Genetic Analysis of Tissue Aging in *Caenorhabditis elegans*: A Role for Heat-Shock Factor and Bacterial Proliferation

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Manuscript received December 3, 2001
Accepted for publication April 11, 2002

ABSTRACT

The genetic analysis of life span has revealed many interesting genes and pathways; however, our understanding of aging has been limited by the lack of a way to assay the aging process itself. Here we show that the tissues of aging worms have a characteristic appearance that is easy to recognize and quantify using Nomarski optics. We have used this assay to determine whether life-span mutations affect the rate of aging, to identify animals that age more rapidly than normal, and to infer the cause of death in *C. elegans*. Mutations that reduce insulin/IGF-1 signaling double the life span of *C. elegans*, and we find that tissue decline is slowed in these mutants. Thus this endocrine system appears to influence the rate at which tissues age. This effect extends even to the germline, which is the only mitotically active tissue in the adult. We find that Nomarski microscopy also allows a ready distinction between short-lived mutants that age more rapidly than normal and those that are simply sick, and we have identified an RNAi clone that confers a dramatic rapid-aging phenotype. This clone encodes the *C. elegans* heat-shock factor (HSF), a transcription factor that regulates the response to heat and oxidative stress. This suggests that heat-shock proteins, many of which act as chaperones, may function in normal animals to slow the rate of aging. Finally, we have identified a cause of death of *C. elegans*: namely, proliferating bacteria. This suggests that increased susceptibility to bacterial infections contributes to mortality in these animals, just as it does in humans.

IN our daily lives, we unconsciously quantify aging by evaluating the physical characteristics of an individual. Signs of aging are easy to recognize in humans: people in their twenties look different from those in their fifties or eighties. Likewise, old and young *Caenorhabditis elegans* look different when examined using a low-power dissecting microscope. Old animals move more slowly and become progressively more flaccid with age. These changes are retarded in long-lived insulin/IGF-1 signaling mutants, which remain active much longer than normal (Kenyon *et al.* 1993; Duhon and Johnson 1995; Gems *et al.* 1998). Almost 30 years ago, electron microscopic examination of *C. briggsae*, which is closely related to *C. elegans*, revealed evidence of tissue degeneration in old individuals (Epstein *et al.* 1972). Although these investigators did not examine animals of intermediate ages, their findings are consistent with the hypothesis that aging in worms involves a progressive decline in tissue integrity. In addition, intestinal autofluorescence, which is thought to be caused by lysosomal deposits of lipofuscin, accumulates with age in *C. elegans* (Klass 1977). Lipofuscin is a pigment that accumulates progressively in aging tissues as a result of the oxidative degradation and autophagocytosis of cellular components. Its ubiquitous occurrence in a variety of organisms makes it a universal marker of aging.

Having a rapid way to monitor additional aspects of the aging process would be useful in several ways. First, it might help to elucidate the means by which mutations affecting life span affect the process of aging itself. In addition, it might provide a way to identify mutants that age more rapidly than normal. Finally, it might provide clues about the causes of death in this animal. In this study, we have found that Nomarski differential interference contrast microscopy provides an effective, rapid, and convenient means of visualizing many features of tissue aging. We find that extensive tissue deterioration takes place during aging, not only in the postmitotic somatic tissues of the animal, but also in a mitotic lineage, the germline. As with old humans, this pattern of deterioration gives the animals a characteristic appearance that becomes easy to recognize.

The life span of *C. elegans* is regulated hormonally by an insulin/IGF-1-like signaling pathway. Wild-type animals live just a few weeks; however, mutants with reduced activity of DAF-2, an insulin/IGF-1-like receptor (Kimura *et al.* 1997), AGE-1, a PI 3 kinase (Morris *et al.* 1996), or downstream signaling components live more than twice as long as normal (reviewed in Guarente and Kenyon 2000). This life-span extension requires the forkhead-family transcription factor DAF-16 (Kenyon *et al.*

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1993; Dorman *et al.* 1995; Larsen *et al.* 1995; Lin *et al.* 1997; Ogg *et al.* 1997). Loss-of-function mutations in *daf-16* suppress the life-span extension of *daf-2* and *age-1* mutants. In addition, they shorten the life span of otherwise wild-type animals (Larsen *et al.* 1995; Lin *et al.* 2001). Thus *daf-16* activity is required for the longevity of wild-type animals, as well as the longevity of insulin/IGF-1 signaling mutants.

At the cellular level, one can imagine several ways in which insulin/IGF-1 signaling mutations could extend life span. If nematodes die from a single lethal event (analogous to a heart attack), then mutations in the insulin/IGF-1 signaling pathway could extend life span by decreasing the probability of this event. Alternatively, if nematodes, like humans, age in a progressive fashion that involves a decline in tissue integrity, a mutation could extend life span by slowing the entire aging process. The final possibility is a trivial one, namely, that the commonly used N2 laboratory strain of *C. elegans* has suffered detrimental mutations that shorten life span and that mutations in the *daf-2* pathway correct this defect. This is unlikely, however, because insulin/IGF-1 pathway mutants live much longer than any of four wild *C. elegans* strains (Hsin and Kenyon 1999).

In this study, we have developed a protocol for evaluating and quantifying age-related changes and used this protocol to compare the tissues of wild-type animals to those of long-lived and short-lived insulin/IGF-1 signaling mutants over the course of their lives. Our findings indicate that insulin/IGF-1 signaling influences life span by changing the rate at which the tissues age and that this pathway governs the aging not only of the postmitotic somatic cells, but of mitotic lineages as well.

The pathways that govern many biological processes, such as the cell cycle, or the timing of developmental events, include genes that function to advance the process, as well as genes that function to retard the process. Because of the difficulty of distinguishing mutations that cause progeria (accelerated aging) from mutations that kill the animal for reasons unrelated to aging, the analysis of life span relies mainly on a single class of regulatory genes: genes whose wild-type function is to shorten life span. Loss-of-function mutations in these genes will extend life span. Thus, having a way to quickly identify rapidly aging mutants (many of which would define genes whose wild-type function extends life span) would be very powerful. Several short-lived mutants that accumulate lipofuscin granules more rapidly than normal have been postulated to age rapidly, on the basis of this one age-related phenotype (Hosokawa *et al.* 1994). In this study, we have asked whether we might be able to use Nomarski microscopy as a way to decide whether a short-lived mutant could have an accelerated-aging syndrome. From a screen of a chromosome I bacterial RNAi library (Fraser *et al.* 2000), we found five clones that shortened adult life span (A. Dillin, D. Garigan, D. Crawford, J. Ramond and C. Kenyon, unpublished results). By examining these animals with Nomarski optics, we could see at once that four produced abnormal phenotypes that did not resemble normal aging. In contrast, the fifth clone caused young animals to look the way they would normally look at a much older age. Thus we believe that this technique does provide a way of eliminating many short-lived mutants from further consideration as aging mutants. Interestingly, this fifth clone encodes the *C. elegans* heat-shock factor (HSF), a transcription factor that regulates the response to heat and oxidative stress. This finding raises the possibility that in normal animals the activation of this stress response may slow down the rate of aging and allow the animals to live much longer than would otherwise be possible.

The changes we observed in old animals may simply correlate with age, as wrinkles do in humans, or they may be a pathology that contributes directly to death. We observed that bacteria, *C. elegans*' food source, often accumulated in the pharynx and gut of worms near death, and we hypothesized that the presence of proliferating bacteria in the body of an aged worm might have a deleterious effect on its health. Accordingly, we grew the animals on live bacteria that were unable to proliferate and observed a sharp decrease in the frequency of constipation and a 30–40% increase in mean life span. We conclude that some feature of proliferating bacteria, possibly their ability to cause infections in older animals, shortens the life span of this organism.

MATERIALS AND METHODS

***C. elegans* strains used:** The following strains were used: N2, *daf-16(mu86)I, daf-2(e1370)III, daf-2(mu150)III, ced-3(n1286)IV, DH1033 bIs1[vit-2::gfp; rol-6]; sqt-1(sc103), daf-2(e1370)III; him-5(e1490)V, CF512 fer-15(b26)II; fem-1(hc17)IV. daf-2(mu150)* was recovered from an EMS mutagenesis screen of >11,000 haploid genomes in a temperature-sensitive sterile background [*CF512 fer-15(b26)II; fem-1(hc17)IV*; J. Apfeld, H. Hsin, B. Albinder, B. Tsung, J. Dorman and C. Kenyon, unpublished data]. Dominance, complementation, and linkage tests using the dauer phenotype were conducted by N. Oliveira, and complementation tests using the life-span phenotype were conducted by N. Libina (Kenyon laboratory). C. Murphy outcrossed the mutant and conducted preliminary life-span analysis.

