fer-15* (mean = 31 days, n = 43, two additional animals died as bags, $\chi^2 = 26.19$, $P \ll 0.0001$) strains. The triple mutant showed a twofold lifespan extension over wild type (mean lifespan = 25 days, n = 44, three additional animals died as bags). A second isolate of the *age-1 fer-15; daf-2* triple mutant also showed an increased lifespan extension (mean lifespan = 53 days, n = 36, five additional animals died as bags, not shown).

daf-2(e1370) single mutants that are shifted to the dauer-inducing temperature (25°) when they are in the L3 stage. Thus it may be that the *age-1* mutation is enhancing this *daf-2* phenotype, by lowering the temperature at which the animals become sterile adults.

We also measured the length of time necessary for the double mutant to exit the dauer state. Dauer formation was induced by growth at 25°, and then the animals were shifted to 15°. At this temperature, *daf-2* single mutants exit the dauer state within 2 to 4 days. The double mutant exited the dauer state much more slowly, often taking several weeks (data not shown). It seems unlikely that this delay in dauer exit is due to an effect of the *age-1* mutation on growth rate *per se*, because at 15° the double mutant progressed to adulthood at a wild-type rate. Thus the *age-1* mutation seemed to have a specific effect on dauer recovery.

DISCUSSION

In this study, we asked whether mutations in *age-1* and *daf-2* are likely to extend lifespan by a common mechanism or by different mechanisms. To do this, we asked whether the lifespan extensions of these two mutants are dependent on the same downstream functions. We identified genes required for the lifespan extension of *daf-2* mutants by testing downstream genes in the dauer formation pathway for effects on lifespan. We then asked whether the longevity of *age-1* mutants depended on these same downstream genes.

The lifespan extension of *daf-2* adults had previously been shown to depend on the function of *daf-16*, a gene also required for *daf-2* mutants to become normal dauers. In addition to *daf-16* mutations, mutations in two other genes in the dauer pathway prevent *daf-2* mutants from forming normal dauers. The first is the *daf-18(e1375)* mutation. We found that, like *daf-16*, *daf-18* mutations suppressed the lifespan extension of *daf-2* mutants, suggesting that *daf-18* gene activity is required for the longevity of *daf-2* mutants. The fact that a fraction of the *daf-18(e1375)* animals are morphologically abnormally was initially a concern to us. However, we found that these abnormal animals explode and die as young adults. The remaining animals appear to be healthy, and undergo a progressive physical decline that is similar to that of aging wild-type animals. Thus we believe that this mutation also has a specific affect on lifespan. Nevertheless, because this gene is defined by a single mutation, we cannot infer the role of the wild-type *daf-18* gene in lifespan control with certainty.

Our central finding is that both *daf-16* and *daf-18* mutations suppress the lifespan extension of *age-1* mutants. Thus, *daf-2* and *age-1* mutants appear to require the same gene activities for their longevity. The simplest interpretation of these findings is that *daf-2* and *age-1* mutations extend lifespan in exactly the same way, by activating a single longevity process that involves the genes *daf-16* and *daf-18*. However, it is also possible that the effects of the two mutations are not identical. For example, unidentified genes could be required for the lifespan extension of *daf-2* but not *age-1* mutants, or vice versa.

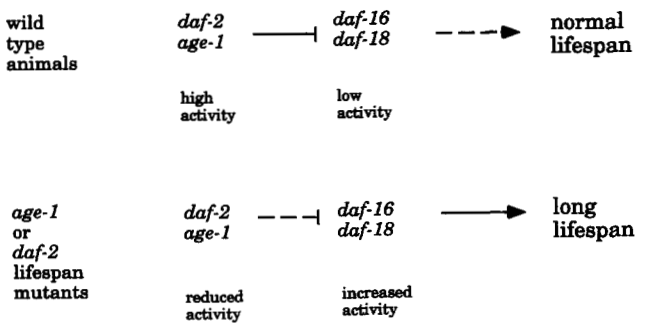

FIGURE 7.—Regulatory relationships between genes that affect adult lifespan in *C. elegans*. In this model, the wild-type *age-1* and *daf-2* gene products shorten adult lifespan; when the activity of either of these genes is reduced, then lifespan is extended in a process that requires *daf-16(+)* and *daf-18(+)* activities. The roles of *age-1* and *daf-18* in lifespan are somewhat uncertain, since only one independent allele of each has been isolated. In the model, we suggest that reduction of *daf-2* function triggers lifespan extension. This is because, whereas putative null alleles of *daf-2* cause nonconditional dauer formation (GOTTLIEB and RUVKUN 1994), temperature-sensitive mutations, which presumably have residual *daf-2* activity, trigger lifespan extension at low temperatures that do not induce dauer formation. However, it may be that complete elimination of *daf-2* activity would also trigger adult lifespan extension as long as the removal occurred at or after the L4 stage. This is because adult lifespan is also extended when *daf-2(ts)* mutants are shifted to the nonpermissive (dauer-inducing) temperature as L4s or young adults.

