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Genetics

Thermotolerance and extended life-span conferred by single-gene mutations and induced by thermal stress

(aging/heat shock response/dauer larvae/stress resistance)

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ABSTRACT **We have discovered that three longevity mutants of the nematode *Caenorhabditis elegans* also exhibit increased intrinsic thermotolerance (Itt) as young adults. Mutation of the *age-1* gene causes not only 65% longer life expectancy but also Itt. The Itt phenotype cosegregates with *age-1*. Long-lived *spe-26* and *daf-2* mutants also exhibit Itt. We investigated the relationship between increased thermotolerance and increased life-span by developing conditions for environmental induction of thermotolerance. Such pretreatments at sublethal temperatures induce significant increases in thermotolerance and small but statistically highly significant increases in life expectancy, consistent with a causal connection between these two traits. Thus, when an animal's resistance to stress is increased, by either genetic or environmental manipulation, we also observe an increase in life expectancy. These results suggest that ability to respond to stress limits the life expectancy of *C. elegans* and might do so in other metazoa as well.**

A mechanistic understanding of the physiology of aging and its relationship to organismic life-span is the subject of considerable speculation. Mutations in any one of several genes in *Caenorhabditis elegans* result in significant extensions of mean and maximum life-span (1–5). The *age-1(hx546)* mutation leads to a 65% increase in mean life-span and a 110% increase in maximum life-span with no detectable effect on development (6) and a marginal effect on fertility (2). Mutations that affect the organism's ability to form dauer larvae (dauers) also extend life-span. The dauer is an alternate developmental stage seen under conditions of poor nutrition or overcrowding (7); dauer-constitutive mutations, such as loss-of-function (lf) temperature-sensitive (ts) mutations in the *daf-2* gene, result in dauer formation at the restrictive temperature under conditions that would not normally cause dauer formation, while dauer-defective lf mutations in other genes cause failure to form normal dauers under any conditions (8). Analysis of these mutations has led to proposals for partially redundant, signal transduction pathways that control entry to and exit from the dauer state (9–12). Kenyon *et al.* (5) demonstrated that *daf-2* mutants had twice the life expectancy of wild type when grown under conditions that allow the formation of normal adults. Life-span extension was suppressed by an lf mutation in the *daf-16* gene. They postulated that mutation of *daf-2* activates a life-span extension mechanism dependent on *daf-16*. This observation has been extended by Larsen *et al.* (13), who showed that the *daf-23(m333)* mutation also confers life-span extension, that the *daf-12(m20)* mutant acts synergistically with *daf-2(e1370)* to extend life-span 4-fold, and that the *daf-16(m26)* mutation is probably epistatic to *daf-2(e1370)* and *daf-12(m20)* mutations. Van Voorhies (4) demonstrated that two mutant alleles of *spe-26(it118* and *hc138)* were long-lived (Age) and postulated that spermatogenesis reduces *C. elegans* life-span.

In addition to Age, mutation of *age-1* results in increased resistance to hydrogen peroxide (14) and paraquat (15), two agents that increase the intracellular levels of reactive oxygen species. The observation that senescent animals exhibit an age-related failure to induce heat shock protein (hsp) genes (16, 17) led us to test the hypothesis that long-lived strains of *C. elegans* may have increased tolerance to thermal stress. We recently reported that *age-1* mutants have significantly increased intrinsic thermotolerance (Itt) as measured by survival at 35°C (18). Mean survival of Age strains is ≈45% longer than that of non-Age strains and, although thermotolerance declines across the life-span, Itt was observed at almost all ages. Here we extend these observations to show that other Age mutants are also resistant to thermal stress: two alleles of *spe-26* and two alleles of *daf-2* result in Itt. Moreover, the Itt phenotype cosegregates with increased longevity (Age) among a series of strains recombinant around the *age-1* locus, suggesting that the two traits (Itt and Age) result from the same mutational event. These observations represent an example of a mutation in a metazoan that increases intrinsic thermotolerance (18).