Life-span analysis: Wild-type, *daf-2*, and *daf-16* animals raised at 20° were shifted to 25° at the L4 molt and transferred to new plates every other day thereafter until progeny production ceased. Animals were judged to be dead when they no longer responded to gentle prodding. Animals that crawled off the plate, became desiccated on the sides of the plate, displayed extruded internal organs, or died from internally hatched progeny were censored. Censored animals were incorporated into the data set until the day of their disqualification, as described previously (Apfeld and Kenyon 1999). Life-span analysis of *ced-3* mutant animals was conducted at 25° using the parental N2 strain from the Horvitz lab, kindly provided by Cori Bargmann, as a control. Life spans of wild-type animals grown on bacteria expressing double-stranded RNA were determined at 20°. We used Statview 5.0.1 (SAS) software to construct life-span curves and to determine means and percen-

tiles. Throughout the article, the ages given refer to days of adulthood.

Photography of autofluorescence: Endogenous gut fluorescence was photographed using a 525-nm bandpass filter. Images were collected without automatic gain control in order to preserve the relative intensity of different animals' fluorescence. Two-, 5-, and 10-day-old adults were photographed on the same day to avoid effects of light source variation on apparent fluorescence intensity.

Visualization of yolk: The *vit-2::GFP* fusion strain (see above) was a kind gift of David Hirsch and Barth Grant. Animals were allowed to age and were photographed using both Nomarski optics and epifluorescence (525 nm).

Nomarski analysis: Animals that had been cultured at 25° were placed on a 2% agarose pad in M9 buffer containing 2 mM sodium azide and covered with a coverslip. Control experiments indicated that sodium azide did not affect the age-related phenotypes we observed. Delicate older animals of all genotypes occasionally ruptured and were lost during this process (~10%). Images were captured using a CCD camera coupled to Universal Imaging's MetaMorph Imaging System (version 3.6). Image files were contrast balanced and rotated when necessary using PhotoShop 5.0.

Quantification of tissue damage: *C. elegans* heads were photographed as described above. In a blind experiment, photographs of heads were given a score of 1–5, with 1 representing a youthful, unsullied appearance and 2–4 denoting low, medium, and high levels of overall deterioration. A rare score of 5 was assigned to animals so deteriorated as to be nearly unrecognizable. Please note that there is no strict quantitative relationship between the extent of damage in these different classes: an animal receiving a score of 2 does not necessarily have twice as much damage as an animal receiving a score of 1. Instead, this scoring system is more analogous to grading of student exams, in which a grade of A–F is assigned on the basis of relative performance. This system makes it possible to carry out statistical analysis by nonparametric methods that simply ask whether members of certain subgroups are more likely to receive low (or high) ranks than are members of other subgroups. Figure 3 shows representative photographs of heads earning these scores.

Photographs of germ cells in the distal gonad also were rated on a scale of 1–5 on the basis of these criteria. In addition, these photographs were also assigned a cumulative value that represented the presence and extent of several of the correlates of aging we often observed: graininess; large, well-separated nuclei; cavities; and fewer nuclei. Each correlate of aging was scored separately and then summed to give the final score.

Scores were assigned without knowledge of the age or genotype of the worm in the photograph. Overall scores were reevaluated at least once, and a naive observer was asked to score a selection of photographs in a double-blind experiment.

Statistical analysis of tissue damage: Nonparametric analysis of head scores was conducted using the Kruskal-Wallis test to determine if there were significant differences between multiple groups, followed by a pairwise comparison, the Mann-Whitney test. All statistical analysis was conducted using Statview 5.0.1 (SAS) software.

RNA interference: Bacterial RNAi clones that shortened adult life span were identified by a procedure described elsewhere (A. Dillin, D. Garigan, D. Crawford, J. Ramond and C. Kenyon, unpublished results). We then used Nomarski optics to observe the tissue phenotypes of *C. elegans* cultured on these bacteria.

Life spans on nonproliferating bacteria: *Antibiotic treatment:* The OP50 strain typically used as a *C. elegans* food source was transformed with a vector containing an ampicillin resistance gene, which also confers resistance to carbenicillin. Conventional or drug-resistant OP50 was seeded onto NG plates and allowed to grow for 2 days before the addition of 80 μl of 0.5 M carbenicillin. *C. elegans* eggs were transferred to these plates, and worms were transferred as necessary to similar plates. Carbenicillin-sensitive bacteria were periodically streaked onto drug-containing and drug-free culture plates to ensure that they were not proliferating but were capable of recovery on drug-free media. An identical procedure was followed for life-span assays on kanamycin-sensitive and -resistant bacteria. After treatment with 80 μl of 10 mM kanamycin, which is a bactericide, drug-sensitive bacteria were streaked onto drug-free plates to confirm that they had been killed. In these experiments, we examined *fer-15(b26); fem-1(hc17)* mutants at 25°. These animals do not produce progeny at this temperature, thus simplifying the procedure.

UV treatment: To kill bacteria, NG plates seeded with OP50 bacteria were exposed to 302 nm ultraviolet light for 30 min. As a control, unseeded plates were also treated in this way and seeded subsequently. Bleached wild-type *C. elegans* eggs were transferred to these plates and incubated at 25°. Worms were transferred as necessary to similar plates.

RESULTS

Decline of tissue integrity in aging wild-type animals: Nomarski microscopy is commonly used to observe the development of *C. elegans*. In young animals, the nuclei and nucleoli of all cells are easy to see using this method. It is also possible to see the boundaries of many cells and tissues, such as the muscles, gonad, epidermal seam cells, and certain neurons. We observed that in young adults the cells and tissues appeared similar to those of late juvenile stages, except that the nuclear boundaries were less distinct. This lack of definition became more pronounced as the animals grew older. For example, by day 10, it was very difficult to see the nuclei of the epidermal cells (Figure 1A). (Throughout the article, the ages given refer to days of adulthood; for example, "day 10" refers to the tenth day of adulthood.) Neuronal nuclei, which have a wrinkled appearance, remained visible throughout the life of the animal, although they, too, grew less distinct with time. In young animals, the cytoplasm and nucleoplasm of most cells was smooth and uniform (Figure 1B). However, as the animals grew older, both began to show signs of deterioration (Figure 1, C and D). Necrotic cavities of various sizes appeared, often containing vibrating particles that appeared to display Brownian motion. Tissues often acquired a curdled texture (Figure 1D).

Older animals also accumulated shiny, mobile patches of a substance that appeared to be yolk. In young animals, yolk is transported from its site of synthesis in the intestine into the gonad, where it is incorporated into embryos. It is possible that yolk accumulates in old animals when the production of embryos ceases. We examined animals expressing a green fluorescent protein (GFP)-tagged yolk protein and confirmed that this substance was in fact yolk (Figure 1, F and G). We also observed shiny but less mobile nonfluorescent material in the bodies of GFP-expressing worms; similar, less mobile

material (presumably something other than yolk) was also present in non-GFP-expressing worms. Finally, as reported previously (Klass 1977; Russell and Seppa 1987), we also observed increased intestinal lipofuscin autofluorescence (Figure 2). The cellular deterioration that we observed during aging was widespread. To quantify these changes, we chose to study two areas of the worm in more detail: the head and the germ cells.

The head is a particularly informative and compact area composed of multiple tissue types. It contains the pharynx, a neuromuscular pump that ingests and grinds bacteria, as well as nervous tissue, muscle, and epidermis. Using Nomarski optics, we were able to evaluate the general character of this body region, but because cellular boundaries can be indistinct, we were often not able to resolve individual tissue types with certainty. Figure 3 shows representative photographs of heads of animals of different ages, illustrating the changes that occur in tissue integrity during aging.

To quantify the changes we observed, we analyzed photographs of 83 individual wild-type worm heads. At every age, some animals exhibited more extensive tissue deterioration than did others. This was not surprising, since some animals of the same genotype live longer than others. Each animal's head received a grade on the basis of the extent of deterioration it exhibited, as described in materials and methods. As shown in the scatterplot in Figure 4, we observed a steady trend toward increasing damage with age. To evaluate these findings quantitatively, we used the Kruskal-Wallis test, a nonparametric statistical test, to ask whether there was a statistically significant relationship between an animal's age and its rank within the overall population. We found that, indeed, the average level of tissue deterioration in the overall population increased with age in a statisically significant manner ($P < 0.0001$; Figures 4 and 5 and legends). These findings imply that the tissues of *C. elegans* deteriorate in a progressive fashion as the animals grow older. This tissue decline correlates with the decreased mobility and flaccid appearance of old worms that are visible with a low-power dissecting microscope.

