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Genetics

Aging and resistance to oxidative damage in *Caenorhabditis elegans*

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ABSTRACT **The dauer larva state and the *age-1* mutation, both of which extend life-span in *Caenorhabditis elegans*, were tested for hyperresistance to cellular damage that may be relevant to aging. The *age-1* strain TJ401 displayed hyperresistance to oxidative stress relative to its parental strain. The activities of two enzymes that protect cells from oxidative damage, superoxide dismutase (SOD) and catalase, showed an age-dependent increase in mutant animals, which was not seen in the parental strain. These increases in activities paralleled the time course of the hyperresistance. The results are consistent with the *age-1* gene product functioning as a negative regulator of SOD and catalase activities. In wild-type and *age-1* dauer larvae, elevated levels of SOD activity, but not of catalase activity, were present when compared with young adults. The common increase in SOD activity prompted cloning the *C. elegans* Cu/Zn SOD gene. Its position on the physical map of the genome was in the region to which the *age-1* gene has been genetically mapped, but it is unlikely that a mutation at the SOD locus confers the Age phenotype. Results support the free radical theory of aging by suggesting that the increased resistance to oxidative stress may be among the causes of increased longevity in both strain TJ401 and in the dauer larva.**

Life-span is a species-specific attribute that is determined by the interaction of the environment with the genetic predisposition of the individual. There are a variety of theories on the mechanisms of aging (1–3). This work tested one mechanism of aging based on the assumption that the level of cellular defense and repair is sufficient yet unlikely to provide the maximum obtainable cellular protection. This assumption is derived from the premise that each species has evolved a unique repertoire of cellular defense and repair mechanisms to maintain the soma (non-germ-cell tissues) while striving to maximize the success of its reproductive strategy. If accumulated cellular damage causes aging, then an increased life-span may result from improved defense and repair capacities (although it may be at the expense of reproductive capacity). It is feasible to test this hypothesis for specific types of damage potentially relevant to aging because protective cellular mechanisms such as DNA repair, heat shock response, and protection against free radical species have been extensively characterized (4–6).

The experiments reported here test the prediction that increased resistance to oxidative damage could lead to increased life-span. The free radical theory of aging postulates that free radical reactions are a basic cause of aging, and these reactions are influenced by genetic and environmental factors (7). The free radical theory is generally supported by correlations between the life-span of various species and either their metabolic rates or their incurred level of oxidative damage (8, 9). In addition, correlations between decreased life-span and decreased levels of enzymes involved in protection from oxidative damage have been documented (10, 11).

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The *Caenorhabditis elegans* model system offers several distinct advantages. This nematode has a rapid life cycle and short life-span; also its pattern of senescence has been characterized (12, 13). Superoxide dismutase (SOD) and, to a lesser extent, catalase have been shown to be crucial for defense against oxygen toxicity in *C. elegans* (14). These two enzymes detoxify the reactive compounds superoxide and hydrogen peroxide. SOD converts superoxide into water and hydrogen peroxide, and catalase produces water and oxygen from hydrogen peroxide.

Increased life-span has been demonstrated in *C. elegans* under two conditions: (*i*) the *age-1* mutation and (*ii*) the dauer larva, which is a developmentally arrested dispersal stage formed under adverse environmental conditions (15). Dauer larvae consume energy but do not feed, and their metabolism differs markedly from all other stages (16, 17). Although the life-span of *C. elegans* is normally ≈2½ weeks, dauer larvae can survive for months with no effect on post-dauer life-span. The dauer larva has thus been described as nonaging (18). The recessive *age-1* mutation increases the mean life-span 65% and increases the maximum life-span 110% (19–21). Mutant traits are well-documented with regard to fertility, movement, rate of development, and rate of aging (20–22). However, function of the *age-1* gene product remains unknown, as neither molecular nor biochemical alterations have been reported.