These observations also led us to test whether a transient exposure to nonlethal thermal stress can also lead to increased thermotolerance; it does. Most surprising is that this single thermal induction also leads to the induction of statistically highly significant increases in mean life-span.

MATERIALS AND METHODS

Media and Strains. Media and standard procedures for the culture of *C. elegans* are described by Sulston and Hodgkin (19). Worms were grown and maintained at 20°C until pretreatment or thermal stress. Life-span was measured as described (2) except that cultures were grown on nutrient growth medium (NGM) agar plates and worms that failed to respond to touch were not cut open to confirm their death but were instead counted as dead. The wild-type Bristol (N2) strain was obtained from David Hirsh (Columbia University). TJ1052 [*age-1(hx546)*II] was isolated from a cross between TJ417 [*dpy-10(e128)fer15(b26ts)age-1(hx546)*] and CB120 [*unc-4(e120)*]. DR63 [*daf-4(m63)*], CB1364 [*daf-4(e1364)*], DR62 [*daf-7(m62)*], CB1372 [*daf-7(e1372)*], BA843 [*spe-26(it118*ts)], and BA821 [*spe-26(hc138*ts)] were obtained from the *Caenorhabditis* Genetics Center at the University of Minnesota.

Age Synchronous Cultures. Populations were established either by moving eggs from a mixed population to a new NGM

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Abbreviations: Itt, increased intrinsic thermotolerance; TS_{50}, time of 50% survival.

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plate or by placing 50–100 gravid adults onto a small, prespotted NGM plate and allowing them to lay for about 8 hr. For strains containing *daf* mutations, normal adults were selected from the large fraction of worms that developed into dauer larvae.

Assay of Thermotolerance. Four-day-old, gravid adult hermaphrodites were placed on small prewarmed (35°C) NGM plates and incubated at 35°C ± 0.8°C. At the indicated times, plates were removed and worms were scored for motility, provoked movement, and pharyngeal pumping. Worms failing to display any of these traits were scored as dead. For the end-point assays, three distinct populations of 20–30 adults were scored at each time point and plates were discarded after scoring. Estimates for time of 50% survival (TS_{50}) were determined following methods for similar pharmacological dose–response assays (20), and statistical comparisons employed a one-tailed Student *t* test. Longitudinal assays were similar except that the same worms were scored repeatedly over the assay period. Mean survival under stress was determined with the Wilcoxon (Gehan) statistic (21). For the single-end-point assays, control wild-type populations were monitored until >50% death was observed; then the fraction surviving was determined for all genotypes. Thermotolerance was induced by exposing 4-day-old adult hermaphrodites to 30°C for 3–24 hr. Worms were then shifted to 35°C for assessment of induced thermotolerance or returned to 20°C for analysis of survival.

RESULTS

***age-1(hx546)* Confers Thermotolerance.** *age-1* mutant and wild-type nematodes were subjected to a 35°C heat shock at 4 days of age (Fig. 1). Two distinct assays were used: dose–response end-point assays and longitudinal assays (see *Materials and Methods*). In the end-point assay (Fig. 1*A*), within 500 min of the start of thermal stress, all wild-type worms were dead, as detected by the loss of touch-provoked movement and pharyngeal pumping. At the same time, under identical conditions, >80% of *age-1* mutant animals were alive, moved spontaneously, and showed normal pharyngeal pumping. The TS_{50} (time of 50% mortality) was significantly different between the *age-1* mutant strain and the wild-type strain ($P < 0.0001$). Highly significant differences were also observed in longitudinal assays (Fig. 1*B*), where thermotolerance was assessed by successively scoring three replicate populations throughout the period of thermal stress until all animals were dead. Mean survival (± SEM) was 539 ± 10 min ($n = 54$) for wild type and 663 ± 17 min ($n = 59$) for *age-1(hx546)*. Survival of *age-1* mutants was significantly longer than that of wild type ($P < 0.0001$). Thermotolerance assays of Age and non-Age strains have been performed 15 times by a more rapid "single-end-point" assay (Table 1). In all but one assay, Age strains survived the heat stress significantly better than did non-Age strains. To ensure that Itt results from a mutation in *age-1*, analysis of life-span and Itt was undertaken on a series of recombinant strains that were used to map the *age-1* gene to a small region of chromosome II and are homozygous for either mutant or wild-type alleles of *age-1* (Fig. 1*C*) (6). The fraction surviving after a 10-hr heat shock varied between 1% and 100%, and mean life-span varied between 18 days and 53 days. Thermotolerance and mean life-span were highly correlated ($r = 0.680, P = 0.001$). All Age strains showed significant increases in intrinsic thermotolerance.

