# A Genetic Pathway Conferring Life Extension and Resistance to UV Stress in *Caenorhabditis elegans*

**Shin Murakami and Thomas E. Johnson**

*Institute for Behavioral Genetics, University of Colorado at Boulder, Boulder, Colorado 80309-0447*

Manuscript received December 4, 1995
Accepted for publication April 8, 1996

ABSTRACT

A variety of mechanisms have been proposed to explain the extension of adult life span (Age) seen in several mutants in *Caenorhabditis elegans* (*age-1*: an altered aging rate; *daf-2* and *daf-23*: activation of a dauer-specific longevity program; *spe-26*: reduced fertility; *clk-1*: an altered biological clock). Using an assay for ultraviolet (UV) resistance in young adult hermaphrodites (survival after UV irradiation), we observed that all these Age mutants show increased resistance to UV. Moreover, mutations in *daf-16* suppressed the UV resistance as well as the increased longevity of all the Age mutants. In contrast to the multiple mechanisms initially proposed, these results suggest that a single, *daf-16*-dependent pathway, specifies both extended life span and increased UV resistance. The mutations in *daf-16* did not alter the reduced fertility of *spe-26* and interestingly a *daf-16* mutant is more fertile than wild type. We propose that life span and some aspects of stress resistance are jointly negatively regulated by a set of gerontogenes (genes whose alteration causes life extension) in *C. elegans*.

RESISTANCE to environmental stress has been repeatedly hypothesized to play a role in longevity (Kirkwood 1977; Finch 1990; Mullaart *et al.* 1990). In this model, exposure to environmental stresses causes numerous alterations in cellular and extracellular components resulting in deleterious physiological changes that affect longevity. Increased resistance to the stress, either by increased prevention of the initial damage or by increased repair of the deleterious events leads to a lower rate of deleterious physiological change and increased longevity.

Ultraviolet (UV) light is a ubiquitous environmental stress and a well-characterized DNA damaging agent. A major component of UV damage is the formation of pyrimidine dimers, which leads to deleterious somatic mutations (Friedberg 1985). UV also causes alterations in various cellular components through formation of free radicals (Black 1987; Mullaart *et al.* 1990). For example, absorption of UV photic energy can produce many reactive oxygen species (*e.g.*, superoxide anions, hydrogen peroxide and hydroxyl radicals) through an energy exchange reaction (Black 1987). These free radical species, in turn, attack cellular components causing DNA and RNA damage, numerous protein modifications and lipid peroxidation, among other damaging events.

Various altered molecules trigger a variety of cellular responses to correct the damage and alleviate toxic effects. For example, in *Escherichia coli*, DNA lesions induce the SOS response, leading to transcriptional activation of about 15 DNA repair genes. In eukaryotes, exposure to UV light induces a set of diverse genes, for example, more than 80 genes are activated by DNA damage, including DNA repair, replication and growth control in yeast (Baker *et al.* 1985; Ruby and Szostak 1985; Johnston *et al.* 1987; Elledge and Davis 1989; Hartwell and Weinert 1989). In mammals, a variety of stresses, including UV light, heat, and cyclohexamide induce a *Ras*-dependent pathway while another, non-*Ras* dependent pathway mediated by stress-activated protein kinases (SAP kinases: Kyriakis *et al.* 1994) is activated by heat, UV, ATP depletion, ischemia, *etc.* (Woodget *et al.* 1995).

Five life-extension (Age) mutations have been reported in the nematode, *Caenorhabditis elegans*. The *age-1* mutation was originally identified in a screen for longer life (Klass 1983; Friedman and Johnson 1988; Johnson 1990). Two mutations, *daf-2* and *daf-23*, showing constitutive formation of dauer larvae (an arrested developmental stage) at 25° (Riddle *et al.* 1981; Gottlieb and Ruvkun 1994; Larsen *et al.* 1995), extend life span under some conditions (Kenyon *et al.* 1993; Larsen *et al.* 1995). Mutations in *spe-26* are defective in sperm formation and two alleles result in extended life (Van Voorhies 1992). Recently, *clk-1* mutants have been found that show delayed embryonic and larval development, reduced fertility, alterations in a variety of timed events such as pharyngeal pumping and defecation and a longer life span (Wong *et al.* 1995). Thus, Age mutants are comprised of subclasses showing a variety of distinct phenotypes in addition to life extension. Since the Age phenotype of *daf-2* (Kenyon *et al.* 1993), *age-1* and perhaps *daf-23* (Dorman *et al.* 1995; Larsen *et al.* 1995; this study) is suppressed by the *daf-16(m26)*

*Corresponding author:* Shin Murakami, Institute for Behavioral Genetics, Box 447, University of Colorado, Boulder, CO 80309-0447. E-mail: murakams@colorado.edu

mutant, an involvement of the dauer-induction pathway has been suggested. Whether *daf-16* might play a role in other classes of Age mutations has not been addressed.

Several groups have investigated the involvement of stress resistance in the specification of extended life span in *C. elegans*. Oxygen toxicity has been implicated in the specification of life span in long-lived strains of *C. elegans* by the discovery that *age-1* mutants are resistant to several environmental toxins including compounds such as methyl viologen and $H_2O_2$ both of which cause lethality through the generation of reactive oxidants (LARSEN 1993; VANFLETEREN 1993). LITHGOW *et al.* (1994, 1995) have shown that *age-1, daf-2* and *spe-26* have increased thermotolerance and have suggested a possible involvement of heat shock proteins in specifying this increase in resistance.

Here we extend this list of resistant phenotypes observed in *age-1* mutants to include resistance to UV light. We also demonstrate that increased resistance to UV light (Uvr) is a common feature of all Age mutants despite the diversity of primary defects initially associated with these mutants. Moreover, we demonstrate, when there is allelic diversity with some alleles showing long life and others not, that there is complete correspondence between increased adult life span and increased UV resistance. This correlation has not been observed in previous studies of either resistance to ROS or thermotolerance. We also show that both the Age and Uvr phenotypes of the diverse array of five long-life mutants are suppressed by *daf-16*; both the Age and Uvr characteristics of mutations in *age-1, daf-2, daf-23, spe-26* and *clk-1* require normal *daf-16* gene function. Thus, regulation by *daf-16* is not a property only of dauer-specific genes; instead, *daf-16* plays a role in other Age mutants and their UV resistance. Our findings are consistent with the hypothesis that the extended life span of all of these mutants is caused by their increased resistance to a variety of stresses (multi-stress resistance) and supports the concept that *daf-16* represents one component modulating this multi-stress resistance pathway in *C. elegans*.

## MATERIALS AND METHODS

**Strains and media:** *C. elegans* strains were maintained and handled on NGM agar with *E. coli*, OP50 (BRENNER 1974; SULSTON and HODGKIN 1988) as a food source. Assessment of life span was performed using either spotted NGM agar or liquid medium as previously described (FRIEDMAN and JOHNSON 1988). N2 was used as a wild-type control in every life-span assessment. The following mutations were also used in this study. LGI: *daf-16(m26), daf-16(m27)*; LGII: *fer-15(b26), age-1(hx542), age-1(hx546), daf-23(m333), daf-23(mg44)*; LGIII: *clk-1(e2519), clk-1(qm30), daf-2(e1370), daf-4(e1364), daf-7(e1372)*; LGIV: *daf-18(e1375), spe-26(hc138), spe-26(it118)*; LGX: *daf-3(e1376), daf-12(m20)*. All but the *fer-15 age-1* and the double mutant strains were obtained from the Caenorhabditis Genetics Center. Abbreviation of phenotypes are as follows: Age (extension of adult life span), Daf-c (dauer formation constitutive), Daf-d (dauer formation defective), Fer (fertilization defective), Spe (spermatogenesis defective), Itt (increased thermotolerance) and Uvr (ultraviolet radiation resistant).

**Construction of double mutants:** For constructing *age-1 fer-15; daf-16*, homozygous *age-1 fer-15* hermaphrodites were crossed with *daf-16* homozygous males. $F_2$ progeny were allowed to self-fertilize and cloned for several generations until they were homozygous. Since *fer-15* is tightly linked to *age-1* (FRIEDMAN and JOHNSON 1988), the Fer phenotype was used to follow *age-1* during stock construction. We classified the Fer strains into two groups, Daf-d and Non-Daf-d. To determine which group is *age-1 fer-15; daf-16*, the Fer strains were backcrossed with wild-type, N2, (or both parental strains as necessary) and the existence of *age-1* and *daf-16* confirmed by recovering these mutants among the $F_2$ progeny of these crosses. For constructing *age-1; daf-16, age-1 fer-15; daf-16* hermaphrodites were mated with *age-1* males and Non-Fer $F_2$ progeny isolated and backcrossed to N2. Strains segregating both Daf-d and Age were kept. The *spe-26; daf-16* and *clk-1; daf-16* strains were constructed similarly and confirmed by progeny testing after backcross with N2 males. In every case, at least two independent double-mutant strains were isolated and checked. The Daf-c; Daf-d double mutants used are a gift from P. ALBERTS and D. RIDDLE.