To determine whether there were biochemical alterations consistent with increased defense or repair mechanisms for oxidative damage, resistance to hydrogen peroxide and SOD and catalase activities were examined in the *age-1* strain TJ401 and in dauer larvae. TJ401 animals are hyperresistant to hydrogen peroxide, and they have increased SOD and catalase activities. Thus, one function of *age-1*(+) may be to negatively regulate SOD and catalase. The catalase activity in dauer larvae was not significantly different from that of young adults, but the SOD activity in dauer larvae was 5-fold higher than in young adults. The increase in dauer larvae is *age-1* independent. The gene encoding the *C. elegans* Cu/Zn SOD was cloned.† The physical map position of the Cu/Zn SOD gene, *sod-1*, is within the interval to which *age-1* has been genetically mapped. But, *age-1* is most likely distinct from *sod-1* based on a recently refined genetic map position (T. Johnson, personal communication). Taken together, the hyperresistance and biochemical results on strain TJ401 and dauer larvae support the free radical theory of aging.

MATERIALS AND METHODS

Growth Conditions and Strains Used. Nematodes were propagated according to standard conditions (23), except that in the liquid medium, water-soluble polyoxyethanyl-cholesteryl sebacate (Sigma) was substituted for cholesterol to reduce crystal formation. The growth rate was not altered

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†The sequence reported in this paper has been deposited in the GenBank data base (accession no. L20135).

by this change. In general, the nematodes were fed *Escherichia coli* OP50. The nematodes for the catalase study were fed a catalase-deficient strain of *E. coli*, PQ65, Δ*oxyR4 katG17*::Tn *10* Δ*katE2* (24). The nematodes for the SOD study were fed a SOD-deficient strain of *E. coli*, GC4468, Φ (*sodA–lacZ*)49 Φ (*sodB-kan*)1-Δ_2 Cm^r Km^r (25).

The *C. elegans* strains used in this study were N2 (var. Bristol), DH26 *fer-15*(*b26*ts), TJ401 *fer-15*(*b26*ts) *age-1*(*hx546*), and BA15 *fer-15*(*hc15*ts). In the original screen for long-lived mutants, DH26 animals were mutagenized (19). The original isolates were backcrossed once to strain N2 to yield various *fer-15 age-1* strains (20, 21). It is likely that the strains contain the same allele of *age-1* because the independence of the original isolates is uncertain (19–22). Strain TJ401 was used because it has been characterized in the greatest detail.

Hydrogen Peroxide Resistance Assays. Synchronous large-scale liquid cultures were initiated from eggs that had been isolated by hypochlorite treatment of gravid adults (23). Aliquots were removed from the culture for the resistance assay. The animals were washed on a 37-μm Nitex screen (using a Hoefer filtration apparatus), resuspended in M9 buffer (23), and distributed into wells of a 96-well microtiter plate with an average of 35 animals per well. At time zero, serially diluted hydrogen peroxide was added to the wells. A set consisted of 12 concentrations from 0 to 44 mM hydrogen peroxide in a final volume of 50 μl. To determine each LD_{50}, four sets of each strain were assayed. The total number of animals and the number of surviving animals were scored for each well after 2 hr. An animal was considered alive when body movement was observed in response to a tap on the plate. Nonswimming animals were scored as alive when pharyngeal pumping was observed. In initial studies catalase was added after the 2-hr incubation with hydrogen peroxide, and the plates were scored at 2 and 24 hr. The number of dead and alive animals was essentially unchanged after 24 hr, so the 2-hr assessment was considered accurate and was used for all assays.

SOD and Catalase Assays as a Function of Age. On the day of the assay, animals from an aliquot of the culture were washed free of bacteria and debris by sucrose flotation (23) followed by washing, as for the bioassay. Lysates were prepared by sonication of the worm suspension intermittently for 10 sec three times with a microtip at 90% duty cycle and output control setting 4.5 (Heat Systems/Ultrasonics). Rate assays were done as described for catalase (26) and total SOD (27), except leupeptin, aprotinin, chymostatin, pepstatin, and antipain were added to a final concentration of 40 μM, just before sonication.

Dauer Larva SOD and Catalase Assays. Densely populated cultures, initiated with synchronized larvae, were harvested after 3 weeks. The dauer larvae were purified by a 2-hr treatment with 1% SDS (15). The dauer larvae were rinsed three times in M9 buffer and then processed as described above for the SOD and catalase assays.