Other Age Mutations Also Confer Itt. To assess the generality of this relationship between Itt and increased life-span we have extended these studies to strains carrying mutant alleles of either the *daf-2* gene or the *spe-26* gene. *daf-2(e1370)* extends mean life-span by up to 100% over the wild-type N2 strain as reported (5); the *e1368* mutant allele of *daf-2* resulted in a 65% extension of life in our laboratory (data not shown).

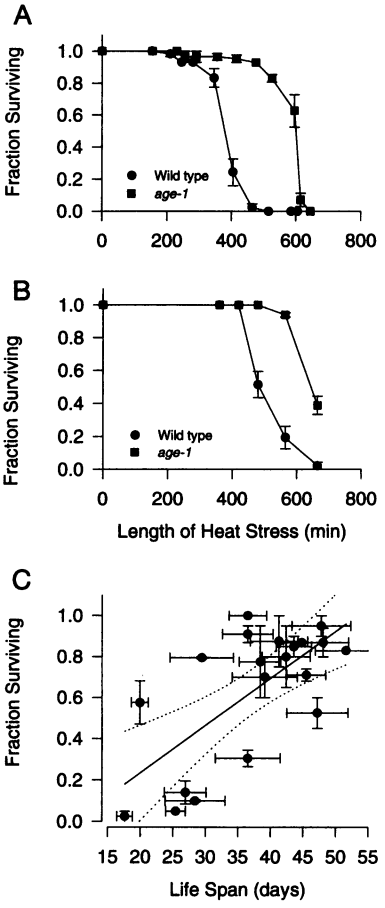

FIG. 1. *age-1(hx546)* confers Itt. (*A*) Survival comparisons of *age-1(hx546)* (TJ1052) and wild-type (N2) hermaphrodites under acute thermal stress at 35°C. TS_{50} of wild type: 382 ± 11 min (SEM); TS_{50} of *age-1(hx546)*: 569 ± 11 min (SEM). The responses of the two genotypes are significantly different ($P < 0.0001$). (*B*) Longitudinal thermotolerance assays. Mean survivals (± SEM): wild type, 539 ± 10 min; *age-1(hx546)*, 663 ± 17 min. Survival of *age-1(hx546)* is significantly longer than that of wild type ($P < 0.0001$). (*C*) Increased thermotolerance (Itt) in long-lived recombinants carrying *age-1(hx546)*. Each point represents the thermotolerance (fraction surviving ± SEM) and life-span (mean ± SEM) for a single recombinant strain. Thermotolerance is highly correlated with mean life-span (multiple correlation coefficient: $r = 0.680$, $P < 0.001$).

Both *daf-2* mutants are Itt (Fig. 2 *A* and *B*) in longitudinal assays. We have also assessed thermotolerance of other dauer-constitutive mutants and find that two alleles of *daf-4* and two alleles of *daf-7* also result in Itt (Fig. 2*C*). Finally, the spermatogenesis-deficient strains carrying either the *spe-26(hc138)* mutation or the *spe-26(it118)* mutation are Itt in longitudinal assays (Fig. 2*D*).