**Assessment of life span:** About 25 adult hermaphrodites were picked 3 days after hatching and were transferred daily until the end of egg lay and every 2 or 3 days thereafter until all were dead. An adult was scored as dead when it did not respond to a mechanical stimulus. All life-span assays were performed two or more times at 20° unless described otherwise. For *spe-26* mutants and controls in those experiments, eggs were collected at 16° and subsequently maintained at 20°. All statistical analyses were performed using SPSS 4.0 (SPSS INC. 1990a,b). Mean life span and standard deviations were calculated using the Wilcoxon (Gehan) statistic (LEE 1992) as implemented in the SPSS survival package (NORUSIS 1992).

**UV-resistance assay:** About 20 adult hermaphrodites were picked 3 to 6 days after hatching and were irradiated on NGM agar medium (no *E. coli*) using a germicidal bulb (254 nm) at 10 $J/m^2/min$ in a UV Stratalinker (Stratagene), followed by transfer to NGM plates with *E. coli*. Liquid medium was not used to avoid both absorption and reflection by the liquid, affecting the dosage. All UV-resistance assays were performed at 20°, unless described otherwise. All assays were replicated in a blind manner. An adult was scored as dead when it did not respond to a mechanical stimulus. For *spe-26* and *daf* mutants and their control strains, eggs were collected at 16° and then maintained at 20° until used. For the UV assay at 25.5°, eggs were collected at 16° and maintained at the same temperature for 2 to 4 days followed by incubation at 25.5° for 2 days. For *daf-23*, because of its maternal effect, homozygous Unc hermaphrodites were picked among the $F_1$ progeny of the heterozygous strains (*daf-23/mnC1 dpy-10 unc-52*). For *clk-1*, which shows delayed development (WONG *et al.* 1995), we synchronized worms both by collecting eggs and by collecting fourth-stage larvae (L4). Both methods produced similar results.

Because the length of survival after UV varies between experiments, we have reported both mean survival times and normalized survival times derived by dividing the observed mean survival time by that of N2 in that experiment. Statistical analyses were performed as in the assessment of life span.

Survival immediately after UV irradiation did not differ significantly between *age-1* and N2, consistent with previous observations (HARTMAN *et al.* 1988). In addition, a slight difference between wild-type and *age-1* frequency of pumping was observed after UV irradiation. For example, in one experiment, the percentage of pumping adults was 32.6% in N2

TABLE 1

**Survival after various doses of UV light**

| UV dose ($J/m^2$) | Mean survivals after UV irradiation (days) | |
|---|---|---|
| | Wild type | *age-1(hx546)* |
| 0 | 13.7 ± 2.4 (123) | 23.6 ± 5.7 (94) |
| 1 | 14.7 ± 1.8 (153) | 24.8 ± 8.1 (90) |
| 5 | 10.4 ± 0.4 (84) | 15.7 ± 0.2 (91) |
| 10 | 4.6 ± 0.6 (39) | 6.6 ± 1.1 (42) |
| 20 | 4.1 ± 0.4 (1105) | 5.8 ± 0.2 (672) |
| 30 | 2.5 ± 0.6 (96) | 3.8 ± 1.0 (90) |
| 40 | 2.4 ± 0.4 (115) | 3.1 ± 0.4 (105) |

Table shows a summary of average survival (±SD) of N2 in all experiments. Values in parentheses are number of the worms irradiated. The mean survivals of N2 and *age-1* strains were significantly different in all experiments ($P < 0.0001$).

and 27.9% in *age-1* 1 day after irradiation at 20 $J/m^2$. Pumping rates were also compared (N2: 6.1 per min, *age-1*: 4.1 per min; the adults not pumping were excluded).

**Fertility assay:** Eggs were collected over the entire fecund period at 16° and were incubated either at 16° or at 25° and counted several days later as adults.

## RESULTS

***age-1* mutants are resistant to ultraviolet irradiation:** To elucidate the basis of the increased longevity in *C. elegans*, we tested whether Age mutants show increased resistance to UV light (Uvr). We first tested mutations in *age-1*. Three- and 4-day-old adult hermaphrodites were irradiated over a range of doses (1–40 $J/m^2$). *age-1(hx546)* survived significantly longer than wild type, *N2* ($P < 0.0001$; Table 1). Increased survival of *age-1* was seen both by measuring fraction alive 2 days after irradiation at a variety of doses (5–40 $J/m^2$; Figure 1A) or by monitoring the entire survival curve (Figure 1B; $P < 0.0001$). At fluences < 10 $J/m^2$, we could not exclude differential survival due to the inherent effects of the Age mutants on length of life, so we chose a dose of 20 $J/m^2$ to avoid this complication. In more than 35 different experiments (Table 1) at total fluences of 20 $J/m^2$, the mean survival of wild type was 4.1 ± 0.4 days, whereas *age-1* survived 5.8 ± 0.2 days, 50% longer than wild type. The increased resistance of *age-1(hx546)* over the wild type, N2, at 20 $J/m^2$ was reproducible in more than 50 independent experiments (Tables 1 and 2 and data not shown). We observed immediate death of the adults at doses over 60 $J/m^2$ and have not investigated further. The mean life span of the other possible allele, *age-1(hx542)*, was also longer than wild type at 20 $J/m^2$ (Table 2). Interestingly, *fer-15(b26)* somewhat increases the UV resistance of *age-1(hx546)* (Table 2). We observed no difference in UV resistance of embryos between *age-1* mutants and wild type (data not shown).

**All life-extension (Age) mutants show increased UV resistance:** All other Age mutants: *daf-2, daf-23, daf-28, spe-26*, and *clk-1* were also Uvr (Figure 2, Table 2; $P < 0.0001$). Both *age-1* and *daf-23* mutants were more resistant to UV than the other mutants. The length of survival after UV irradiation was strongly correlated with the amount of increase of adult life span. We observed Uvr in *clk-1(e2519)*, one allele which extends adult life, but not in another allele, *clk-1(qm30)*, which does not show such an extension. Since only *clk-1* alleles show a lengthened cell cycle and longer development, there is no necessary association of the Clk phenotype with Uvr

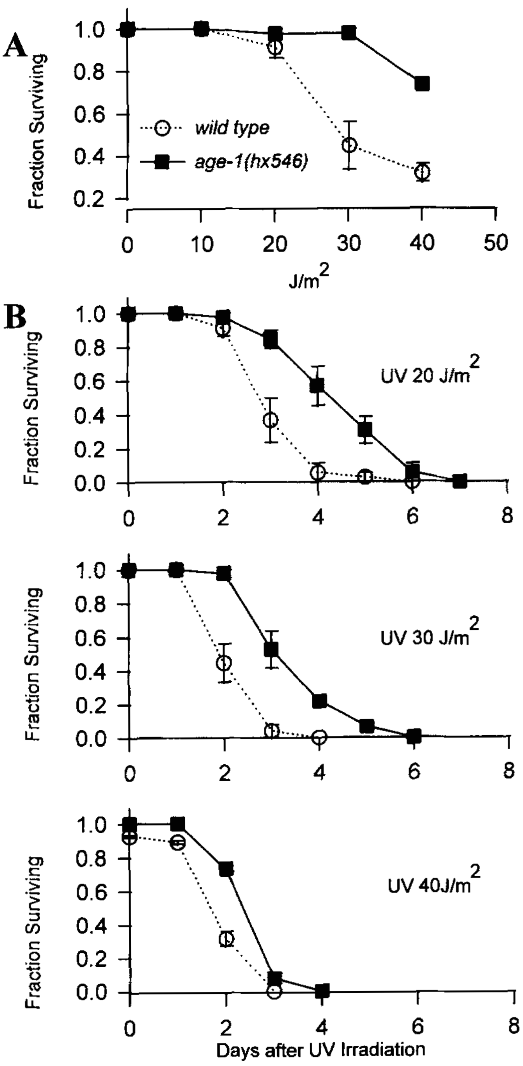


FIGURE 1.—A typical experiment showing survival after UV irradiation. (A) Dose response curve showing fraction of worms surviving (mean ± SEM) 2 days after UV irradiation (0–40 $J/m^2$). The sample sizes of wild type were 40 (0 $J/m^2$), 31 (10 $J/m^2$), 48 (20 $J/m^2$), 52 (30 $J/m^2$) and 47 (40 $J/m^2$). The sample sizes of *age-1* were 37, 35, 45, 45 and 49, respectively. (B) Survival curves after UV irradiation (20, 30 or 40 $J/m^2$; $P < 0.0001$). The mean survivals (±SD) at 20 $J/m^2$ were 3.6 ± 0.7 days (wild type; $n = 54$) and 5.3 ± 1.1 days (*age-1*; $n = 43$), at 30 $J/m^2$ survivals were 2.5 ± 0.7 days (wild type; $n = 48$) and 3.8 ± 1.0 days (*age-1*; $n = 45$), and at 40 $J/m^2$ survivals were 2.1 ± 0.8 days (wild type; $n = 55$) and 2.8 ± 0.6 days (*age-1*; $n = 49$). The survival of *age-1(hx546)* is significantly different from wild type, N2, ($P < 0.0001$; Wilcoxon (Gehan) statistic).