Isolation of the *C. elegans* Cu/Zn SOD cDNA. Degenerate oligonucleotides were used to screen a λgt10 cDNA library for plaques that hybridized to both oligonucleotides (28). The oligonucleotides used were derived from the highly conserved amino acid sequences GPHFNP and DDLGKG from eukaryotic Cu/Zn SODs and were 32- and 48-fold degenerate, respectively. These oligonucleotides were specific for the Cu/Zn SOD and should not detect the Mn SOD. The 5′-end of the cDNA was isolated by rapid amplification of cDNA ends (29). Standard protocols for recombinant DNA manipulations were followed (30) to generate subclones of the cDNA, using unique restriction sites in the cDNA. Sequencing of the clones was performed by using the Sequenase version 2.0 kit (United States Biochemical). Each strand was sequenced, and the coverage was at least three times per base pair. The chromosomal location of the Cu/Zn SOD cDNA was determined by hybridization to a filter that contained an ordered array of yeast artificial chromosome clones of the *C. elegans* genome (31).

RESULTS

***age-1* Mutation Confers Resistance to Hydrogen Peroxide.** The sensitivity of *C. elegans* to oxidative stress was tested with an acute assay. Hydrogen peroxide was chosen as the stress-inducing agent because it is a small molecule, easy to administer, and can cause oxidative damage directly, without enzymatic conversion (14, 32). The wild-type (N2), DH26, and TJ401 strains displayed equivalent resistance to hydrogen peroxide as young gravid adults. Because both the *age-1* strain TJ401 and its parent strain carry a temperature-sensitive mutation, *fer-15*, that prevents hermaphrodite self-fertilization, offspring are absent from a population of animals raised at the restrictive temperature (25°C). Age-synchronized cultures were maintained at 25°C. For each experiment the TJ401 and DH26 cultures were grown simultaneously to control for environmental fluctuations in growth conditions. In each of the experiments presented the population was assayed until no survivors remained. There were no surviving DH26 animals at the later times and, thus, no data points. This result confirmed the age synchrony of the cultures and the difference in life-spans of the strains.

Resistance to hydrogen peroxide as a function of age is presented in Fig. 1. The sensitivity of DH26 animals to hydrogen peroxide is essentially unchanged throughout adult life. By contrast, there is a statistically significant relationship between age and LD_{50} for TJ401 adults ($P < 0.001$); they showed an ≈7-fold increase in resistance to hydrogen peroxide at the advanced ages measured.

The resistance of dauer larvae to hydrogen peroxide was also tested. The dauer larva LD_{50} is 0.38 ± 0.01 M H_2O_2. This level of resistance is nearly 20 times higher than in any adults tested. Resistance was not unexpected because dauer larvae are particularly resistant to chemical insults, owing to the dauer-specific cuticle and the lack of feeding (15). Similarly, it was possible that the resistance trend observed in the TJ401 animals could be due to cuticular changes that only occur at old ages when all DH26 animals have died, and so there are

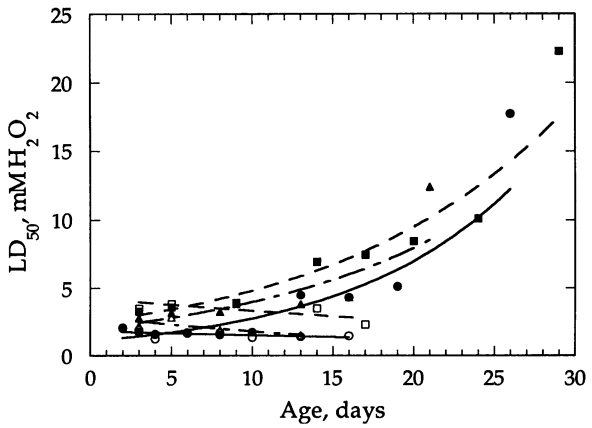

FIG. 1. Resistance to hydrogen peroxide as a function of age in DH26 (open symbols) and in TJ401 (closed symbols). Day 1 corresponds to the first day of adulthood. The resistance was followed for three independent paired cultures, experiments 1, 2, and 3 (solid, dashed, and dot-dashed lines, respectively). The day 3 mean hydrogen peroxide concentration was 2.6 ± 0.78 mM and 2.6 ± 0.81 mM for strains TJ401 and DH26, respectively. The significance probability for the correlation coefficient (33) showed that no statistically significant relationship exists between age and LD_{50} ($P > 0.1$) for strain DH26.