Tissue deterioration occurs in the germline of aging wild-type animals: The only cells that are able to divide in *C. elegans* adults are the germline stem cells located near the distal tip of the gonad (Kimble and White 1981). Thus it was particularly interesting to ask whether signs of age-related tissue deterioration were present in this tissue. We found that the germlines of older animals showed dramatic signs of aging (Figure 1, I–K). The mitotic germline of *C. elegans* is a multinucleate syncytium. In older animals, the nuclei were spaced more widely and the nucleoplasm was disrupted by cavities and grainy material. In addition, cavities were often apparent (Figure 1I). Occasionally nuclei appeared to be severely

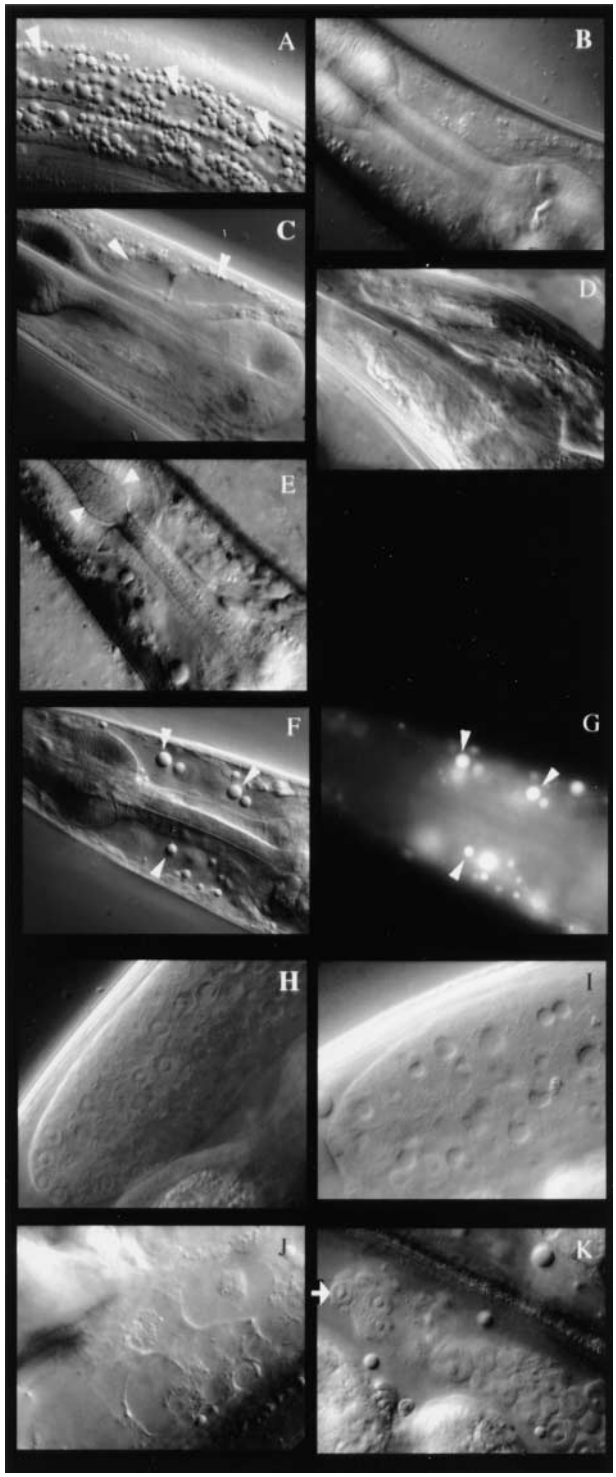

Figure 1.—Age-related phenotypes. (A) Epidermal nuclei of a young adult. (B) The head of a young adult. (C) Cavities (arrowheads). (D) "Curdled," disordered tissue in the head. (E) Bacterial packing in the pharynx. (F) Oily droplets (arrowheads) in the head of an animal containing a yolk::GFP fusion protein; GFP fluorescence of same head (G) shows that the substance is yolk. (H) Intact distal end of the gonad in a young adult. Pathologies in the distal gonad include cavities (I) and swollen, granular nuclei (J). (K) "Shriveled" distal gonad containing nuclei that appear cellularized. Arrow indicates distal tip.

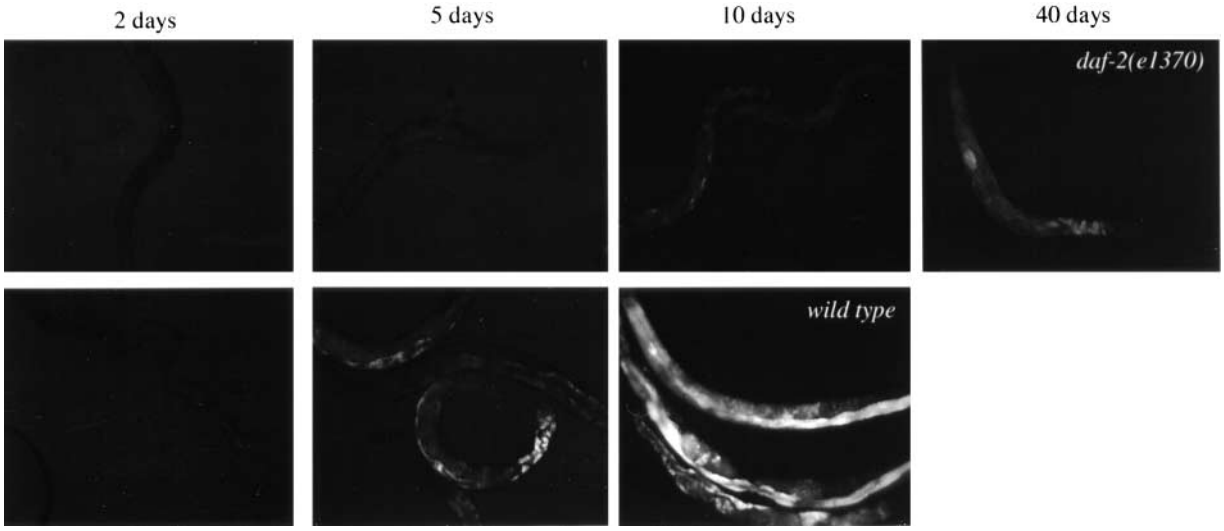

FIGURE 2.—Accumulation of lipofuscin autofluorescence with age. Wild-type and *daf-2(e1370)* animals at 2, 5, and 10 days of adulthood were photographed on the same day under identical conditions.

swollen and grainy (Figure 1J). We frequently observed gonads in which the nuclei appeared to be cellularized (Figure 1K). These same gonads also contained relatively few nuclei, giving the structure an overall "shriveled" appearance (Figure 1K). This deterioration of the germline began to be apparent at the fifth day of adulthood and increased with age. Germline deterioration was quantified as described above: photographs of distal gonad arms were scored blind, that is, without knowledge of their identities. Each received a value that reflected the condition of the tissue. Two-day, 5-day, and 10-day-old animals exhibited progressively more extensive degeneration (Figure 4) and these differences were statistically significant (Mann-Whitney test: 2 days *vs.* 5 days, $P = 0.0011$; 5 days *vs.* 10 days, $P = 0.018$). Thus, our findings indicate that the integrity of the germline declines during the life of the animal.

Mutations in the insulin/IGF-1 pathway change the rate at which both mitotic and postmitotic tissues age: Next, we asked whether mutations in the insulin/IGF-1 signaling system influence tissue aging. To do this, we examined two long-lived but otherwise quite dissimilar *daf-2* mutants, *daf-2(e1370)* and *daf-2(mu150)*, as well as the short-lived *daf-16(mu86)* null mutant, at different ages using Nomarski optics. The *daf-2(e1370)* allele has been characterized previously (KENYON *et al.* 1993; DORMAN *et al.* 1995; LARSEN *et al.* 1995; KIMURA *et al.* 1997; GEMS *et al.* 1998). This mutant has a temperature-sensitive dauer-constitutive phenotype. When shifted to the restrictive temperature (25°) at the L4 molt, it becomes a long-lived adult but has a pleiotropic phenotype. The animals are uncoordinated and produce progeny late in life (up to day 40; GEMS *et al.* 1998; D. GARIGAN and C. KENYON, data not shown). They also shift metabolism toward fat production (KIMURA *et al.* 1997; WOLKOW *et al.* 2000) and appear dark when viewed with a dissecting microscope (VOWELS and THOMAS 1992). We found that these animals were shorter than normal (1.0 *vs.* 1.4 mm, $P < 0.0001$), and their bodies were thinner (47 *vs.* 54 μm, $P = 0.002$). In contrast, the *mu150* allele, which was isolated in our lab in a screen for long-lived mutants (see MATERIALS AND METHODS), causes animals to appear much more similar to wild type under the dissecting microscope than does *e1370*. Unlike *daf-2(e1370)* animals, which become dauers permanently at 25°, *daf-2(mu150)* animals did so only transiently and recovered after about a day. In addition, *mu150* animals moved normally and did not produce progeny late in life (0/105 animals had progeny after day 6 of adulthood). Their bodies were not dark like those of *e1370* mutants (we examined them in double-blind experiments), but resembled wild type, indicating that *daf-2* mutations can uncouple fat production and longevity. They were only slightly shorter than normal (1.2 *vs.* 1.4 mm, $P = 0.01$) and not thinner (53 *vs.* 54 μm, $P = 0.45$). All measurements were made 2 days after animals were shifted from 20° to 25° at the L4 molt. Overall, this mutant appears remarkably similar to wild type in terms of its behavior and morphology. In spite of their different phenotypes, *mu150* and *e1370* mutants displayed similar mean lifespan extensions, 121 and 124% longer than those of wild type, respectively.