**TABLE 2**

**Survival of Age mutants or of Age *daf-16* double mutants after UV irradiation**

| | | Mutation alone | | | | Mutation with *daf-16*[a] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Phenotypes | Survival after UV (days)[b] | Ratio *vs.* wt[c] | $N$[d] | $P$ *vs.* wt[e] | Survival after UV (days)[b] | Ratio *vs.* wt[c] | $N$[d] | $P$ *vs.* wt[e] | Uvr | Suppressed by *daf-16* |
| 4-day-old worms | | | | | | | | | | | |
| Wild type | | 4.1 ± 0.4 | 1.00 | 1105 | 1.0 | 4.0 ± 0.1 | 0.98 | 603 | 0.573 | Non-Uvr | |
| | | | | | | 4.1 ± 0.3* | 1.00* | 59* | 0.749* | | |
| *age-1(hx542) fer-15(b26)* | Age | 7.2 ± 0.7 | 1.76 | 36 | <0.0001 | 4.4 ± 0.5* | 1.07* | 128* | 0.067* | Uvr | Yes |
| *age-1(hx546)* | Age | 5.8 ± 0.2 | 1.41 | 672 | <0.0001 | 4.0 ± 0.0 | 0.98 | 170 | 0.759 | Uvr | Yes |
| *age-1(hx546) fer-15(b26)* | Age | 6.9 ± 0.3 | 1.68 | 105 | <0.0001 | 4.7 ± 0.4 | 1.15 | 80 | 0.001 | Uvr | Yes |
| *daf-2(e1370)* | Age, Daf-c | 5.4 ± 0.2 | 1.31 | 269 | <0.0001 | 4.2 ± 0.2 | 1.02 | 216 | 0.224 | Uvr | Yes |
| *daf-23(m333)* | Age, Daf-c | 6.0 ± 0.4 | 1.46 | 42 | <0.0001 | 4.3 ± 0.1 | 1.05 | 41 | 0.287 | Uvr | Yes |
| *daf-28(sa191)* | Age, Daf-c | 4.9 ± 0.2 | 1.20 | 295 | <0.0001 | ND | | | | Uvr | ND |
| *spe-26(it118)* | Age, Spe | 5.4 ± 0.3 | 1.32 | 128 | <0.0001 | 4.7 ± 0.4* | 1.15* | 86* | 0.002* | Uvr | Yes |
| *spe-26(hc138)* | Age, Spe | 5.6 ± 0.0 | 1.37 | 273 | <0.0001 | 4.1 ± 0.1 | 1.00 | 404 | 0.614 | Uvr | Yes |
| *let-60(n1046)* | Let | 3.9 ± 0.2 | 0.95 | 63 | 0.096 | ND | | | | Non-Uvr | |
| 6-day-old worms | | | | | | | | | | | |
| Wild type | | 5.4 ± 0.5 | 1.00 | 175 | 1.0 | 5.4 ± 0.5 | 1.00 | 63 | 0.741 | Non-Uvr | |
| | | | | | | 5.1 ± 0.2* | 0.91* | 130* | 0.504 | | |
| *clk-1(e2519)* | Age, Clk | 6.8 ± 0.4 | 1.26 | 87 | <0.0001 | 5.4 ± 0.5 | 1.00 | 127 | 0.705 | Uvr | Yes |
| | | | | | | 5.4 ± 0.5* | 1.00* | 56* | 0.785* | Uvr | Yes |
| *clk-1(qm30)* | Clk | 5.8 ± 0.0 | 1.07 | 88 | 0.089 | ND | | | | Non-Uvr | |
| *age-1(hx546)* | Age | 7.6 ± 0.1 | 1.41 | 108 | <0.0001 | ND | | | | Uvr | ND |
| *age-1(hx542) fer-15(b26)* | Age | 7.3 ± 0.2 | 1.35 | 44 | <0.0001 | ND | | | | Uvr | ND |
| *daf-4(e1364)* | Daf-c | 3.0 ± 0.5 | 0.55 | 20 | <0.0001 | ND | | | | UV sensitive | |
| *daf-7(e1372)* | Daf-c | 5.7 ± 0.1 | 1.06 | 27 | 0.702 | 4.2 ± 0.2 | 0.78 | 60 | <0.0001 | Non-Uvr | |

wt, wild type, N2; ND, not determined.

[a] The mutations shown in the left column were used to construct the double or triple mutations with either of two alleles of *daf-16;* asterisk indicates *daf-16(m27);* others are *daf-16(m26).*

[b] Values are means ± SEM.

[c] The mean survivals normalized by dividing by the mean life span of wild type.

[d] Total number of hermaphrodites used in all experiments.

[e] Probability of survival being different from wild type.

or Age. Moreover, a Daf-c (dauer formation constitutive), gain of function mutation in *daf-28* (Malone and Thomas 1994) extends adult life by 30% (G. J. Lithgow and TEJ, unpublished results). This weakest Age mutant also showed the weakest Uvr. All of these results have been replicated in at least one independent experiment. Finally, we observed a strong correlation between the relative amount of Uvr (Figure 3) and mean life span ($r^2 = 0.80$; $P < 0.001$).

We tested whether Uvr is observed in mutations resistant to other types of environmental stress. Two Non-Age mutations, *daf-4(e1364)* and *daf-7(e1372),* show increased resistance to thermal stress (Lithgow *et al.* 1995) and are Daf-c (Riddle *et al.* 1981; also see Figure 6A). We used both semi-permissive and nonpermissive temperatures (20 and 25.5°, respectively), to determine if any difference in Uvr might result from a partial induction of the dauer pathway by growth at these temperatures. The *daf-7(e1372)* mutant was indistinguishable from wild type for Uvr, and *daf-4(e1364)* was more sensitive than wild type [Table 2; at 25.5°, mean survivals of wild type, *daf-4* and *daf-7,* were 3.6 ± 0.16 days ($n$ = 86), 2.2 ± 0.09 days ($n$ = 22), and 3.6 ± 0.03 days ($n$ = 84), respectively]. The data suggest that Uvr is more strongly correlated with life extension than either increased thermotolerance or dauer constitutiveness at 25.5°. Similar to its effects on Uvr, *fer-15(b26)* appears to enhance the *age-1* life extension about 20% (Table 2).

**daf-16 mutants suppress UV resistance and life extension:** If a common pathway confers increased UV resistance and longer life, mutations in a gene required for the pathway would suppress both phenotypes of all the mutants. We tested this hypothesis by constructing double mutants with *daf-16* mutations that are defective in dauer-formation (Daf-d) and suppress the Daf-c (Riddle *et al.* 1981; Gottlieb and Ruvkun 1994; Larsen *et al.* 1995) and the Age phenotypes of *daf-2* (Kenyon *et al.* 1993) and perhaps of *daf-23* (Larsen *et al.* 1995) and *age-1* (Dorman *et al.* 1995; Larsen *et al.* 1995; this study). The Age phenotype of these mutants was suppressed, as previously reported; surprisingly, the longer-life phenotype of both the *clk-1* and *spe-26* mutant strains was also suppressed by *daf-16* (Figure 4; Table 3). Both mean and maximum life span are not different