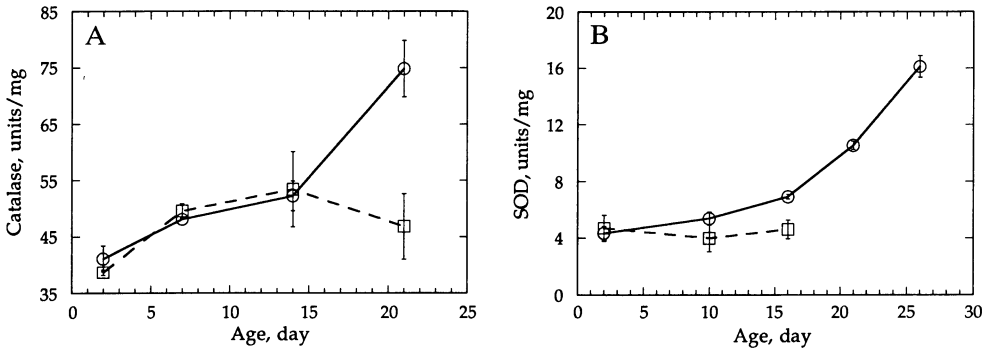

FIG. 2. Catalase and SOD activities as a function of age. Cultures of age-synchronous strains DH26 (□) and TJ401 (○). Vertical bars represent SDs from four to seven replicate assays. (*A*) Catalase activity increases significantly after day 7 in both strains but then decreases in strain DH26 and increases in strain TJ401. (*B*) Increase in SOD activity is statistically significant (*t* test, ref. 33) at the 0.05 level, starting with day 10 for strain TJ401. There is no significant change in strain DH26 SOD activity with age.

no control animals to test. Thus, hydrogen peroxide resistance was not necessarily indicative of increased intracellular resistance to oxidative damage. Further tests were necessary to ascertain this.

Catalase and SOD Activity as a Function of Age. Age-synchronous cultures were prepared and followed, as for the hydrogen peroxide-resistance bioassay. The *E. coli* strains used as a food source were mutant in the catalase or SOD genes (24, 25), so that the enzymatic activity measured would be solely due to the *C. elegans* enzymes. There is an increase in catalase activity with increasing age in both extracts until mid-life, when the TJ401 activity increases further, whereas the activity may decrease slightly near the end of the DH26 life span (Fig. 2*A*). The SOD activity is relatively constant in DH26 (Fig. 2*B*). Strain TJ401 displays a 4-fold increase in SOD activity from early adulthood to 26 days of age (Fig. 2*B*). Both enzyme activities show a time course similar to that of the hydrogen peroxide resistance. That is, the age at which the LD_{50} for hydrogen peroxide begins to increase for TJ401 is approximately the same age that the SOD and catalase activities also begin to increase, as compared with DH26. The most obvious interpretation of these data is that the increase in SOD and catalase activities enables the observed oxidative stress resistance. Furthermore, each of these three measured increases is *age-1* dependent and is thereby related to increased life-span. The increases are unlikely to be due to background mutations in strain TJ401 because a recent independent study that used different *age-1* strains also detected increases in SOD and catalase activities with age, as well as resistance to paraquat (34).