We found that the quality of tissue deterioration that took place in both *daf-2* and *daf-16* mutants resembled that of wild type, suggesting that both types of mutants age in a normal way. All of the mutants displayed increased lipofuscin-like intestinal fluorescence at relatively old ages (Figure 2, and data not shown). We found that both the somatic tissues and the germlines of *daf-2(mu150)* and *daf-16(mu86)* mutants exhibited age-related damage that appeared identical to that seen in wild type (Figure 3 and data not shown). *daf-2(e1370)*

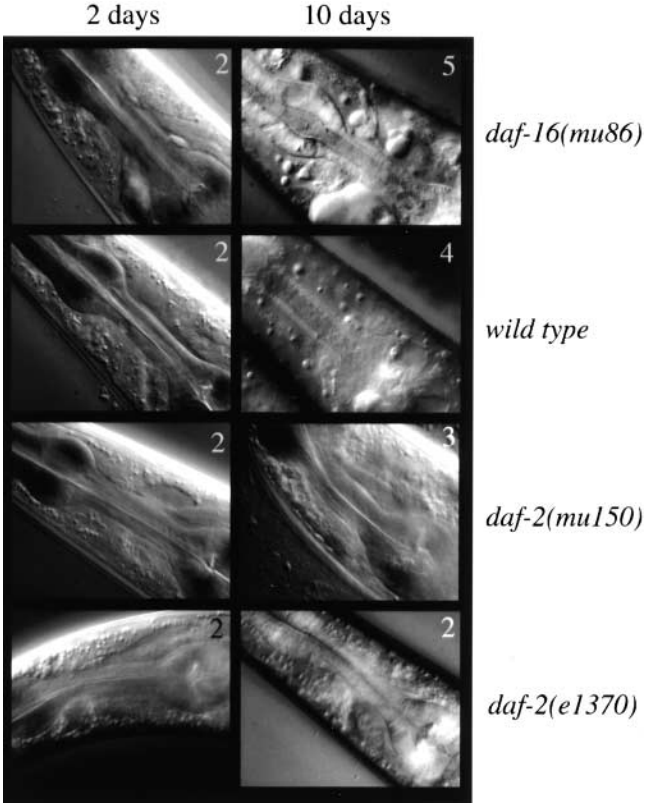

FIGURE 3.—Heads of 2-day and 10-day-old adult wild-type, *daf-16*, and *daf-2* animals. Representative animals of each genotype are shown here, although the appearances of individual animals within each group varied considerably (see Figure 4). The score each head received (top right) reflects its overall extent of damage (see MATERIALS AND METHODS). In all photos, anterior is to the left.

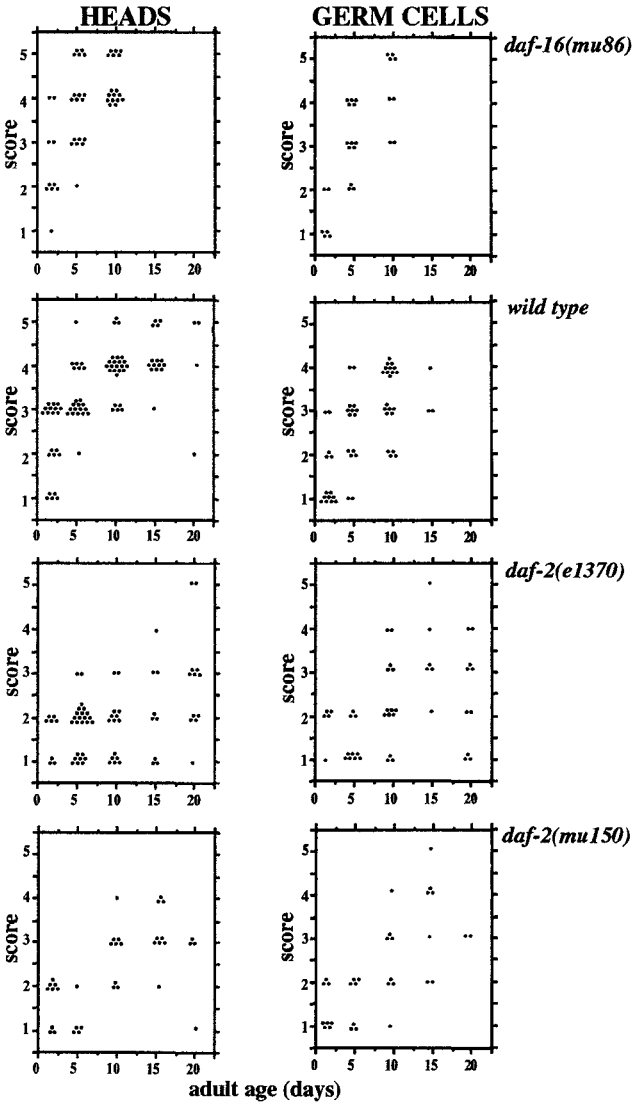

FIGURE 4.—Scatter diagrams of values assigned to heads and germ cells. Each dot corresponds to a single animal. All animals were evaluated in two separate sittings. At least one of these evaluations was made without knowledge of the animal's age or genotype. Note that this is a nonparametric assessment: the scores are essentially grades, and they indicate increasing deterioration but do not have a particular numerical or quantitative relationship to one another. (For example, scoring 4 is not necessarily twice as bad as scoring 2.) We also analyzed the heads of several older *daf-2(e1370)* animals. The scores of these animals were: day 25, 1, 2, 3, 3; day 30, 1, 4; day 34, 2.

mutants tended to look less damaged even very late in life; however, because they were prone to die from internally hatching progeny, their germlines were difficult to evaluate because they were often obscured by quiescent L1 progeny. Their bodies were somewhat opaque, and they had less yolk in the body cavity than did wild type, even at advanced ages (perhaps because yolk was still exported into progeny). Also, the frequency of constipation and bacterial packing in the pharynx was markedly reduced in *daf-2(e1370)* mutants (see Figures 1E and 3), although it was present in some infirm individuals. Nevertheless, overall, the bodies of aging *e1370* mutants resembled those of wild type. Interestingly, the tissues of aging *daf-2(mu150)* animals were indistinguishable from those of wild type.

Although the character of the tissue deterioration we observed in *daf-2* mutants was similar to that of wild type, we found that the rate of this deterioration was dramatically slowed (Figures 3–5). For example, in *daf-2(e1370)* mutants, the outlines of epidermal nuclei (shown in Figure 1A) were clearly visible until at least 20 days of adulthood, compared to ~5 days in wild type. In addition, it was not until *daf-2* animals were ~20 days old that we began to see cavities and "curdled" tissues.

We quantified the extent of tissue damage by scoring photographs of individual heads and germ cells in the distal gonad (Figure 4). Nonparametric analysis of scores assigned to *daf-2* and wild-type animals of the same age confirmed our impression that *daf-2* animals exhibited significantly less age-related damage in both body regions. For example, in 5-day-old adults, comparison of scores by the Mann-Whitney statistical test showed *daf-2* and wild-type tissue to be significantly different. (N2 *vs. e1370* heads, $P < 0.0001$; N2 *vs. mu150* heads, $P =$