Induction of Thermotolerance Also Extends Life-Span. The correlation between increased thermotolerance and extended life-span in mutant strains could imply a causal relationship between Itt and Age. A test of this hypothesis can be performed by inducing an increase in thermotolerance environmentally. In a variety of organisms increased thermotolerance can be induced by treatment with a mild thermal stress (23). We have found conditions in *C. elegans* that result in induced thermotolerance. Four-day-old adult hermaphrodites of *age-1(hx546)* (TJ1052) and wild type (N2) were pretreated by exposure to 30°C for 3–24 hr. Following this induction, thermotolerance was assessed by shifting to 35°C (Fig. 3 *A–C*). Pretreatment for 3 hr (Fig. 3*A*) significantly induced thermotolerance in N2 ($P < 0.0001$) but not in TJ1052. Thermotolerance of the Age strain was significantly greater than that of

Table 1. End-point assay of survival after thermal stress

Exp.	Length of stress, hr	Fraction surviving: Wild type Mean ± SEM	*n*	*age-1* mutant Mean ± SEM	*n*	*P*
		N2		TJ1052		
1	8	0.04 ± 0.04	60	0.52 ± 0.06	60	<0.001*
2	8	0.00 ± 0.0	60	0.37 ± 0.08	60	<0.0001
3	8	0.00 ± 0.0	111	0.45 ± 0.06	135	<0.0001
4	8	0.50 ± 0.10	30	0.86 ± 0.00	30	<0.121
5	10	0.26 ± 0.04	37	0.95 ± 0.0	40	<0.0001
6	6	0.11 ± 0.06	38	0.61 ± 0.04	39	0.017*
7	8	0.15 ± 0.14	33	0.97 ± 0.03	33	0.028*
8	7.5	0.05 ± 0.00	40	0.75 ± 0.10	37	<0.0001
9	7.8	0.06 ± 0.01	40	0.88 ± 0.02	40	<0.001*
10	13	0.01 ± 0.01	40	0.64 ± 0.05	40	<0.006*
11	10.5	0.06 ± 0.04	60	0.50 ± 0.04	60	<0.001*
12	10	0.19 ± 0.10	60	0.94 ± 0.02	60	<0.008*
		BA713		TJ401		
13	8	0.10 ± 0.03	100	0.88 ± 0.08	100	<0.001*
14	10	0.39 ± 0.22	40	1.00 ± 0.0	40	0.003
15†	6	0.26 ± 0.06	100	0.85 ± 0.00	100	<0.0001

A series of replicate experiments were conducted in which wild-type and *age-1(hx546)* animals, raised at 20°C, were subjected to thermal stress at 35°C. Worms were scored immediately after 35°C heat stress. *P* values are from unpaired Student *t* tests (*) or from χ^2 tests. *n* is the total number of hermaphrodites for each strain scored in two or three subgroups. Four genotypes were employed in these studies: N2 (wild type), TJ1052 [*age-1(hx546)*], BA713 [*fer-15(b26)*], and TJ401 [*fer-15(b26) age-1(hx546)*]. Fraction surviving was scored when the viability of N2 had decreased to 50% or less; thus, the length of the thermal stress varied between 6 and 13 hr among experiments. Combining the significance tests following Sokal and Rohlf (22), Age strains are significantly more resistant to the thermal stress than non-Age strains ($P \ll 0.000001$). The single-end-point assays described here may facilitate screening of large numbers of strains for Itt. However, a more robust assessment of intrinsic thermotolerance results from the determination of mean survival during the course of thermal stress by scoring the same worms at regular intervals throughout as described in the legend to Fig. 1*B*.