Catalase and SOD Activity in Dauer Larvae. The SOD activity in N2 dauer larvae was previously reported to be 17.1 ± 4.4 units/mg of protein (35). Determination of the SOD activity for this report revealed that N2, DH26, and TJ401 dauer larvae levels were 22.7 ± 2.3, 21.5 ± 2.7, and 23.1 ± 7.6 units/mg of protein, respectively, a 5-fold increase of SOD activity in dauer larvae relative to 2-day old adults (4.53 ± 0.7 units/mg of protein). This increase appeared to be independent of *age-1*. In this regard it differed from the 4-fold increase in very old TJ401 animals, implying that increase of SOD activity can result from a mechanism that does not involve *age-1*. The catalase activities in N2, DH26, and TJ401 dauer larvae were 42.3 ± 2.0, 40.5 ± 4.6, and 38.9 ± 1.7 units/mg of protein, respectively. These levels were not elevated with regard to that measured in young adults.

Isolation of the Cu/Zn SOD Gene. An increase in SOD activity was detected in both dauer larvae and old TJ401 animals, the two conditions that have a positive affect on life-span. Eighty percent of the SOD activity in *C. elegans* is due to the Cu/Zn form (14). The wild-type *C. elegans* Cu/Zn SOD gene was cloned by using degenerate oligonucleotides. The nucleotide sequence of the *C. elegans* Cu/Zn SOD cDNA is shown in Fig. 3*A*. The deduced coding region is shown in Fig. 3*B* aligned with Cu/Zn SOD protein sequences

A
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acaaaaattattttattacaagctcataatagaataaaatttcaaaacaaaaaaaaaagtgatgtttatgaatcttctcactcaggtctccaacgcgatttttccgcagg  110
tcgaagccgctcaaaaaATGTCGAACCGTGCTGTCGCTGTTCTTCGTGGAGAAACTGTTACCGGTACTATCTGGATCACACAGAAGTCCGAAAATGACCAGGCAGTTATT  220
                  M  S  N  R  A  V  A  V  L  R  G  E  T  V  T  G  T  I  W  I  T  Q  K  S  E  N  D  Q  A  V  I
GAAGGAGAAATCAAGGGACTTACTCCCGGTCTTCATGGATTCCACGTTCACCAATATGGTGATTCCACCAACGGATGCATTTCTGCCGGTCCACACTTCAATCCATTTGG  330
E  G  E  I  K  G  L  T  P  G  L  H  G  F  H  V  H  Q  Y  G  D  S  T  N  G  C  I  S  A  G  P  H  F  N  P  F  G
AAAGACTCATGGTGGACCAAAATCCGAGATCCGTCACGTAGGCGATCTAGGAAATGTGGAAGCTGGAGCCGATGGAGTGGCAAAAATCAAGCTCACCGACACGCTCGTCA  440
 K  T  H  G  G  P  K  S  E  I  R  H  V  G  D  L  G  N  V  E  A  G  A  D  G  V  A  K  I  K  L  T  D  T  L  V  T
CGCTTTACGGTCCAAACACTGTCGTTGGCCGATCTATGGTTGTTCATGCCGGACAAGACGACCTCGGCGAGGGAGTCGGAGACAAGGCAGAAGAGTCCAAGAAGACTGGA  550
  L  Y  G  P  N  T  V  V  G  R  S  M  V  V  H  A  G  Q  D  D  L  G  E  G  V  G  D  K  A  E  E  S  K  K  T  G
AACGCCGGAGCTCGTGCTGCCTGCGGTGTCATTGCTCTCGCTGCTCCCCAGtgactacctgaatcgcgtctctgaatctccacacaattcctactaaagacaatttttca  660
N  A  G  A  R  A  A  C  G  V  I  A  L  A  A  P  Q
tttcttgctttgtcgttatattcttaagaatcccgttgttcctactcctactactgtatattttcacataaaatttcttcaaaatttcaaataaaggttgtagttcc     767
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B
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                1       10        20        30        40        50        60        70
C. elegans:     MSNRAVAVLRGET-VTGTIWITQKSENDQA-VIEGEIKGLTPGL-HGFHVHQYGDSTNGCISAGPHFNPFGKTHGGPKSEI
Human:           ATKAVCVLKGDGPVQGIINFEQKESNGPVKV-WGSIKGLTEGL-HGFHVHEFGDNTAGCTSAGPHFNPLSRKHGGPKDEE
D. melanogaster: VVKAVCVINGDD-AKGTVFFEQESSGTPVKV-SGEVCGLAKGL-HGFHVHEFGDNTNGCMSSGPHFNPYGKEHGAPVDEN
Maize:           MVKAVAVLAG-TDVKGTIFFSQEG-DGPTTV-TGSISGLKPGL-HGFHVHALGDTTNGCMSTGPHFNPVGKEHGAPEDED
S. cerevisiae:    VQAVAVLKGDAGVSGVVKFEQASESEPTTV-SYEIAGNSPNAERGFHIHEFGDATNGCVSAGPHFNPFKKTHGAPTDEV