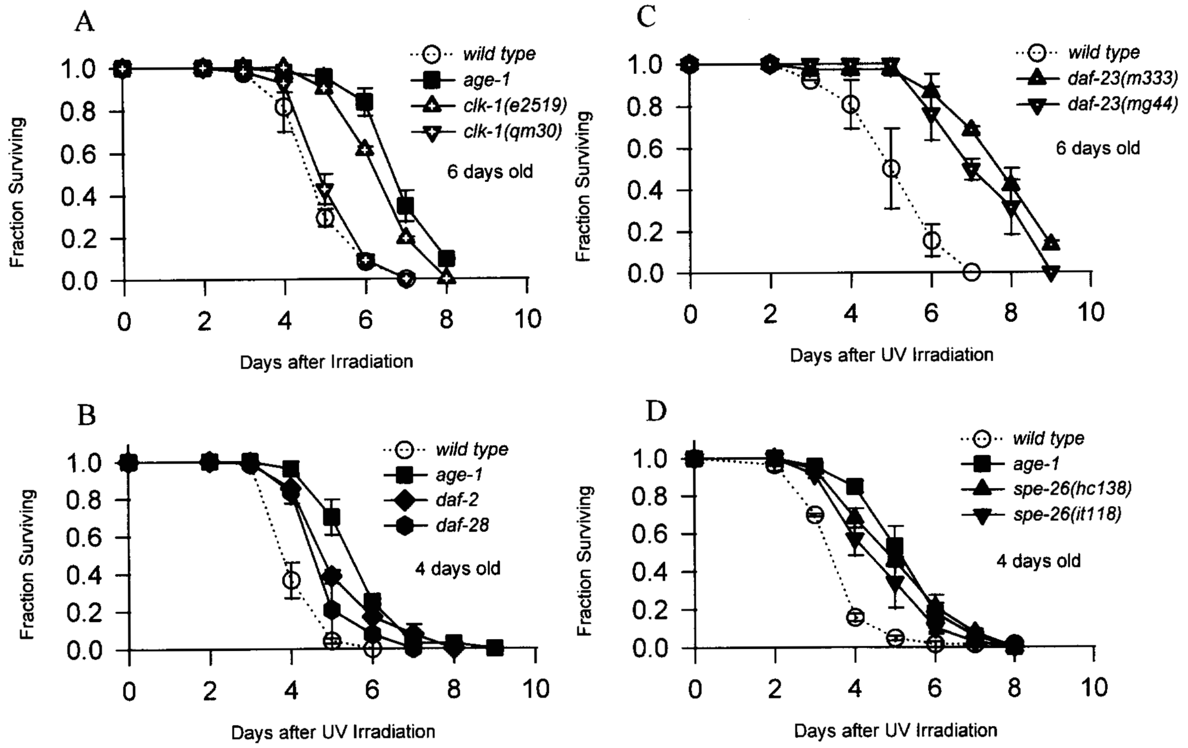

FIGURE 2.—Increased UV resistance of Age mutants in one experiment after UV irradiation at 20 J/m². Survival of the Age strains was significantly longer than wild type, N2 ($p < 0.0001$). The Uvr of the non-Age mutant, *clk-1(qm30)* was not increased. (A) Survival of *age-1(hx546)*, *clk-1(e2519)* and *clk-1(qm30)*. (B) Survival of *age-1(hx546)*, *daf-2(e1370)* and *daf-28(sa191)*. (C) Survival of *daf-23(m333)* and *daf-23(mg44)*. (D) Survival of *age-1(hx546)* and the *hc138* and *it118* alleles of *spe-26*. A summary of mean length of survival after UV over all experiments is shown in Table 2.

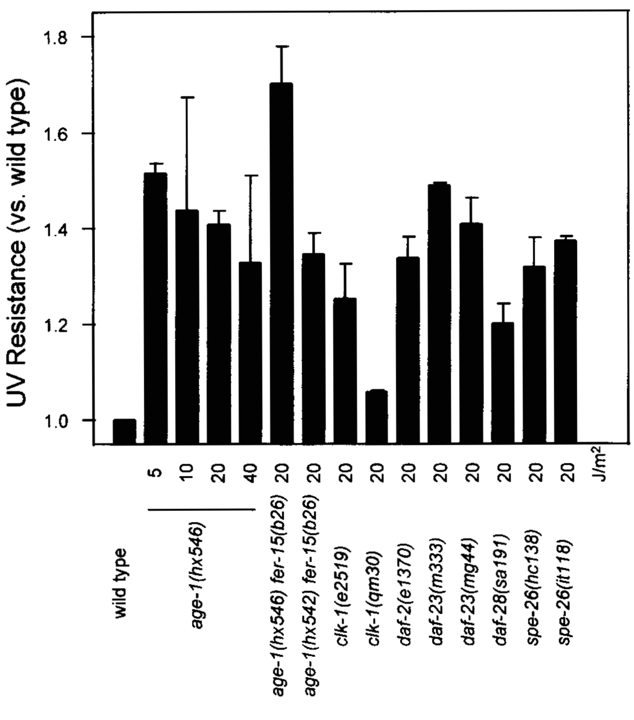

FIGURE 3.—Summary showing the resistance of Age mutants after UV irradiation, normalized by the wild type seen in that experiment (mean ± SEM, $P < 0.0001$). Data are averages of multiple experiments.

from wild type. The increased UV resistance of all the recessive mutants (*age-1, daf-2, daf-23, spe-26* and *clk-1*) was also suppressed by *daf-16* (Figure 5; Table 2). One problem with the Age phenotype is that any life-shortening mutation could appear to "suppress" it by shortening life, while the suppression effect is actually nonspecific, *i.e.*, *daf-16* could be shortening the life of these Age mutants not by blocking the action at the molecular level but by shortening life span independent of any molecular interaction. Previous studies (KENYON *et al.* 1993; DORMAN *et al.* 1995; LARSEN *et al.* 1995) did not address this possibility by demonstrating, for example, that Daf-d mutants earlier in the dauer-formation pathway, may similarly suppress the Age trait of *daf-2, age-1* and *daf-23*. As a control against such nonspecific effects, we tested *daf-3*; Daf-c double mutants. *daf-3* is upstream, perhaps on a separate branch of the pathway, from *daf-2* and downstream of *daf-4* (Figure 6A). *daf-3* has no effect on Uvr itself (Table 4) and in double mutants *daf-3* did not suppress Uvr of *daf-2(e1376)*. *daf-3* even enhances the Uvr effect of *daf-2(e1370)* (Table 4) but did not enhance Uvr in another Daf-c mutant, *daf-7(e1372)*, showing that *daf-3* mutants do not enhance all Daf-c mutants.

Moreover, to avoid the possibility of nonspecific suppression of longer-life phenotype, we have not used Daf-d mutants with shorter life spans. For example, *daf-18*, which is 20% more sensitive to UV than wild type, did

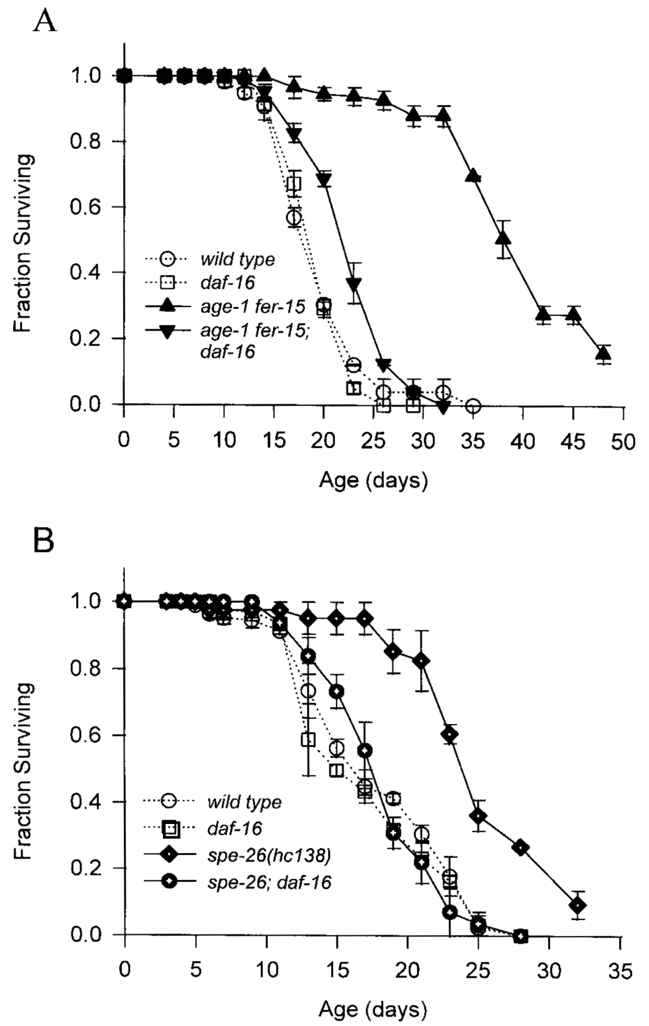


FIGURE 4.—Results from a typical experiment showing that the increased longevity of the Age mutants was suppressed by *daf-16(m26)*. (A) Suppression of life-extension of *age-1* by *daf-16*. The *age-1; daf-16* double-mutant was shorter-lived than *age-1* ($P < 0.0001$). Mean life spans ± SD (days) are shown in Table 3. Assays were on NGM plates. (B) Suppression of life-extension of *spe-26(hc138)*. The *spe-26; daf-16* double-mutant was shorter-lived than the *spe-26* mutant strain ($P < 0.0001$). Assays were in liquid media but similar results were obtained also using NGM plates but the difference between wild type and *spe-26* was much smaller on plates than in liquid.

suppress UV resistance of *daf-2* mutants by 10% and that of *daf-23* mutants by 25% (Table 4). However, it is not clear whether this suppression is specific.

***daf-16* mutants do not suppress the reduced fertility of *spe-26*:** We further tested whether the reduced fertility of *spe-26* might also be suppressed by the *daf-16* mutation. Compared with the wild type, worms carrying *daf-16(m26)* show both a 50% increase in total fertility and increased fertility on days 8, 9 and 10 at the permissive temperature of 16° (Figure 7 and Table 5), and this increase was replicated in an independent assessment of fertility. In contrast to the situation for Uvr, we observed no suppression of the fertility of *spe-26* by *daf-16* (Figure 7 and Table 5). Therefore, reduced fertility alone is not responsible for the increased longevity; instead, long-lived *spe-26* mutant alleles probably cause life-extension by altering a *daf-16*-dependent Uvr pathway. Moreover, a large number of other mutations that reduce fertility show no or little effect on life span (Johnson 1984; Fabian and Johnson 1994; S. A. Duhon and TEJ, unpublished results).