†Animals scored 24 hr after heat stress.

wild type before and after pretreatment ($P < 0.0001$). Pretreatments for 6.5 hr (Fig. 3*B*) induced thermotolerance in N2 and TJ1052; thermotolerance of the pretreated Age strain was still significantly greater than that of pretreated wild type ($P < 0.0001$). Pretreatment for 12 hr 30 min (Fig. 3*C*) induced thermotolerance in N2 and TJ1052 to even higher levels and then the thermotolerance of the pretreated Age strain was not significantly different from that of the pretreated wild-type strain.

The survival of wild-type animals at 20°C was also increased by these pretreatments (Fig. 3 *D–G*). In the first experiment, two of three pretreatments led to significant increases in wild-type life-span of >25%. In the second experiment, all

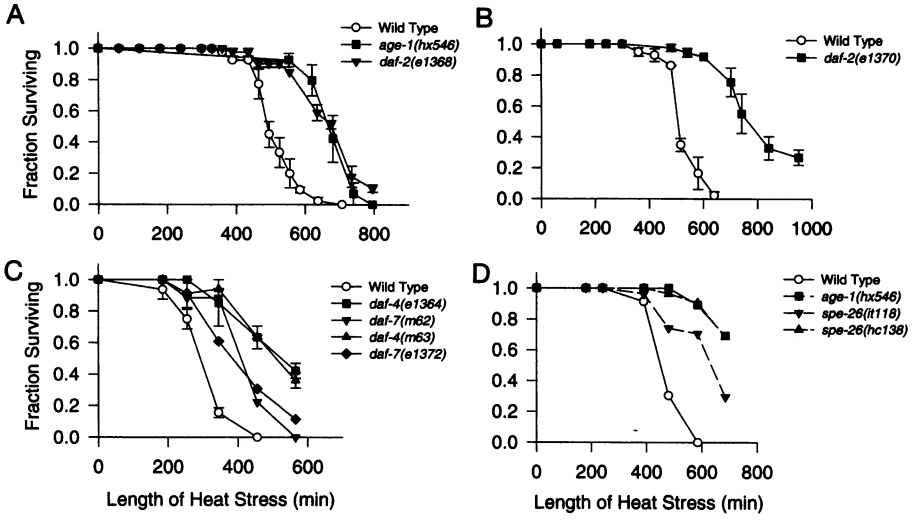

FIG. 2. Longitudinal thermotolerance assays demonstrating that other mutations of *C. elegans* that extend life also exhibit Itt at 35°C. (*A*) Mean survivals (± SEM): wild type, 523 ± 11 min; *daf-2(e1368)*, 668 ± 17 min; *age-1(hx546)*, 693 ± 9 min. Mutants were significantly different from wild type [$P < 0.0001$, Wilcoxon (Gehan) statistic (21)]. (*B*) Mean survivals (± SEM): wild type, 535 ± 10 min; *daf-2(e1370)*, 789 ± 21 min. Wild type and *daf-2(e1370)* differ significantly in response to thermal stress ($P < 0.0001$). (*C*) Mean survivals (± SEM): wild type, 444 ± 14 min; *age-1(hx546)*, 537 ± 12 min; *daf-4(m63)*, 589 ± 14 min; *daf-4(e1364)*, 594 ± 12 min; *daf-7(m62)*, 563 ± 16 min; *daf-7(e1372)*, 541 ± 20 min. Both *daf-4* mutant strains and both *daf-7* mutant strains exhibited Itt ($P < 0.0001$). (*D*) Mean survivals (± SEM): wild type, 508 ± 15 min; *age-1(hx546)*, 678 ± 4.34 min; *spe-26(it118*ts), 600 ± 18 min; *spe-26(hc138*ts), 676 ± 6 min. Both *spe-26* mutant strains exhibited Itt ($P < 0.001$).