                80        90        100       110       120       130       140       150       158
C. elegans:     RHVGDLGNVEAGADGVAKIKLTDTLVTLYGPNTVVGRSMVVHAGQDDLGEGVGDKAEESKKTGNAGARAACGVIALAAPQ
Human:          RHVGDLGNVTADKDGVADVSIEDSVISLSGDHCIIGRTLVVHEKADDLGKG-G--NEESTKTGNAGSRLACGVIGI---Q
D. melanogaster: RHLGDLGNIEATGDCPTKVNITDSKITLFGADSIIGRTVVVHADADDLGQG-G--HELSKSTGNAGARIGCGVIGIAK
Maize:          RHAGDLGNVTAGEDGVVNVNITDSQIPLAGPHSIIGRAVVVHADPDDLGKG-G--HELSKSTGNAGGRVACGIIGL---QG
S. cerevisiae:  RHVGDMGNVKTDENGVAKGTFKDSLIKLIGPTSVVGRSVVIHAGQDDLGKG--D-TEESLKTGNAGPRPACGVIGLTN
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FIG. 3. Nucleic acid and deduced protein sequence of *C. elegans* Cu/Zn SOD gene. (*A*) cDNA sequence. The open reading frame is in uppercase letters, and the corresponding amino acid sequence is shown directly below. The regions corresponding to the degenerate oligonucleotides, used as hybridization probes to isolate the gene, are underlined. (*B*) Alignment of the deduced coding region of the *C. elegans* Cu/Zn SOD gene with the gene from four other organisms, as noted (36–39). The shaded amino acids are identical to that of *C. elegans*; the regions corresponding to the degenerate oligonucleotides are underlined.

from other species. The *C. elegans* Cu/Zn SOD is 57% identical to the *Saccharomyces cerevisiae* and maize Cu/Zn SOD, 56% to that of human, and 53% to that of *Drosophila melanogaster*. This amino acid identity verified that the sequenced *C. elegans* cDNA encoded a Cu/Zn SOD.

Physical Map Position of *sod-1*. A single Cu/Zn SOD locus, *sod-1*, was detected on linkage group II by hybridization to a yeast artificial chromosome filter (31). The physical position of *sod-1* was further delimited to a region (≈35 kb) between the previously cloned genes *tra-2* and *unc-104* (Fig. 4*A*) by hybridization to cosmids from this region of the physical map (31). The SOD cDNA hybridized to the cosmids C47A5 and C15F1. Mutations in *tra-2* and *unc-104* have been genetically mapped (Fig. 4*B*) and are within the 1.2 map unit interval to which *age-1* has been mapped (20, 40). This raised the possibility that *age-1* is a mutation in the Cu/Zn SOD gene.

Southern hybridization of the SOD cDNA to genomic DNA from N2, DH26, TJ401, and BA15 *fer-15*(*hc15*ts) detected no restriction fragment length polymorphism in 12 restriction enzyme digestions. Hybridization with the cosmids to the same DNA digests did detect some restriction fragment length polymorphisms, but it showed no correlation to increased life-span (data not shown). Furthermore, the *age-1*-increased longevity appears distinct from *sod-1*, according to recent genetic mapping data that places *age-1* to the right of *fer-15* (T. Johnson, personal communication). Owing to the close linkage of *age-1* and *sod-1*, it remains possible that the *age-1*-containing strain could carry a secondary mutation at *sod-1* that was not separated in genetic crosses. Hence, a lesion at the SOD locus might enhance the longevity of strain TJ401, although it is not the major cause of increased life-span. It will be important to see whether genetic recombinants that carry *age-1* but do not carry *fer-15* are equally as long-lived as strain TJ401. Recently, a *C. elegans* catalase gene has been cloned (41) and found to map on linkage group II at a site distant from *age-1* and *sod-1*.