## DISCUSSION

We have demonstrated that a novel phenotype, increased UV resistance (Uvr), is a common feature of the life-extension (Age) mutants. All Age mutations show resistance to this type of environmental stress. All Age mutants are Uvr at a dose of 20 J/m$^2$, including an allele of *clk-1*, which extends adult life (Table 2). In addition, *age-1* shows resistance to UV at various doses from 10 to 40 J/m$^2$. It appears that *daf-16* plays a role in the specification of Uvr and life extension of the Age mutants (*age-1, daf-2, daf-23, spe-26* and *clk-1*), suggesting a common molecular pathway uniting all five Age loci. Moreover, we present data showing that reduced fertility in *spe-26* is not correlated with its life extension.

UV resistance does not appear to correlate with other phenotypes in some of the Age mutants; for example, the Uvr phenotype was seen only in the *e2519* allele of *clk-1*, which shows both Clk (delayed cell cycle and development) and Age phenotype but not in the *qm30* allele, which is only Clk (the life-extension of this allele results primarily from a prolonged developmental period and not from a significant extension of the adult life span). Similarly, the Daf-c mutants, *daf-2, daf-23* and *daf-28*, all of which are Age, are also Uvr, while other Daf-c mutants, such as *daf-4* and *daf-7*, are neither Uvr nor Age. The Age and Uvr phenotypes of the *spe-26* mutant were suppressed by *daf-16* mutants but the reduced fertility trait was not affected. This finding essentially eliminates reduced fertility as a direct cause of the life extension. It is worth noting that *daf-16* mutants that show no increase in UV sensitivity are replicably 50% more fertile than wild type at 16°; at 25°, there is also an increase in fertility but this increase is not as replicable.

**Survival after UV irradiation is a measure of resistance to UV light:** We have interpreted the longer survival of *age-1* after UV irradiation to indicate an underlying resistance to UV at the time of irradiation. Alternatively, the longer survival after UV irradiation of *age-1* and the strains carrying mutations in other gerontogenes could result from the fact that these strains are longer lived. Then, UV irradiation would reduce the remaining period of life. By this model, UV irradiation accelerates aging. Arguing against this interpretation are the following three facts. First, the Uvr phenotype does not result from innate differential mortality rates between the two strains; over the 7 days following irradi-

**TABLE 3**

**Mean life span of various mutants and combinations**

| Expt | *Genotype* | Mean life span (days) | Ratio *vs.* wt[a] | Maximum life span (days)[b] | *N* | *P vs.* wt[c] |
|---|---|---|---|---|---|---|
| 1 | *wild type* | 19.8 ± 4.5 | | 26, 33 | 50 | |
| | *daf-16(m26)* | 19.7 ± 3.1 | 0.99 | 26, 26 | 59 | 0.678 |
| | *age-1(hx546) fer-15(b26)* | 38.3 ± 9.5 | 1.93 | 57, 57 | 39 | <0.0001 |
| | *age-1(hx546) fer-15(b26); daf-16(m26)* | 23.0 ± 4.4 | 1.16 | 33, 33 | 71 | <0.0001[d] |
| 2 | *wild type* | 21.2 ± 8.9 | | 37, 37 | 53 | |
| | *daf-16(m26)* | 20.3 ± 8.0 | 0.95 | 40, 40 | 54 | 0.536 |
| | *age-1(hx546)* | 30.2 ± 11.8 | 1.42 | 50, 56 | 49 | <0.001 |
| | *age-1(hx546); daf-16(m26)* | 20.7 ± 8.0 | 0.97 | 28, 37 | 41 | 0.652 |
| 3 | *wild type* | 19.3 ± 6.4 | | 28, 31 | 47 | |
| | *daf-16(m26)* | 18.9 ± 5.9 | 0.98 | 28, 31 | 60 | 0.473 |
| | *spe-26(hc138)* | 28.0 ± 6.9 | 1.45 | 38, 38 | 33 | <0.0001 |
| | *spe-26(hc138); daf-16(m26)* | 19.9 ± 4.6 | 1.03 | 25, 31 | 41 | 0.675 |
| 4 | *wild type* | 19.8 ± 5.0 | | 24, 27 | 36 | |
| | *daf-16(m26)* | 19.2 ± 4.8 | 0.97 | 24, 24 | 31 | 0.583 |
| | *daf-16(m27)* | 20.7 ± 3.4 | 1.05 | 24, 27 | 36 | 0.705 |
| | *age-1(hx542) fer-15(b26)* | 42.0 ± 7.9 | 2.12 | 45, 45 | 37 | <0.0001 |
| | *clk-1(e2519)* | 33.6 ± 13.0 | 1.70 | 40, 40 | 24 | <0.001 |
| | *spe-26(it118)* | 28.9 ± 12.7 | 1.46 | 40, 40 | 45 | 0.005 |
| | *age-1(hx542) fer-15(b26); daf-16(m27)* | 22.1 ± 4.6 | 1.12 | 27, 27 | 43 | 0.019 |
| | *clk-1(e2519); daf-16(m26)* | 21.4 ± 5.2 | 1.08 | 30, 30 | 46 | 0.047 |
| | *clk-1(e2519); daf-16(m27)* | 20.7 ± 5.6 | 1.05 | 27, 30 | 51 | 0.338 |
| | *spe-26(it118); daf-16(m27)* | 16.7 ± 5.0 | 0.84 | 24, 24 | 39 | 0.008 |

Results of one typical experiment are shown. wt, wild type.

[a] The ratio of mean life span divided by wild type.

[b] Maximum life span in duplicated plates.

[c] Probability of survival being different from wild type.

[d] Probability of survival being different than *age-1 fer-15* was also <0.0001. See Table 2 for details.

ation, there is almost no mortality in nonirradiated controls. The different life expectancies of *age-1* and wild type result from a lower rate of increase in mortality rate over the entire life span of the worm; at either 4 or 6 days of age there is almost no difference in mortality rate (JOHNSON 1990). Second, 6-day-old adults were about 30% more resistant to 20 J/m$^2$ UV light than were 4-day-old adults. If we were monitoring an accelerated aging process, the 6-day-old worms should be more sensitive because they are older and have a shorter remaining life span to begin with. Third, numerous tests of "radiation induced life shortening" have been performed in a variety of species and conclude that "extensive reassessment of all phases of radiation-induced life shortening has suggested that this phenomenon is substantially different from the normal aging process" (TICE and SETLOW 1985; p. 199). Thus, we conclude that it is likely that the measure of survival after UV irradiation is a measure of UV resistance.

**Multi-stress resistance is associated with life extension:** The strong correlation between the Age and Uvr phenotypes is consistent with the theory that the ability to withstand environmental stress is an important component of the aging process (KIRKWOOD 1977; FINCH 1990; MULLAART *et al.* 1990). The fact that all available Age mutants are more resistant to UV stress suggests that this is a good indicator of the major or perhaps the only mechanism that can mediate life-extension. It also points to the fact that the "rate-determining event" specifying the longevity of *C. elegans* may be its ability to withstand some aspects of environmental stress.

It seems probable that the Age mutations confer resistance to a variety of environmental insults (multi-stress resistance) and are not specific to UV radiation. Resistance to reactive oxygen species (ROS) was demonstrated earlier for the canonical allele *age-1(hx546)* (LARSEN 1993; VANFLETEREN 1993). LITHGOW *et al.* (1994, 1995) showed that three Age mutants (*age-1, daf-2* and *spe-26*) show increased thermotolerance (Itt). However, Itt was also observed in two non-Age Daf-c mutants: *daf-4* and *daf-7.* These non-Age mutants do not show multi-stress resistance, because they are not Uvr (this study) nor are they resistant to ROS (S. HONDA and Y. HONDA, personal communication). Since all alleles of *daf-4* and *daf-7* are Ts, it is possible that the Itt of *daf-4* and *daf-7* could result from inducing a "dauer-like" response by the elevated temperature to which these worms were exposed to assay Itt. If so, such an induction shows that some aspects of the dauer pathway can be induced in the adult phase of *C. elegans* in Daf-c mutants that do not confer life extension. Two other Age mutants, *daf-28* and *daf-23,* are also thermotolerant