These findings can be summarized in a simple pathway for lifespan control (Figure 7). In this model, in the wild type, both *age-1* and *daf-2* products accelerate the aging process of *C. elegans*. When either gene is defective, lifespan is increased by a mechanism that depends on the activities of both *daf-16* and *daf-18*. Our data do not allow us to order *age-1* and *daf-2*, or *daf-16* and *daf-18* relative to one another. There are two important caveats to this model. First, because only one allele of *daf-18* has been identified, it is not possible to infer the wild-type function of this gene with certainty. Second, because the *fer-15* mutation was present in all the *age-1* mutants we analyzed, it is possible that the lifespan extension we have attributed to *age-1* is actually modified by *fer-15*.

It is interesting that *daf-16* and *daf-18(e1375)* mutations prevent some but not all aspects of dauer formation. They prevent the remodeling of the pharynx and the cessation of pharyngeal pumping that occurs during normal dauer formation. *daf-16* and *daf-18(e1375)* mutations also prevent the maintenance of the dauer state. However, they do not prevent formation of a dauer-specific cuticle and some slimming of the body. This suggests that lifespan can be controlled separately from other aspects of dauer formation. In addition, although *daf-16, daf-18* and *daf-20(m25)* mutations all have similar dauer phenotypes, only *daf-16* and *daf-18* mutations shorten the lifespan of *daf-2* mutant animals. Thus there seems to be a surprising degree of regulatory specificity in the system.

We found that the *daf-20(m25)* mutation did not suppress the lifespan extension of *daf-2(e1370)*. This mutation has recently been shown to be a likely allele of the gene *daf-12*, which is required for dauer formation (LARSEN *et al.* 1995). To ask whether a stronger *daf-12* mutation might suppress the lifespan extension of *daf-2* mutants, we have examined the lifespans of *daf-2(e1370); daf-12(m20)* animals at 20°, where *daf-2(e1370)* single mutants have extended lifespans. At 20° we observed a biphasic lifespan curve: all the animals appeared unhealthy, and the great majority of double mutants died much sooner than wild type, whereas a small fraction lived for a much longer period (data not shown). This raises the possibility that the wild-type *daf-12* gene actually inhibits the longevity of *daf-2* mutants. The wild-type *daf-12* gene could initiate some aspects of dauer formation that limit lifespan; alternatively, the wild-type *daf-12* gene could shorten lifespan in a way that has nothing to do with its role in dauer formation. The interpretation of this result is complicated by the strikingly biphasic nature of the lifespan curves. An extensive analysis of the role of *daf-12* in lifespan control has been carried out by LARSEN *et al.* (1995).

Does *age-1* play a role in dauer formation? There is no evidence that the *age-1* gene regulates entry into the dauer state. However, we found that *age-1(hx546)* did retard the rate of exit from the dauer state in a *daf-2* mutant background. In addition, the *age-1* mutation caused *daf-2* mutants to become sterile adults at 20°. This does not appear to be a completely novel phenotype, because *daf-2(e1370)* single mutants enter this state when shifted to the dauer-inducing temperature during L2 or L3. These animals have many characteristics of dauers: in particular, sterility, motionless posture, and a dark intestinal color. Thus it is possible that the *age-1(hx546)* mutation is potentiating the expression of some processes normally coupled to dauer formation. It would be quite informative to isolate null alleles of *age-1* and to learn whether they have a stronger effect on dauer formation. From the analysis of the single existing allele, it appears that the *age-1* gene may play at least a minor role in the process.

We found that the *age-1 fer-15* double mutant had a longer mean lifespan relative to wild type than the 65% extension reported previously. In our experiments at 25°, the mean lifespan of the *age-1 fer-15* double mutants was often twofold greater than wild type. The reason for this is unclear; one possibility is that in our experiments, the worms are grown on solid media, whereas in previous work they were grown in liquid (FRIEDMAN and JOHNSON 1988).

The lifespan of the *age-1 fer-15; daf-2* triple mutant was similar to that of *daf-2* at 25°, and slightly longer than that of *daf-2* at 15°. How can we explain this? Unfortunately, there is no clear-cut interpretation of this experiment, because neither allele is known to be null. However, the fact that the triple mutant had a lifespan

similar to that of *daf-2(e1370)* mutants at 25° is consistent with the idea that during wild-type development, the presence of both wild-type gene products is necessary to prevent lifespan extension, and that lowering the activity level of either gene produces the same effect. The slight lengthening of lifespan at 15° could occur if the either the *daf-2* or *age-1* genes retain significant lifespan-reducing activity at that temperature.

In summary, we have shown that the longevity of both *daf-2* and *age-1* mutants is abolished by mutations in the same two genes, *daf-16* and *daf-18*. Thus our findings suggest that both *age-1* and *daf-2* mutations extend life in similar or identical ways; namely, by triggering a process that is dependent on the wild-type *daf-16* and *daf-18* genes. Learning what types of proteins are encoded by these genes and where they act may help us to understand how the lifespan of an animal is determined.

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