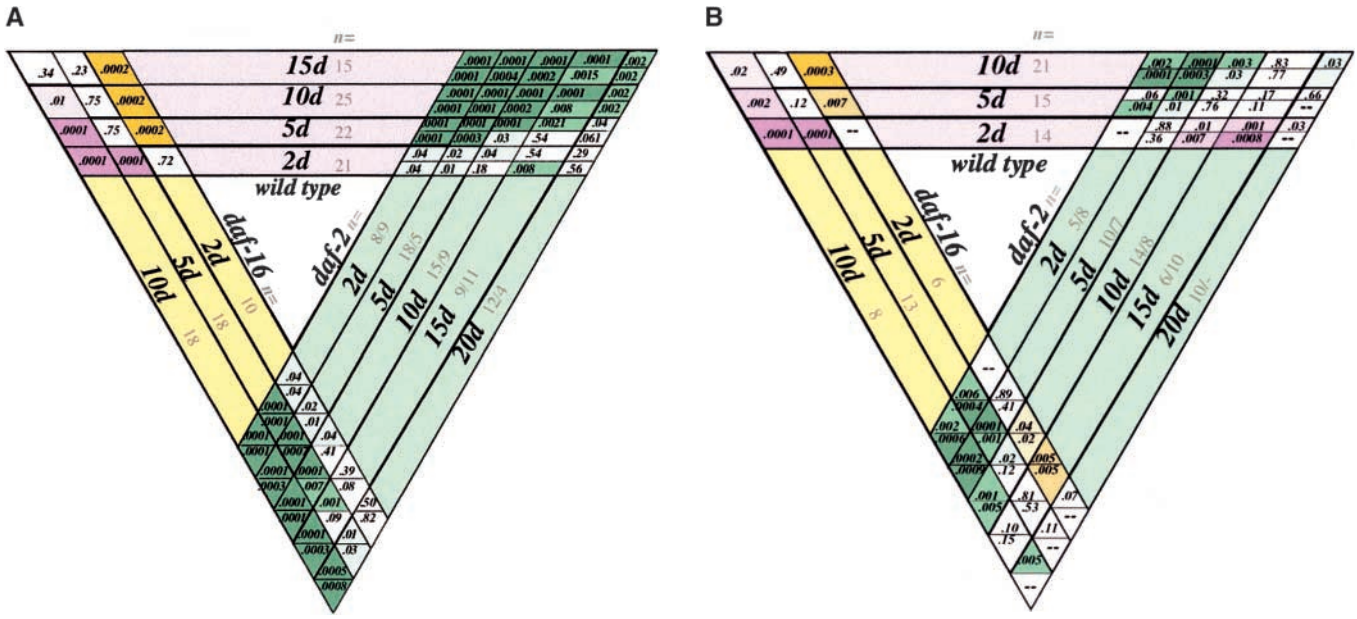

FIGURE 5.—Pairwise comparisons between heads (A) and germ cells (B) of animals of different genotypes and ages. Each set of animals of a particular age and genotype shown in Figure 4 was compared to every other set. First, the Kruskal-Wallis test was used to determine whether a significant difference was present between animals of different genotypes that were the same age. This test was also used to look for significant differences between animals of the same genotype at different ages (see text). If a significant difference was observed, then the Mann-Whitney test was used to carry out pairwise comparisons between the individual groups within that set, assigning *P*-values to each pair. The *P*-values are listed in the vertices of the triangle, at the intersection between the two groups compared. Each cell is color coded. The color of the cell corresponds to the group that appeared more youthful, that is, the group that exhibited a lower level of deterioration. The shading of the color indicates the relative significance of that difference. *daf-2* cells are split in half to accommodate the *e1370* (top half) and *mu150* (bottom half) alleles. The *P*-value is influenced by the number of animals present in each group; this number (*n*) is given in the central column of each side. When heads of all four genotypes of 2-day-old animals were treated as a group, no significant difference emerged by the Kruskal-Wallis test. Therefore we did not perform Mann-Whitney pairwise comparisons between these groups.

0.0003; N2 *vs. e1370* germ cells, $P = 0.001$; N2 *vs. mu150* germ cells, $P = 0.001$; Figure 5). We also examined the tissues of *daf-16* mutants, whose mean life spans were 10–20% shorter than normal at 25°. *daf-16* animals did not look dramatically older than age-matched wild-type animals until the tenth day of adulthood. These differences were apparent for both heads (Mann-Whitney, $P = 0.01$) and germ cells (Mann-Whitney, $P = 0.02$). *daf-16* animals at this age were predicted to be dead within 2 days, whereas wild-type animals have a longer life expectancy (maximum *daf-16* life span, 12 days; maximum wild-type life span, 19 days). In contrast, *daf-2* mutants, which significantly outlive both wild-type and *daf-16* animals, began to look younger than *daf-16* mutants as early as 2 days of adulthood (Mann-Whitney, $P = 0.04$).

Nomarski optics can be used to assay for progeria: A major goal in the study of *C. elegans*' aging is to be able to identify mutations that accelerate the aging process. From a screen of 2445 individual clones of a chromosome I bacterial RNAi library (FRASER *et al.* 2000), we identified five bacterial RNAi strains that shortened the life span of *C. elegans* (A. DILLIN, D. GARIGAN, D. CRAWFORD, J. RAMOND and C. KENYON, unpublished results). In our screen, the animals were subjected to RNAi from hatching, and young adults appeared active and healthy. The *C. elegans* genes expressed as dsRNA by these bacteria were B0205.10, which encodes a novel protein with a proline-rich region and a transmembrane domain; *gld-1*, which encodes a germline tumor suppressor protein (FRANCIS *et al.* 1995a,b); Y53C10A.1, which encodes a protein with coiled-coil domains; M01G12.4, which encodes a rhodopsin-like G-protein-coupled receptor homolog; and Y53C10A.12, which encodes the only homolog of the *C. elegans* heat-shock factor. Worms cultured on these bacteria were all dead by 10 days of adulthood, whereas ~80% of control animals were still alive. To ask which of these RNAi clones might accelerate aging, we examined 5-day-old RNAi-treated adults by Nomarski optics. Four of the five clones clearly did not cause 5-day-old worms to look older than normal. For example, the *gld-1(RNAi)* clone, which causes ectopic germline proliferation, caused germ cells to accumulate in the body cavity of day-5 adults but did not affect tissue morphology. Another clone, Y53C10A.1, caused a tissue degradation phenotype that did not resemble that of normal aging (Figure 6). However, one clone, Y53C10A.12, produced a truly striking progeric phenotype. When viewed by Nomarski optics, 5-day-old animals grown on Y53C10A.12 dsRNA at 25° looked like normal animals at a much older age; they resembled wild-type animals that were 10–15 days old. They contained cavities and deterio-

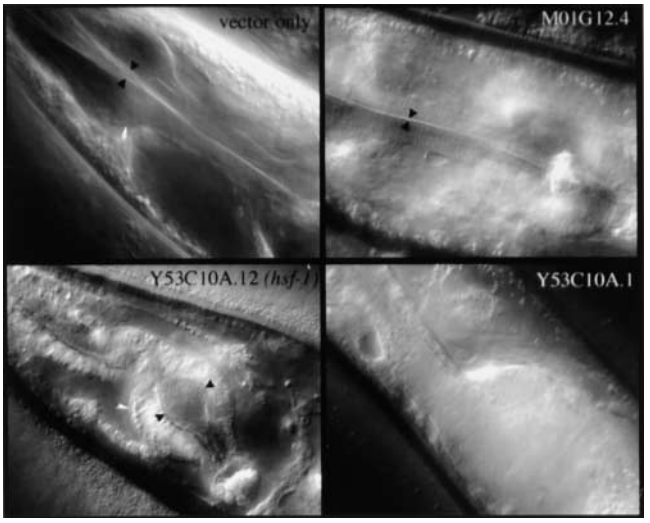

FIGURE 6.—Phenotypes of wild-type 5-day-old adult *C. elegans* on RNA bacteria. Unlike animals cultured on bacteria expressing vector alone, animals cultured on bacteria expressing Y53C10A.12 *(hsf-1)* dsRNA displayed bacteria packing the pharynx (black arrowheads) and degradation of the pharyngeal muscle (white arrowheads). See Figures 1 and 3 for comparison with old animals. The other two images demonstrate a relatively normal phenotype (M01G12.4) or a sick-but-not-old phenotype (Y53C10A.1). Unlike normal old animals, those cultured on Y53C10A.1 dsRNA displayed a bizarre degradation of the structure of pharynx muscle, so that structures including the grinder and muscle fibers were not visible. We never observed this in normal old animals. Animals were cultured at 25°.

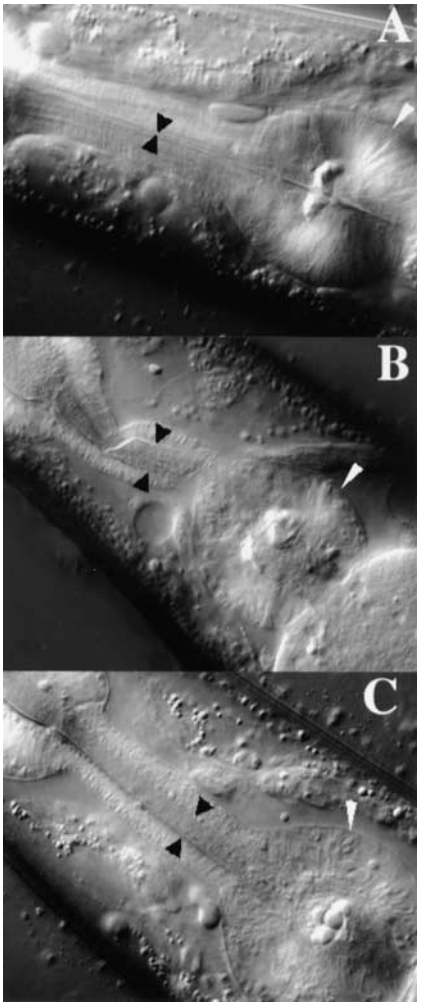

FIGURE 7.—Treatment with *hsf-1* dsRNA phenocopies normal aging. Seven-day-old adults cultured on control bacteria look reasonably youthful (A), whereas age-matched animals cultured on bacteria expressing *hsf-1* dsRNA (B) have many features in common with non-RNAi-treated 15-day-old animals (C). Among these features are bacteria packing the pharynx (black arrowheads) and degradation of the pharyngeal tissue (white arrowheads). Constipation of the anterior gastrointestinal tract is also visible in B. All animals are wild type and were cultured at 20°.

rated tissue that had the curdled texture characteristic of old animals. In addition, their pharynx and gastrointestinal tracts were packed with bacteria (Figures 6 and 7). These animals were also short lived and progeric at 20°, where they had a mean life span that was 38% shorter than that of control animals (10.7 *vs.* 17.2 days; Figure 8). We quantified the phenotypes of day-7 animals grown at 20° by photographing them and scoring them blind, as described above. Of 13 control animals examined, 1 received a score of 1, 11 received a score of 2, and 1 received a score of 3. In contrast, 5/14 animals cultured on *hsf-1* dsRNA received a score of 3 and 9/14 received a score of 4 ($P < 0.0001$; Mann-Whitney test). Thus, unlike the other four genes whose RNAi phenotypes we examined, this gene, which encodes the *C. elegans* heat-shock factor, is a candidate for a gene that functions in normal animals to prolong youthfulness and retard the aging process.