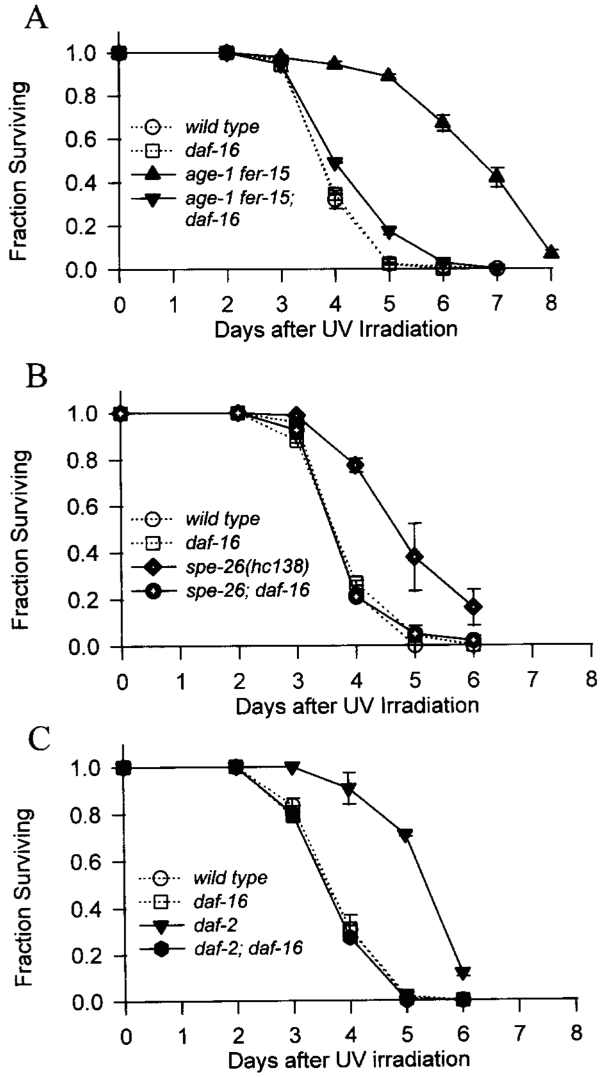


FIGURE 5.—Results from a typical experiment showing that the UV resistance of Age mutants was suppressed by *daf-16(m26)*. Suppression of UV resistance of (A) *age-1*, (B) of *spe-26(hc138)* and (C) of *daf-2*. Mean survivals ± SD (days) were: (A) wild type (4.1 ± 0.8: $n =$ 43), *daf-16* (4.1 ± 0.8: $n =$ 40), *age-1* (5.7 ± 0.6: $n =$ 45), and *age-1; daf-16* (4.6 ± 1.1: $n =$ 40); (B) wild type (4.2 ± 0.5: $n =$ 75), *daf-16* (4.2 ± 0.7: $n =$ 75), *spe-26* (5.3 ± 1.0: $n =$ 91), and *spe-26; daf-16* (4.2 ± 0.6: $n =$ 105); and (C) wild type (4.1 ± 0.7: $n =$ 129), *daf-16* (4.1 ± 0.8: $n =$ 97), *daf-2* (5.7 ± 0.8 : $n =$ 79) and *daf-2; daf-16* (4.1 ± 0.7: $n$ = 104). The double-mutants were significantly less resistant to UV than *age-1, daf-2* or *spe-26*, respectively ($P < 0.0001$). At least two independent double mutants, *age-1; daf-16* or *spe-26; daf-16*, showed similar results. For other Age mutants, see Table 2.

(G. L. LITHGOW and TEJ, unpublished results), and we show here that they are also Uvr. All of our data suggest that the Uvr phenotype is only observed in Age mutants. Similar correlations between Age mutants and resistance to oxidative stress as measured by high oxygen tension, also have been observed (S. HONDA and Y. HONDA, personal communication). Therefore, life extension appears to correlate with multi-stress resistance but not with resistance specific to one type of stress. Moreover, DUHON *et al.* (1996) have shown that several new Age mutants (possibly allelic to *age-1*) are also Uvr, Itt and ROS resistant. These new mutants map to chromosome *2* and fail to complement *age-1(hx546)* for Age, Uvr, Itt and ROS resistance, and are Daf-c at 27°. Recent data show that *age-1(hx546)* is Daf-c at 27° and fails to complement *daf-23* for the Daf-c phenotype (T. INOUE and J. THOMAS, personal communication). However, these complementation tests must be interpreted cautiously because the interactions between these mutants are complex involving rescue of a *daf-23* maternal effect, because a variety of other mutants, for example *unc-4(e130)*, are Daf-c at 27° and because no mutations in the *daf-23* open reading frame have been found in *age-1(hx546)* or *age-1(hx542)* (J. MORRIS, H. TISSENBAUM and G. RUVKUN, personal communication). Thus, the conclusion that *age-1* and *daf-23* are allelic is not yet warranted.

It is surprising that a series of Age mutations in several genes with distinct physiological effects ranging from sperm activation (VARKEY *et al.* 1995) to control of the dauer pathway (RIDDLE *et al.* 1981; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995) show resistance to stresses. The molecular basis of these stress resistances is not yet known. UV light causes both the formation of pyrimidine dimers (FRIEDBERG 1985) and ROS, which attack various cellular and extracellular components (BLACK 1987); UV resistance could be at either or both levels. Heat stress presumably works through protein denaturation by thermal energy and results in the induction of multiple heat shock proteins (for a review see, PARSELL and LINDQUIST 1994) and many of the Age mutants show an elevated accumulation of the small heat shock protein HSP-16 (G. J. LITHGOW and T. E. JOHNSON, unpublished data). The finding that all Age mutations show resistance to multiple distinct stresses suggests the possibility that a common molecular mechanism may regulate the response to all three stressors. Such coordinate regulation is only beginning to be understood.

Increased resistance to one or more forms of environmental stress has also been observed in association with life extension in selected lines of *Drosophila melanogaster* (starvation, SERVICE *et al.* 1985, and oxidative stress resistance, ARKING *et al.* 1991) and in mutations in *Saccharomyces cerevisiae* (resistance to starvation and heat; KENNEDY *et al.* 1995). In *C. elegans*, HARTMAN *et al.* (1995) showed that both methyl viologen and high oxygen tension inhibited development of recombinant inbred strains in a manner proportional to their mean life span, suggesting that some of the genes responsible for the longer life of these strains may also specify resistance to oxygen radicals. Moreover, dietary restriction in several species is associated with increased resistance to oxidative and thermal stress (WEINDRUCH and WALFORD 1988; HEYDARI *et al.* 1993; E. MASORO and S. AUSTAD, unpublished) and with prolonged retention of the

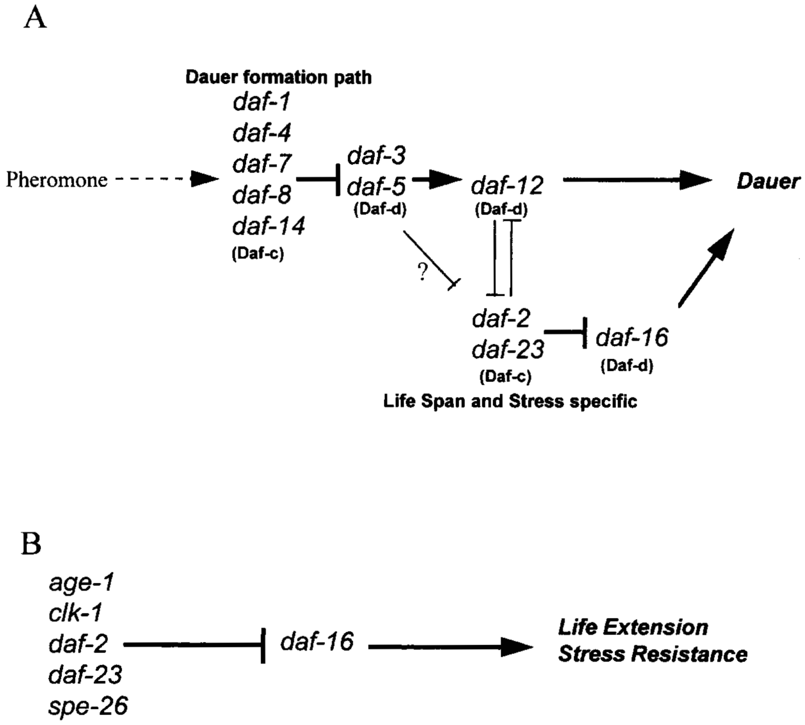


FIGURE 6.—Genetic pathway models. (A) A partial genetic pathway for dauer formation (abstracted from THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995) consistent with the results presented in this study. The lower branch of the pathway mediates both extended life span and increased stress resistance while the upper branch controls dauer formation. All mutants in *daf-4* and *daf-7* are Ts and are also Itt but not Uvr or Age. These facts are consistent with the suggestion that these genes are only used at 25° and also suggest that the Itt phenotype of these mutants (LITHGOW *et al.* 1995) could result from the temperature-sensitive nature of the pathway (THOMAS *et al.* 1993; GOTTLIEB and RUVKUN 1995). (B) A genetic pathway integrating the five Age mutants, consistent with the results of this study.

ability to induce heat shock proteins (HEYDARI *et al.* 1993). Taken as a whole, these results support the hypothesis that increased resistance to environmental stress is necessary for life extension. Whether increased stress resistance is sufficient is still not clear, although preliminary data suggest that increased resistance to ROS may be sufficient in the fruit fly (ORR and SOHAL 1994).