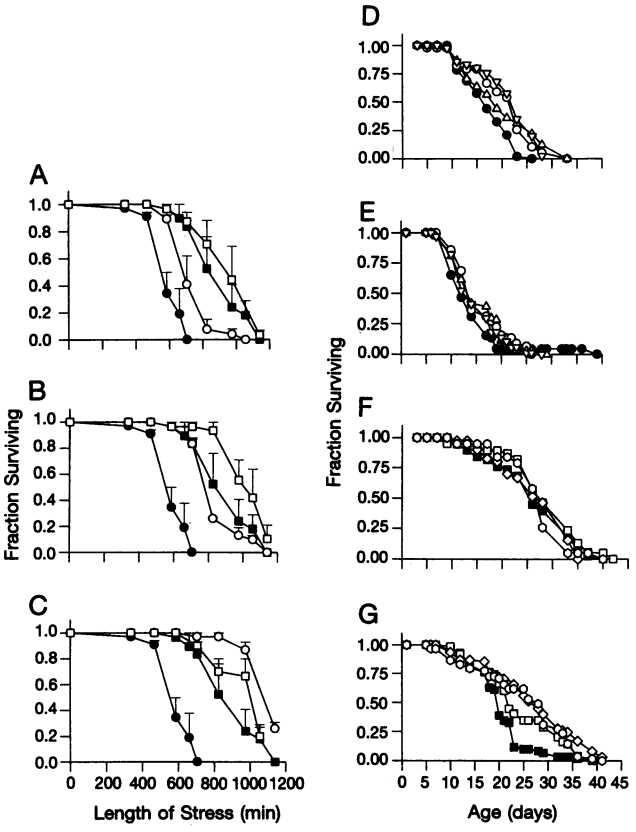

FIG. 3. Pretreatment induces thermotolerance and life-span extension. Young adult hermaphrodite *age-1(hx546)* (TJ1052; squares) and wild-type (N2; circles) animals were pretreated (open symbols) by exposure to 30°C for 3–24 hr or maintained at 20°C (filled symbols). Animals were then shifted to 35°C for assessment of induced thermotolerance (*A–C*) or returned to 20°C for survival analysis (*D–G*). (*A*) Thermotolerance after pretreatment for 3 hr for N2 gave 754 ± 21 hr (mean ± SEM) and for TJ1052 gave 986 ± 31 min. N2 was significantly more thermotolerant than the controls ($P < 0.0001$). (*B*) Thermotolerance after pretreatment for 6 hr 45 min for N2 gave 851 ± 25 min and for TJ1052 gave 1034 ± 22 min. Both pretreated populations were significantly more thermotolerant than the controls (N2, $P < 0.0001$; TJ1052, $P < 0.002$). (*C*) Thermotolerance after pretreatment for 12 hr 30 min for N2 gave 1097 ± 17 min and for TJ1052 gave 1018 ± 31 min (N2, $P < 0.0001$; TJ1052, $P < 0.006$). (*D*) Life-spans (mean days ± SD) of N2 after pretreatments at 30°C for 4.5 hr (open circles), 8 hr (triangles), or 12 hr (inverted triangles). Controls (no pretreatment), 19.2 ± 4.6 ($n = 43$); 4.5-hr pretreatment, 22.1 ± 6.6 ($P = 0.015$, $n = 40$); 8-hr pretreatment, 21.2 ± 6.4 [*P* not significant (NS); $n = 42$]; 12-hr pretreatment, 24.5 ± 6.8 ($P < 0.0001$, $n = 41$). (*E*) Life-spans of N2 after pretreatments at 30°C for 6 hr (open circles), 12 hr (triangles), and 24 hr (inverted triangles). Controls (no pretreatment), 13.2 ± 4.5; 6-hr pretreatment, 14.7 ± 4.3 ($P = 0.021$, $n = 51$); 12-hr pretreatment, 15.2 ± 4.8 ($P = 0.009$, $n = 60$); 24-hr pretreatment, 14.5 ± 4.5 days ($P = 0.046$, $n = 62$). (*F*) Life-spans of TJ1052 after pretreatments at 30°C for 4.5 hr (open squares), 8 hr (diamonds), or 12 hr (hexagons). Control, 28.6 ± 8.3 ($n = 35$); 4.5-hr pretreatment, 30.6 ± 7.6 days (NS, $n = 39$); 8-hr pretreatment, 29.0 ± 7.3 days (NS, $n = 39$); 12-hr pretreatment, 29.2 ± 6.3 days (NS, $n = 19$). (*G*) Life-spans of TJ1052 after pretreatments at 30°C for 6 hr (open squares), 12 hr (diamonds), and 24 hr (hexagons). Control, 19.7 ± 6.3 ($n = 41$); 6-hr pretreatment, 23.4 ± 7.8 ($P = 0.023$, $n = 49$); 12-hr pretreatment, 26.1 ± 9.4 ($P < 0.0005$, $n = 58$); 24-hr pretreatment, 25.1 ± 10.2 days ($P = 0.012$, $n = 55$). Combining probabilities of independent tests of significance (22) shows that pretreatments significantly extended life-spans of N2 ($P < 0.001$) and TJ1052 ($P < 0.001$).