Apoptosis is unlikely to influence aging in *C. elegans*: One of the goals of this study was to identify possible causes of death in *C. elegans*. In *C. elegans*, 131 cells undergo programmed cell death, or apoptosis, during development, and additional apoptosis occurs among the meiotic precursor cells in the germline (SULSTON and HORVITZ 1977; SULSTON *et al.* 1983; GUMIENNY *et al.* 1999). An important question is whether apoptosis influences life span in *C. elegans*. We looked for apoptotic cells, which have a characteristic refractile appearance (SULSTON *et al.* 1983), in the somatic tissues of wild-type and mutant animals during aging and failed to observe them. However, to test the role of apoptosis in a more definitive way, we examined the life spans of *ced-3* mutants, which lack a caspase that is required for apoptosis in *C. elegans*. In *ced-3* mutants, cells that should die instead remain alive and, at least in some cases, differentiate into functional cells. We reasoned that if programmed cell death influences organismal death, mutants defective in apoptosis should have abnormal, possibly extended, life spans. However, we found that *ced-3* mutants had life spans that were indistinguishable from those of wild type (Figure 9). Similar results have been observed previously by the Horvitz lab and by T.

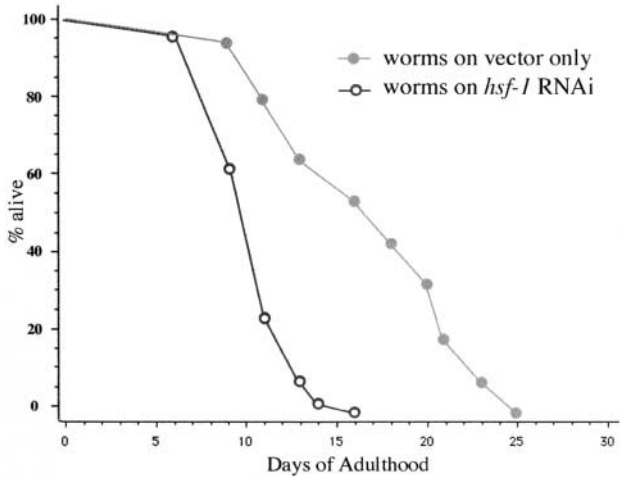

FIGURE 8.—Life spans of wild-type animals cultured at 20° on bacteria expressing *hsf-1* double-stranded RNA ($n = 62$) or vector only ($n = 58$; Mantel-Cox Logrank $P < 0.0001$).

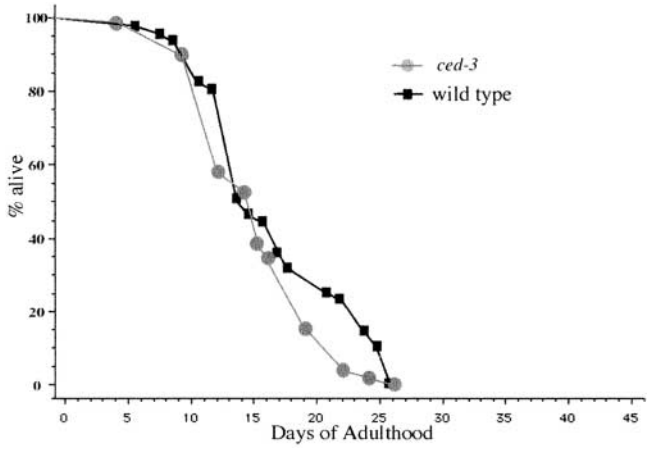

FIGURE 9.—*ced-3(n1286)* mutants, which lack programmed cell death, have normal life spans. The wild-type strain ($n = 67$) is the isogenic parent of the *ced-3(n1286)* mutant ($n = 47$; Mantel-Cox Logrank $P = 0.21$).

JOHNSON (personal communication). We conclude that apoptosis does not play a significant role in determining wild-type life span.

***C. elegans* is killed by its food:** *C. elegans* is normally cultured on a lawn of OP50 bacteria (BRENNER 1974). In wild-type and *daf-16* animals older than 5 days of adulthood and in *mu150* animals older than 15 days of adulthood, the pharynx and both the anterior and posterior portions of the intestine were frequently distended and packed with bacteria (Figure 1E and data not shown). We hypothesized that bacterial proliferation within the gastrointestinal tract might contribute to this packing. To test this, we grew worms on bacteria that were unable to proliferate, including bacteria killed by UV treatment or by the antibiotic kanamycin, as well as live bacteria whose growth was arrested by the bacteriostatic agent carbenicillin. We found that worms grown on these three kinds of nonproliferating bacteria had much lower rates of constipation during their lifetime, although at least some of these animals did become constipated shortly before death (Figure 10). Thus we conclude that bacterial proliferation within the gastrointestinal tract is the major cause of constipation in old animals.

Previously, GEMS and RIDDLE (2000) showed that culturing worms on UV-killed bacteria extended mean life span of hermaphrodites by ~16%. We also determined the life spans of animals grown on UV-irradiated bacteria and found that, under our conditions, mean life span was increased 30–40% (Figure 10). Why do UV-irradiated bacteria extend life span? UV irradiation damages cellular components and may also induce the bacterial SOS response, which is associated with many physiological changes that could conceivably trigger a life-span-extension response. To ask whether the effect was specific to UV, we also determined the life spans of animals grown on bacteria killed with kanamycin. These, too, lived longer than normal (Figure 10). Finally, to ask whether life-span extension required the death of the bacteria, we determined the life spans of animals grown on live bacteria whose growth was arrested with carbenicillin. We found that this, too, increased life span (Figure 10). Thus we conclude that live bacteria *per se* are not harmful to *C. elegans*; instead, something associated with bacterial growth and proliferation kills them.

DISCUSSION

We have found that Nomarski microscopy provides a powerful way to monitor and quantify the aging process in *C. elegans*. This technique does not permit a detailed study of individual tissue types, because many of the boundaries between cells and tissues cannot be resolved. It does, however, permit a general assessment of the quality of cells and tissues that make up most of the body mass. Using this method, we find that tissues of wild-type animals deteriorate progressively during aging. Like old humans, old worms have a very characteristic appearance, which is easily recognized by the experienced eye.

One objective of this study was to learn how mutations in the insulin/IGF-1 signaling pathway influence the aging of the body. The relevance of this pathway to aging has been questioned by some who have asserted that mutations in this pathway do not affect the rate at which the aging process takes place (HAYFLICK 1999). Our findings contradict this view. By defining the phenotype of tissue aging and comparing photographs of normal and mutant *C. elegans*, we were able to infer that age-related deterioration proceeds at different rates in normal *vs.* mutant animals. The rate of tissue deterioration is slowed by mutations that lengthen life span and is accelerated by mutations that shorten life span. Thus, we propose that this endocrine system influences life span by changing the rate at which the tissues of the animal age.