The UV resistance reported here may well not utilize the DNA repair system identified in developmental stages of *C. elegans* (HARTMAN 1984; HARTMAN *et al.* 1989). We did not observe any differential UV sensitivity between wild-type and *age-1* embryos. No alteration in survival after ionizing irradiation was observed in several *rad* mutants selected for radiation-sensitivity during development (JOHNSON and HARTMAN 1988). Moreover, long-lived RI strains showed no increased repair capability in embryos and larvae after treatment with any of three DNA damaging agents: UV light, γ-radiation, and methyl methanesulfonate (HARTMAN *et al.* 1988).

**Life-extension pathway and function of the gerontogenes:** Both life extension and UV resistance of five Age mutants (*age-1, daf-2, daf-23, spe-26* and *clk-1*) were suppressed by mutations in *daf-16*. Previous studies revealed an involvement of *daf-16* in the transduction of the dauer formation signal (RIDDLE *et al.* 1981; ALBERTS and RIDDLE 1988; GOTTLIEB and RUVKUN 1994; LARSEN *et al.* 1995). Recently, two Daf-c mutants, *daf-2* (KENYON *et al.* 1993) and *daf-23* (LARSEN *et al.* 1995), have been shown to be Age under appropriate environmental conditions, and the Age phenotype of both can be blocked

TABLE 4

**The effect of the other Daf-d (dauer formation defective) mutations on UV resistance**

| | | Single mutation | | | | Double mutant with *daf-3*[a] | | | | Double mutant with *daf-18* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genotypes | Phenotypes | Survivals after UV (days) | Ratio *vs.* wt[b] | N[c] | P *vs.* wt[d] | Survivals after UV (days) | Ratio *vs.* wt[b] | N[c] | P *vs.* wt[d] | Survivals after UV (days) | Ratio *vs.* wt[b] | N[c] | P *vs.* wt[d] |
| Wild type | | 5.4 ± 0.5 | 1.0 | 175 | 1.0 | 5.1 ± 0.1 | 0.94 | 66 | 0.055 | 4.5 ± 0.0 | 0.83 | 69 | <0.0001 |
| *daf-2(e1370)* | Age, Daf-c | 6.5 ± 0.5 | 1.2 | 269 | <0.0001 | 8.6 ± 1.2 | 1.6 | 37 | <0.0001 | 5.9 ± 0.1 | 1.1 | 48 | 0.143 |
| *daf-7(e1372)* | Daf-c | 5.7 ± 0.1 | 1.1 | 27 | 0.702 | 5.0 ± 0.2 | 0.93 | 64 | 0.005 | ND | | | |
| *daf-23(m333)* | Age, Daf-c | 8.1 ± 0.4 | 1.5 | 38 | <0.0001 | ND | | | | 6.1 ± 0.4 | 1.1 | 21 | 0.353 |
| *daf-23(mg44)* | Age, Daf-c | 7.7 ± 0.3 | 1.4 | 20 | <0.0001 | ND | | | | ND | | | |

wt, wild type, N2; ND, not determined.
[a] The mutations shown in the left column were used to construct the double or triple mutations with either *daf-3* or *daf-18*.
[b] The mean survivals normalized by dividing by the mean life span of wild type.
[c] Total number of hermaphrodites used in all experiments.
[d] Probability of survival being different from wild type.

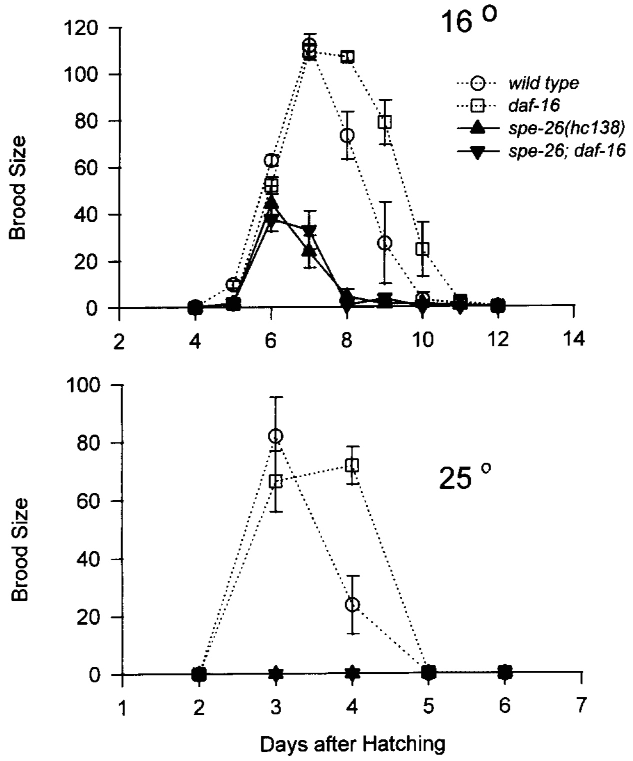


FIGURE 7.—Measurement of fertility. The *daf-16(m26)* mutation had no effect on *spe-26(hc138)* fertility. Self-fertility was measured at 16° Cor at 25°. No difference was observed in the fertility between *spe-26(hc138)* and *spe-26(hc138); daf-16* at either temperature ($0.92 < P < 1.00$). All results were replicated in at least one more independent experiment and similar results were obtained except that for *daf-16* at 25° where the total number of progeny was not significantly different from wild type in one experiment.

by a mutation in *daf-16*. Our results show that the involvement of *daf-16* is not limited to dauer genes, but that *daf-16* plays a role in other types of Age mutants and their UV resistance, as well. Whether *daf-16* mediates the other stress resistance phenotypes is still not clear but is a formal possibility consistent with all of our data as well as the other data cited above.

Our results can be summarized as shown in Figure 6B: (1) the increased longevity of all five disparate mutants (*age-1, daf-2, daf-23, spe-26* and *clk-1*) is mediated by a common, *daf-16*-dependent genetic pathway; (2) this *daf-16*-dependent pathway also confers increased UV resistance. LARSEN *et al.* (1995) also proposed that *daf-18* is involved in the dauer life-extension pathway. However, the *daf-18* mutant is sensitive to UV as well as shorter lived (this study; DORMAN *et al.* 1995; LARSEN *et al.* 1995) and exhibits a swollen midbody region (DORMAN *et al.* 1995) that may be responsible for early deaths even when this mode of death is corrected for by removing animals with visible swelling. Therefore, it is not clear whether *daf-18* is a specific suppresser of *daf-2* or *daf-23* or whether it may be acting nonspecifically. To clarify the role of *daf-18* in stress resistance and life prolongation, *daf-18* mutant alleles that are not UV sensitive and do not shorten life must be isolated and tested.

**TABLE 5**

**Self-fertility of *spe-26* was not suppressed by *daf-16(m26)***

| Genotype | Brood size 16° | Brood size 25° |
|---|---|---|
| *N2* | 258 ± 19 | 102 ± 24 |
| *daf-16(m26)* | 375 ± 38 | 139 ± 28 |
| *spe-26(hc138)* | 67 ± 18 | 0 |
| *spe-26(hc138); daf-16(m26)* | 66 ± 32 | 0 |

Interestingly, *fer-15* and *daf-3* appear to enhance both the UV resistance and life extension of *age-1* and *daf-2*, respectively. The enhancement by *fer-15* is not due to inhibiting death by eggs hatching inside the bodies of adult hermaphrodites (bagging), because we did not include such bagging in our analyses. The *fer-15* mutation alone has little or no effect on life span. Similarly, *daf-3* mutations alone also have little effect on either UV resistance or life span. It is unclear why the enhancement by *fer-15* and by *daf-3* was observed but there may be some genetic interaction between *fer-15* and *age-1* or between *daf-2* and *daf-3*. Similar genetic interactions have been reported previously for *daf-2* and *daf-12* (LARSEN *et al.* 1995).

We propose that a normal function of these gerontogenes (the genes whose alteration cause life extension) is to negatively regulate both life extension and multistress resistance in adults. The gerontogenes may participate in the regulation of various stress-response mechanisms, including molecular-defense or cellular-suicide systems. Interestingly, the signal transduction pathways involving RAS/MAP kinases, SAP kinases or PI-3 kinases have been reported to affect both radiation resistance and cellular immortality (KAEPELLER and CANTLEY 1994; HILL and TREISMAN 1995; JUNG *et al.* 1995; MARSHALL 1995; SAVITSKY *et al.* 1995). Consistent with these facts, the *daf-23* gerontogene is a member of the PI-3 kinase family (G. RUVKUN, personal communication). The causal relation between the developmental defects and increased longevity in the Dafs, *spe-26* and *clk-1* mutants remains unclear but these mutations reveal a group of genes that play a role both in development and longevity. Our findings lend support to the hypothesis that the evolution of longevity involves a trade-off between the level of stress resistance and length of life (KIRKWOOD 1977).

We thank M. HAN, P. HARTMAN, C. LINK, E. STONE and members of the JOHNSON lab, especially P. TEDESCO, for comments. We also thank the Caenorhabditis Genetics Center, D. RIDDLE and P. ALBERTS for the strains. This work was supported by grants from the National Institutes of Health (AG-08322, AG-08761 and AA-00195), by a gift from the Glenn Foundation for Medical Research and by the Japan Society for the Promotion of Science (JSPS). S.M. is a fellow of JSPS, Research Abroad.