pretreatments led to a statistically significant increase in the life-span of wild-type animals. For the *age-1(hx546)* animals, two of three pretreatments resulted in increased mean life-span in the first experiment but none reached statistical significance. In the second experiment, all pretreatments resulted in significant increases of mean life-span of up to 30%. In summary, five of the six wild-type cohorts and three of six *age-1(hx546)* cohorts showed significant ($P < 0.05$) increases of life-span after pretreatments and all pretreatments caused increased mean life-spans. Combining probabilities of independent tests of significance (22) shows that the same pretreatment regimen that induces increased thermotolerance also significantly extends the life-span of N2 by 14% ($P < 0.001$) and TJ1052 by 16% ($P < 0.001$). The finding that an increase in mean life expectancy results from an induction of thermotolerance is consistent with a causal relationship between these two events.

DISCUSSION

We have investigated a stress response phenotype associated with longevity in *C. elegans* and discovered that at least five mutational events in three genes leading to increased life-span also confer Itt. *age-1(hx546)*, the first *C. elegans* Age mutation identified (2), causes a reduction in the acceleration of mortality rate with increasing age (3). We demonstrate that *age-1(hx546)* confers resistance to lethal thermal stress. We therefore conclude that the wild-type *age-1* gene product negatively influences not only longevity but also intrinsic thermotolerance. Moreover, since Itt can be assessed by a relatively quick and highly reproducible biological assay that is carried out on young adults, this assay is a useful addition to methods for assessing the Age phenotype.

Two mutant alleles of *spe-26(it118*ts and *hc138*ts) and two mutant alleles of *daf-2(e1368* and *e1370)* also confer Age and Itt. We have assessed thermotolerance of other constitutive, dauer-forming mutations and find that two alleles of *daf-4* and two alleles of *daf-7* also confer Itt. The *daf-4* gene encodes a homologue of transforming growth factor β receptor protein kinase (24) and, like the *daf-7* gene product, forms part of a signal transduction pathway that appears to be independent of and parallel to the pathway containing the *daf-2* gene product (9–11). Our results suggest that both arms of the partially redundant dauer-formation pathway are involved in the regulation of thermotolerance. The increased thermotolerance of these dauer-constitutive mutants is consistent with a model in which a mechanism leading to dauer thermotolerance (25) is induced in adults. Transgenic strains carrying extra copies of certain heat shock protein genes can lead to increased thermotolerance in other systems (26, 27), but, to our knowledge, there is no previous report of single-gene mutations leading to increased thermotolerance in any metazoan species.

The association between Itt and Age in all three extant Age mutants raises the possibility that enhanced tolerance to thermal stress is mechanistically linked to the extension of life-span. To pursue this correlation we devised methods for inducing thermotolerance by means of a nonlethal thermal stress of young adults. Populations treated in this fashion showed increased thermotolerance as young adults and displayed a modest but highly significant increase in mean life-span averaging 14% ± 3% (mean ± SEM) over six experiments in the wild type and 16% ± 5% in an *age-1* mutant. Recent experiments of Khazaeli *et al.* (28) could also be interpreted to show an increased life-span after a semilethal thermal stress.