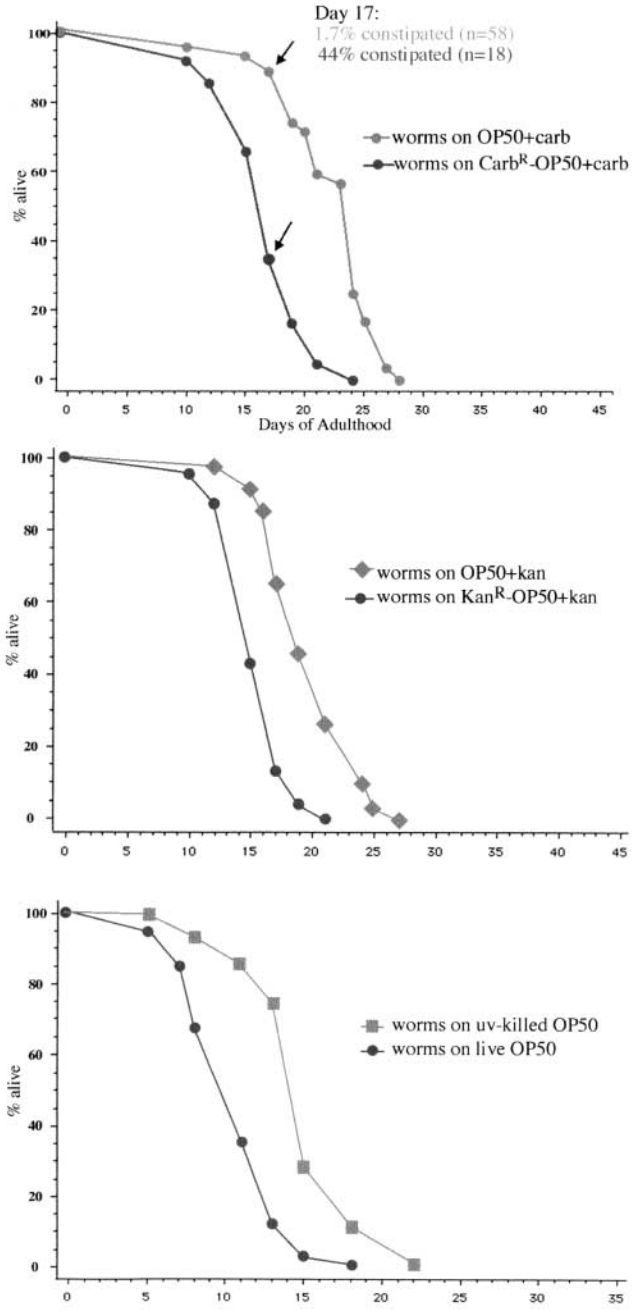

FIGURE 10.—Life spans of *C. elegans* cultured on proliferating OP50 bacteria *vs.* either arrested or dead OP50 bacteria. (Top) The life span of *C. elegans* cultured on carbenicillin-sensitive (arrested) OP50 ($n = 40$) and OP50 containing a carbenicillin-resistance plasmid ($n = 49$), both in the presence of carbenicillin. (Middle) The life span of *C. elegans* cultured on kanamycin-sensitive (killed) OP50 ($n = 31$) and OP50 containing a kanamycin-resistance plasmid ($n = 52$), both in the presence of kanamycin. (Bottom) The life span of *C. elegans* cultured on UV-killed ($n = 34$) and untreated OP50 ($n = 58$) bacteria.

Some have argued that the genetic analysis of aging in *C. elegans* will not reveal universal mechanisms of aging control, because, unlike many higher organisms, cells in the adult are postmitotic. This criticism has been tempered by the finding that *SIR2* protein, which regulates the mitotic life span of yeast, also functions in the *C. elegans* insulin/IGF-1 system (TISSENBAUM and GUARENTE 2001). Our findings, too, argue against this possibility, since insulin/IGF-1 mutations affect the tissue quality of the mitotic germline lineages as well as the postmitotic somatic lineages. One caveat about this conclusion is that it is not clear how long during adulthood the germline remains mitotic. It is possible that at some point germline cells stop dividing and that the insulin/IGF-1 pathway mutations affect their integrity after that time.

Another goal of this study was to learn whether Nomarski optics would allow us to distinguish short-lived mutants that were simply sick from those that might be aging prematurely. This type of analysis seemed likely to be informative: in the case of humans, a young person with a terminal illness, who has a short expected life span, would never be confused with old person. We were pleased to find that a cursory examination allowed us to discard, immediately, four of five short-lived RNAi-treated strains from further consideration as progeric animals. Interestingly, one clone did cause animals that were young to resemble normal, old animals. These animals' tissues displayed the curdled texture and cavity-ridden appearance so characteristic of old animals. Furthermore, they were constipated, suggesting that their physiology, too, was similar to that of older animals. Thus we conclude that this RNAi clone is an excellent candidate for a gene that prevents progeria.

Nomarski analysis will never provide definitive proof that a short-lived mutant is aging precociously. Specifically, it is possible that these animals are in a physiological state that is not the same as that of normal old animals but nevertheless causes them to resemble old animals when viewed with Nomarski optics. The case for progeria would be strengthened by examining gene expression profiles to confirm that animals that appear older than normal also display gene expression patterns characteristic of old animals. In addition, if a gene encodes a protein that carries out a rate-limiting step in the aging process, then overexpression should lengthen life span. In spite of these caveats, we would like to emphasize that the morphology of these animals was quite striking. As shown in Figure 1, the Nomarski aging phenotype is quite complex, involving many different morphological features (tissue texture, constipation, etc.), and these RNAi-treated young animals closely resembled normal old animals. They were analogous to 45-year-old people who looked 70.

The identity of this progeric RNAi clone was very interesting: it encodes a homolog of HSF, which is the transcription factor that activates expression of heat-shock genes in response to heat shock and oxidative stress. Thus the finding that *hsf-1(RNAi)* shortens life span and produces a progeric Nomarski phenotype suggests the hypothesis that this transcription factor, and

thus presumably the stress response it regulates, acts in normal animals to prolong youthfulness and extend life span by retarding the aging process. One possibility is that improperly folded proteins that accumulate during aging activate heat-shock factor, which in turn would be expected to activate the expression of heat-shock genes (Freeman *et al.* 1999). Many of these genes are known to encode chaperones, which could bind to and stabilize, or help to refold, improperly folded proteins.

Although this is the first demonstration that the heat-shock system acts in normal animals to prevent a syndrome that resembles precocious aging, a link between the heat-shock response and aging has been demonstrated previously. The expression of heat-shock proteins has been shown to increase during aging in wild-type *C. elegans* (Cherkasova *et al.* 2000) and in some long-lived mutants (Walker *et al.* 2001). In addition, many long-lived *C. elegans* mutants are thermotolerant (Lithgow *et al.* 1995; Cypser and Johnson 1999; Yang and Wilson 1999, 2000; Johnson *et al.* 2000; Murakami *et al.* 2000), and heat pretreatment can extend *C. elegans* life span by up to 23% (Lithgow *et al.* 1995; Cypser and Johnson 2002). Overexpression of Hsp70 in Drosophila has been shown to cause a small (4%) increase in mean life span (but no increase in maximum life span; Tatar *et al.* 1997). It will be interesting to learn whether overexpression of *C. elegans hsf-1*, which is predicted to regulate the entire heat-shock response, might extend life span to a greater extent.

In addition to providing a way of examining the aging process in long- and short-lived mutants, Nomarski examination also suggested possible causes of death. One of the most striking phenotypes exhibited by older animals was bacterial packing in the pharynx and the anterior and posterior regions of the intestine. This packing was never observed in young animals, which must therefore be resistant to it. We found that bacterial packing was greatly reduced if the bacteria were incapable of proliferation. This suggests that, as the animal ages, it loses the capacity to prevent bacterial proliferation in its gastrointestinal tract. Bacterial packing in the pharynx could be due to the decreased rate of pumping observed in older animals (Kenyon *et al.* 1993), which might give bacteria time to proliferate before expulsion. Alternatively, young (but not old) animals might produce an antibacterial substance that renders ingested bacteria incapable of division.

In addition to reducing bacterial packing, feeding worms bacteria that were incapable of proliferation extended life span substantially—by 30–40% (Figure 10). A trivial explanation for this is that the animals cannot use the nonproliferating bacteria as a food source and become calorically restricted as a result. Caloric restriction is known to extend life span (Klass 1977; Lakowski and Hekimi 1998); however, we believe that these animals are not calorically restricted for three reasons. First, calorically restricted animals are thin and these are not. Second, the schedule of progeny production, which is delayed in calorically restricted animals (D. Crawford and C. Kenyon, unpublished results), is normal in these animals (data not shown). Likewise, Gems and Riddle (2000) showed that UV-irradiated bacteria do not affect the morphology or reproduction of *C. elegans* and thus do not cause caloric restriction. Finally, bacteria treated with carbenicillin are still alive; their growth is simply arrested by the antibiotic. Thus their nutritional value should be the same as (or similar to) normal. Thus we conclude that none of these treatments cause caloric restriction. Instead, something about proliferating bacteria *per se* seems to be detrimental to the animals.

Interestingly, when we examined the tissues of five long-lived animals shown at the top of Figure 10 at 15 days of adulthood, we found that they looked very old, just as old as age-matched controls (data not shown). This suggests that bacteria may not influence the rate of tissue aging of the animals, but instead that they may cause a catastrophic death once the animals' health has declined sufficiently. One possibility is that the bacterial packing we saw in the gastrointestinal tract kills the animals. Since this packing is markedly reduced in the absence of bacterial proliferation, this is a plausible explanation. A related explanation, also suggested by Gems and Riddle (2000), is that proliferating bacteria actually enter the tissues of the old worms, causing infections that kill the animal. In general, we did not observe bacteria in the tissues of old animals; however, we may not have noted small but nevertheless lethal amounts. It is also possible that once the bacteria enter the tissues, they proliferate and kill the animal rapidly (the tissues of dead animals almost always contain bacteria). If this is the case, then simply by chance we may have failed to view any animals during this short time window. Finally, we cannot rule out a completely different possibility: that proliferating bacteria produce a toxin that kills the animals.

Because *daf-2* mutants live 100% longer than normal, much longer than the 30–40% extension conferred by nonproliferating bacteria, it is unlikely that they are long-lived simply because they are able to resist bacterial growth or infections. However, many life-span mutants in *C. elegans* do live ~30% longer than normal. It should be possible to determine whether any of these mutants are long-lived because they are resistant to bacteria, since their life spans should not be increased further by growth on nonproliferating bacteria.