## LITERATURE CITED


ALBERTS, P. S., and D. L. RIDDLE, 1988 Mutants of *Caenorhabditis elegans* that form dauer-like larvae. Dev. Biol. **126:** 270–293.

ARKING, R., S. BUCK, A. BERRIOS, S. DWYER AND G. T. BAKER III, 1991 Elevated paraquat resistance can be used as a bioassay for longevity in a genetically based long-lived strain of *Drosophila*. Dev. Genet. **12:** 362–370.

BAKER, D. G., J. M. WHITE and L. H. JOHNSTON, 1985 The nucleotide sequence of the DNA ligase gene (CDC9) from *Saccharomyces cerevisiae*: a gene which is cell-cycle regulated and induced in response to DNA damage. Nucleic Acids Res. **13:** 8323–8337.

BLACK, H. S., 1987 Potential involvement of free radical reactions in ultraviolet light-mediated cutaneous damage. Photochem. Photobiol. **46:** 213–221.

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

DORMAN, J. P., 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., MURAKAMI, S., and JOHNSON, T. E., 1996 Direct isolation of longevity mutants in the nematode *Caenorhabditis elegans*. Dev. Genet. (in press).

ELLEDGE, S. J., and R. W. DAVIS, 1989 DNA damage induction of ribonucleotide reductase. Mol. Cell. Biol. **9:** 4932–4940.

FABIAN, T. J., and T. E. JOHNSON, 1994 Production of age-synchronous mass cultures of *Caenorhabditis elegans*. J. Gerontol. Bio. Sci. **49:** B145-B156.

FINCH, C. E., 1990 *Longevity, Senescence and the Genome*, Chicago Press, Chicago.

FRIEDBERG, E. C., 1985 *DNA Repair*, W. H. Freeman and Company, New York.

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

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

HARTMAN, P. S., 1984 Effects of age and liquid holding on the UV-radiation sensitivities of wild-type and mutant *Caenorhabditis elegans* dauer larvae. Mutat. Res. **132:** 95–100.

HARTMAN, P. S., V. J. SIMPSON, T. E. JOHNSON and D. R. MITCHELL, 1988 Radiation sensitivity and DNA repair in *Caenorhabditis elegans* strains with different mean life spans. Mutat. Res. **208:** 77–82.

HARTMAN, P. S., J. HEVELONE, V. D. DWARAKANATH and D. L. MITCHELL, 1989 Excision repair of UV-induced DNA damage in *Caenorhabditis elegans*. Genetics **122:** 379–385.

HARTMAN, P., E. CHILDRESS and T. BEYER, 1995 Nematode development is inhibited by methyl viologen and high oxygen concentrations at a rate inversely proportional to life span. J. Gerontol. Biol. Sci. **6:** B322-B326.

HARTWELL, L. H., and T. A. WEINERT, 1989 Check points: controls that ensure the order of cell cycle events. Science **246:** 629–634.

HEYDARI, A. R., B. WU, R. TAKAHASHI, R. STRONG and A. RICHARDSON, 1993 Expression of heat shock protein 70 is altered by age and diet at the level of transcription. Mol. Cell. Biol. **13:** 2909–2918.

HILL, C. S., and R. TREISMAN, 1995 Transcriptional regulation by extracellular signals: mechanisms and specificity. Cell **80:** 199–211.

JOHNSON, T. E., 1984 Analysis of the biological basis of aging in the nematode with special emphasis on *Caenorhabditis elegans*, pp. 59–93 in *Invertebrate Models in Aging Research*, edited by T. E. JOHNSON and D. H. MITCHELL. CRC, Boca Raton, FL.

JOHNSON, T. E., 1990 Increased life-span of *age-1* mutants in *Caenorhabditis elegans* and lower gomperz rate of aging. Science **249:** 908–912.

JOHNSON, T. E., and P. S. HARTMAN, 1988 Radiation effects on life span in *Caenorhabditis elegans*. J. Gerontol. Biol. Sci. **43:** B137–141.

JOHNSTON, L. H., J. M. WHITE, A. L. JOHNSON, G. LUCCHINI and P. PLEVANI, 1987 The yeast polomerase I transcript is regulated in both mitotic cell cycle and in meiosis and is also induced after DNA damage. Nucleic Acids Res. **15:** 5017–5030.

JUNG, M., Y. ZANG, S. LEE and A. DRISCHILLO, 1995 Correction of radiation sensivity in ataxia telangiectasia cells by a truncated IκB-α. Science **268:** 1619–1621.

KAPELLER, R., and L. C. CANTLEY, 1994 Phosphatidylinositol 3-kinase. Bioessays **80:** 565–576.

KENNEDY, B. K., N. R. AUSTRIACO, JR., J. ZHANG and GURARENTE L., 1995 Mutation in the silencing gene *SIR4* can delay aging in *S. cerevisiae*. Cell **80:** 485–496.

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

KIRKWOOD, T. B. L., 1977 Evolution of aging. Nature **270:** 301–304.

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

KYRIAKIS, J. M., P. BANERJEE, E. NIKOLAKAKI, T. DAI, E. A. RUBIE, *et al.*, 1994 The stress-activated protein kinase subfamily of c-Jun kinases. Nature **369:** 156–160.

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

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

LEE, E. T., 1992 *Statistical Methods for Survival Data Analysis*. John Wiley and Sons, New York.

LITHGOW, G. J., T. M. WHITE, D. A. HINERFELD and T. E. JOHNSON, 1994 Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. J. Gerontol. Biol. Sci. **49:** B270-B276.

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.

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

MARSHALL, C. J., 1995 Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. Cell **80:** 179–185.

MULLAART, E., P. H. M. LOHMAN, F. BERENDS and J. VIJG, 1990 DNA damage metabolism and aging. Mutat. Res. **237:** 189–210.

NORUSIS, M. J., 1992 *SPSS for Windows. Advanced Statistics. Release 5.* SPSS Inc., Chicago, IL.

PARSELL, D. A., and S. LINDQUIST, 1994 Heat shock proteins and stress tolerance, pp. 457–494 in *The Biology Of Heat Shock Protein And Molecular Chaperones*, edited by R. I. MORIMOTO, A. TISSIERES, C. GEORGOPOULOS. Cold Spring Harbor Laboratory Press, Plainview, NY.

RIDDLE, D. L., M. M. SWANSON and P. S. ALBERT, 1981 Interacting genes in nematode dauer larva formation. Nature **290:** 268–271.

RUBY, S. W., and J. W. SZOSTAK, 1985 Specific *Saccaromyces cerevisiae* genes are expressed in response to DNA-damaging agents. Mol. Cell. Biol. **5:** 75–84.

SAVITSKY, K., A. BAR-SHIRA, S. GILAD, G. ROTMAN, Y. ZIV, *et al.*, 1995 A single ataxia telangiectasia gene with a product similar to PI-3 kinase. Science **268:** 1749–1753.

SERVICE, P. M., E. W. HUCHINSON, M. D. MACKINLEY, and M. R. ROSE, 1985 Resistance to environmental stress in *Drosohila melanogaster* selected for postponed senescence. Physiol. Zool. **58:** 380–389.

SPSS INC., 1990a *SPSS Reference Guide*. SPSS, Inc., Chicago, IL.

SPSS INC., 1990b *SPSS Advanced Statistics Users Guide*. SPSS, Inc., Chicago, IL.

SULSTON, J., and J. HODGKIN, 1988 Methods, pp. 587–606 in *The Nematode Caenorhabditis elegans*, edited by W. B. WOOD. Cold Spring Harbor Laboratory, Plainview, NY.

TICE, R. R., and R. B. SETLOW, 1985 DNA repair and replication in aging organisms and cells, pp. 173–224 in *Handbook of the Biology of Aging*, Ed. 2, edited by. C. E. FINCH and E. L. SCHNEIDER. Van Nostrand Reinhold, New York.

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

Van Voorhies, W. A., 1992 Production of sperm reduces nematode life span. Nature **360:** 456–458.

Varkey, J. P., P. J. Muhlrad, A. N. Minniti, B. Do, and Ward, S. 1995 The *Caenorhabditis elegans spe26* gene is necessary to form spermatids and encodes a protein similar to the actin-associated proteins kelsh and scruin. *Genes Dev.* **9:** 1074–1086.

Weindruch, R., and R. L. Walford, 1988 *The Retardation of Aging and Disease by Dietary Restriction.* Charles C Thomas, Springfield, IL.

Wong, A., P. Boutis, P., and S. Hekimi, 1995 Mutations in *clk-1* gene of *Caenorhabditis elegans* affect developmental and behavioral timing. Genetics **139:** 1247–1259.

Woodget, J. R., J. Avruch and J. M. Kyriakis, 1995 Regulation of nuclear transcription factors by stress signals. Clin. Exp. Pharm. Phys. **22:** 281–283.

Communicating editor: I. Greenwald