Our results are reminiscent of the larger increases in life-span obtained by Maynard Smith with female *Drosophila subobscura* after transient exposure to elevated temperature (29, 30). Maynard Smith proposed that the enhanced life-span was a consequence of a reduction in egg laying, but another explanation, that of induced thermotolerance, should also be considered. In *C. elegans*, progeny production is reduced by mild thermal stress (18), but neither the Age nor the Itt phenotypes are dependent on progeny production (18), as shown by the following observations. (*i*) A sterilized *age-1*

strain remains significantly more thermotolerant than a sterile, non-Age strain (18). (*ii*) Among recombinant strains segregating *age-1(hx546)* and *fer-15(b26)*, a sperm-defective temperature-sensitive mutation causing a significantly reduced brood size at 20°C, there was no effect of *fer-15(b26)* on Itt and there was no significant correlation between the mean number of progeny produced by each strain and its thermotolerance (G.J.L., unpublished data).

We have demonstrated that increased thermotolerance in *C. elegans* results from mutations in the *age-1, daf-2, daf-4, daf-7,* and *spe-26* genes and we have demonstrated a correlation between environmentally induced thermotolerance and mean life-span. A number of previous observations influence our interpretation of these experiments. We are intrigued by similarities between dauer larvae and *age-1* mutant strains. *age-1* mutants overexpress superoxide dismutase (SOD) and catalase late in life and are resistant to oxidative stress (14, 15), while dauer larvae also exhibit a high specific activity for SOD and are resistant to thermal stress (25, 31). We postulate that the processes that confer increased thermotolerance in dauers also function in *daf-2, daf-4, daf-7, spe-26,* and *age-1* mutant adults. Thermotolerance is in large part determined by the synthesis of heat shock proteins (23). Since genes regulating response to oxidative stress are, in many circumstances, coordinately expressed with heat shock protein genes (32, 33), we postulate that Itt and Age phenotypes may result from a single physiological alteration that enhances several forms of stress resistance. Consistent with this notion is the observation that *age-1, daf-2,* and *spe-26* mutant strains have increased resistence to ultraviolet radiation (S. Murakami and T.E.J., unpublished data). As no long-lived alleles of *daf-4* or *daf-7* have yet been identified (5, 13), we conclude that Itt is necessary but not sufficient for the Age phenotype.

Kenyon *et al.* (5) suggested that dauers express a regulated mechanism of life-span extension, which is activated in *daf-2* mutant adults. Although evolutionary theory is inconsistent with the evolution of such a mechanism by direct selection on adult life-span (34), a mechanism that extends life-span has been activated in Age mutants or worms subject to mild thermal stress. Our results are consistent with a model in which the coordinate overexpression of antioxidant-enzyme genes (14, 15) and heat shock protein genes leads to an extended life-span. Also consistent with this model is the recent observation of a causal relationship between the expression of antioxidant-enzyme genes and age-specific mortality rates in *Drosophila* (35) and our earlier observations of increased hermaphrodite life-span as a result of exposure to ^{137}Cs irradiation (36).

Two important predictions can be made from these results: (*i*) a subset of mutant strains selected for Itt should exhibit extended life-span and (*ii*) genetic or environmental manipulations that result in overexpression of stress protein genes should extend life. These predictions must be tested to establish a causal relationship between stress response and longevity. It has been noted that caloric restriction, which dramatically increases rodent life-span, also partially compensates for the age-related decline of induction of heat shock protein genes (37). These observations may imply that since stress-response genes are conserved in diverse species (38), the relationship we have demonstrated between stress resistance and life-span in *C. elegans* could also be found in mammals.

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