We thank Paige Nittler, Douglas Crawford, Andrew Dillin, Lisa Williams, Natasha Libina, Joy Alcedo, and Coleen Murphy for helpful conversations. We also thank Peter Bacchetti of the UCSF Biostatistics Consulting Service for statistical expertise. The *mu150* mutant was isolated in a mutagenesis screen conducted in the Kenyon lab by J. Apfeld, H. Hsin, B. Albinder, B. Tsung, and J. Dorman; outcross and complementation tests were performed by N. Oliveira, N. Libina, and C. Murphy. J. Alcedo initially analyzed the life span of cell death mutants. A. Dillin directed, and D. Garigan, D. Crawford, and J. Ramond assisted with, the RNAi library screen in the Kenyon lab that

yielded the five clones that decreased life span. We thank Barth Grant and David Hirsch, the Caenorhabditis Genetics Center, and Cori Bargmann for strains. This work was supported by an American Federation for Aging Research Scholarship to D.G., a Canadian Institutes of Health Research fellowship to A-L. H., and a grant from the Ellison Foundation to C.K., who is the Herbert Boyer Professor of Biochemistry and Biophysics. A.G.F. was supported by a U.S. Army Breast Cancer Research Fellowship, R.S.K. by a Howard Hughes Medical Institute Predoctoral Fellowship, and J.A. by a Wellcome Trust Senior Research Fellowship (054523).

LITERATURE CITED

Apfeld, J., and C. Kenyon, 1999 Regulation of lifespan by sensory perception in *Caenorhabditis elegans*. Nature **402:** 804–809.

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

Cherkasova, V., S. Ayyadevara, N. Egilmez and R. Shmookler Reis, 2000 Diverse *Caenorhabditis elegans* genes that are upregulated in dauer larvae also show elevated transcript levels in long-lived, aged, or starved adults. J. Mol. Biol. **300:** 433–448.

Cypser, J. R., and T. E. Johnson, 1999 The *spe-10* mutant has longer life and increased stress resistance. Neurobiol. Aging **20:** 503–512.

Cypser, J. R., and T. E. Johnson, 2002 Multiple stressors in *Caenorhabditis elegans* induce stress hormesis and extended longevity. J. Gerontol. A Biol. Sci. Med. Sci. **57:** B109–114.

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.

Duhon, S. A., and T. E. Johnson, 1995 Movement as an index of vitality: comparing wild type and the *age-1* mutant of *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. **50:** B254–261.

Epstein, J., S. Himmelhoch and D. Gershon, 1972 Studies on aging in nematodes. III. Electronmicroscopical studies on aging-associated cellular damage. Mech. Ageing Dev. **1:** 245–255.

Francis, R., M. K. Barton, J. Kimble and T. Schedl, 1995a *gld-1*, a tumor suppressor gene required for oocyte development in *Caenorhabditis elegans*. Genetics **139:** 579–606.

Francis, R., E. Maine and T. Schedl, 1995b Analysis of the multiple roles of *gld-1* in germline development: interactions with the sex determination cascade and the *glp-1* signaling pathway. Genetics **139:** 607–630.

Fraser, A. G., R. S. Kamath, P. Zipperlen, M. Martinez-Campos, M. Sohrmann and J. Ahringer, 2000 Functional genomic analysis of C. elegans chromosome I by systematic RNA interference. Nature **408:** 325–330.

Freeman, M. L., M. J. Borrelli, M. J. Meredith and J. R. Lepock, 1999 On the path to the heat shock response: destabilization and formation of partially folded protein intermediates, a consequence of protein thiol modification. Free Rad. Biol. Med. **26:** 737–745.

Gems, D., and D. L. Riddle, 2000 Genetic, behavioral and environmental determinants of male longevity in *Caenorhabditis elegans*. Genetics **154:** 1597–1610.

Gems, D., A. J. Sutton, M. L. Sundermeyer, P. S. Albert, K. V. King *et al.*, 1998 Two pleiotropic classes of *daf-2* mutation affect larval arrest, adult behavior, reproduction and longevity in *Caenorhabditis elegans*. Genetics **150:** 129–155.

Guarente, L., and C. Kenyon, 2000 Genetic pathways that regulate aging in model organisms. Nature **408:** 255–262.

Gumienny, T. L., E. Lambie, E. Hartwieg, H. R. Horvitz and M. O. Hengartner, 1999 Genetic control of programmed cell death in the *Caenorhabditis elegans* hermaphrodite germline. Development **126** (Suppl.): 1011–1022.

Hayflick, L., 1999 Aging and the genome. Science **283:** 2019. (erratum: Science **285:** 838.)

Hosokawa, H., N. Ishii, H. Ishida, K. Ichimori, H. Nakazawa *et al.*, 1994 Rapid accumulation of fluorescent material with aging in an oxygen-sensitive mutant *mev-1* of *Caenorhabditis elegans*. Mech. Ageing Dev. **74:** 161–170.

Hsin, H., and C. Kenyon, 1999 Signals from the reproductive system regulate the lifespan of *C. elegans*. Nature **399:** 362–366.

Johnson, T. E., J. Cypser, E. de Castro, S. de Castro, S. Henderson *et al.*, 2000 Gerontogenes mediate health and longevity in nematodes through increasing resistance to environmental toxins and stressors. Exp. Gerontol. **35:** 687–694.

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.

Kimble, J. E., and J. G. White, 1981 On the control of germ cell development in *Caenorhabditis elegans*. Dev. Biol. **81:** 208–219.

Kimura, K. D., H. A. Tissenbaum, Y. Liu and G. Ruvkun, 1997 daf-2, an insulin receptor-like gene that regulates longevity and diapause in *Caenorhabditis elegans*. Science **277:** 942–946.

Klass, M. R., 1977 Aging in the nematode *Caenorhabditis elegans*: major biological and environmental factors influencing life span. Mech. Ageing Dev. **6:** 413–429.

Lakowski, B., and S. Hekimi, 1998 The genetics of caloric restriction in *Caenorhabditis elegans*. Proc. Natl. Acad. Sci. USA **95:** 13091–13096.

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.

Lin, K., J. B. Dorman, A. Rodan and C. Kenyon, 1997 *daf-16*: an HNF-3/forkhead family member that can function to double the life-span of *Caenorhabditis elegans*. *Science* **278:** 1319–1322.

Lin, K., H. Hsin, N. Libina and C. Kenyon, 2001 Regulation of the *Caenorhabditis elegans* longevity protein DAF-16 by insulin/IGF-1 and germline signaling. Nat. Genet. **28:** 139–145.

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.

Morris, J. Z., H. A. Tissenbaum and G. Ruvkun, 1996 A phosphatidylinositol-3-OH kinase family member regulating longevity and diapause in *Caenorhabditis elegans*. Nature **382:** 536–539.

Murakami, S., P. M. Tedesco, J. R. Cypser and T. E. Johnson, 2000 Molecular genetic mechanisms of life span manipulation in *Caenorhabditis elegans*. Ann. NY Acad. Sci. **908:** 40–49.

Ogg, S., S. Paradis, S. Gottlieb, G. I. Patterson, L. Lee *et al.*, 1997 The fork head transcription factor DAF-16 transduces insulin-like metabolic and longevity signals in *C. elegans*. Nature **389:** 994–999.

Russell, R. L., and R. I. Seppa, 1987 Genetic and environmental manipulation of aging in *Caenorhabditis elegans*. Basic Life Sci. **42:** 35–48.

Sulston, J. E., and H. R. Horvitz, 1977 Post-embryonic cell lineages of the nematode, *Caenorhabditis elegans*. Dev. Biol. **56:** 110–156.

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

Tatar, M., A. A. Khazaeli and J. W. Curtsinger, 1997 Chaperoning extended life. Nature **390:** 30.

Tissenbaum, H. A., and L. Guarente, 2001 Increased dosage of a *sir-2* gene extends lifespan in *Caenorhabditis elegans*. Nature **410:** 227–230.

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

Walker, G. A., T. M. White, G. McColl, N. L. Jenkins, S. Babich *et al.*, 2001 Heat shock protein accumulation is upregulated in a long-lived mutant of *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. **56:** B281–287.

Wolkow, C. A., K. D. Kimura, M. S. Lee and G. Ruvkun, 2000 Regulation of *C. elegans* life-span by insulinlike signaling in the nervous system. Science **290:** 147–150.

Yang, Y., and D. L. Wilson, 1999 Characterization of a life-extending mutation in *age-2*, a new aging gene in *Caenorhabditis elegans*. J. Gerontol. A Biol. Sci. Med. Sci. **54:** B137–142.

Yang, Y., and D. L. Wilson, 2000 Isolating aging mutants: A novel method yields three strains of the nematode *Caenorhabditis elegans* with extended life spans. Mech. Ageing Dev. **113:** 101–116.

Communicating editor: P. Anderson