DISCUSSION

In support of the hypothesis that oxidative damage may play a role in limiting life-span in *C. elegans*, evidence for increased resistance to an oxidative damage-inducing agent and increased SOD and catalase activities was detected in both the *age-1* strain TJ401 and the dauer larva. Increased resistance to hydrogen peroxide and accompanying biochemical alterations were detected in the *age-1* strain TJ401 and not in the parental strain DH26. The *age-1* mutation was not isolated in a manner that assumed a relationship between life-span and response to oxidative stress. Rather, it was isolated in a screen for mutants displaying an increased life-span (19). In accordance with the free radical theory of aging (7), the coincidence of increased life-span and increased level of SOD and catalase activities implies that this improved level of defense against intracellular oxidative damage might be instrumental in affecting the increased life-span.

The time courses of the increased resistance to hydrogen peroxide and increased SOD and catalase activities in the *age-1* strain TJ401 are similar to one another, and they begin in mid-life rather than in early adulthood. Consistent with this is the lack of an obvious effect on development or reproductive period by the *age-1* mutation (20, 21). There are at least two interpretations for the observed late onset. (*i*) The time course for the population is representative of each individual animal. (*ii*) There is a range of SOD and catalase activities in the TJ401 animals due to variable expressivity, and the animals with higher enzyme levels live longer. Either interpretation supports the free radical theory of aging. To construe the changes as causal in the latter case is straightforward. In the former case, the boost (induced or programmed) in protection conferred by SOD and catalase could slow the accumulation of oxidative damage in *age-1*-carrying animals, thereby increasing life-span at a time when the abilities of *age-1*(+) animals are declining.

As a dispersal stage, the dauer larva is likely to encounter harsh conditions for long periods, during which it is essential to avoid cellular damage that would interfere with development into a fertile adult. This naturally occurring stage of diapause can survive for months, yet when it resumes development the post-dauer life-span is unaltered (18). The dauer larva stage thereby represents an extremely efficient life-maintenance model system for the study of mechanisms of aging. Dauer larvae are extraordinarily resistant to external stress by virtue of their specialized cuticle and absence of feeding (15), and yet they have highly elevated levels of SOD activity. This fact may reflect that efficient scavenging of internally generated free radicals is critical for long-term survival.

Previous work in *D. melanogaster* investigated whether overexpression of the SOD gene was sufficient to prolong life-span. Either no significant increase in life-span resulted (42) or a modest but significant increase of 2%–18% resulted, depending on the number and configuration of the SOD genes in the strain (43). By contrast, the increase in mean life-span displayed by animals carrying the *age-1* mutation is 65% (20). An important difference may be that the SOD activity increase in *age-1*-carrying animals was initiated late in life and rose gradually, whereas in the *D. melanogaster* experiments the SOD overexpression was continuous. Overexpression of SOD alone can be deleterious (44, 45), and this may confound any positive effect on life-span.

The changes in catalase and SOD activities in TJ401 animals suggest that the *age-1* gene product may be a negative regulator of these activities, either directly or indirectly. The negative regulation by the *age-1* gene product could act directly on each gene individually, or act only on the SOD gene, if increased catalase activity were a secondary consequence of elevated intracellular levels of hydrogen peroxide produced by SOD. However, high SOD activity in dauer larvae is not paralleled by an increase in catalase activity, which suggests that indirect induction of catalase does not occur. An *E. coli* strain that has 10 times the normal SOD activity level has normal catalase levels, although this renders that strain more sensitive to oxidative damage-inducing agents than the normal strain (44). The more probable direct mode of regulation by the *age-1* gene product

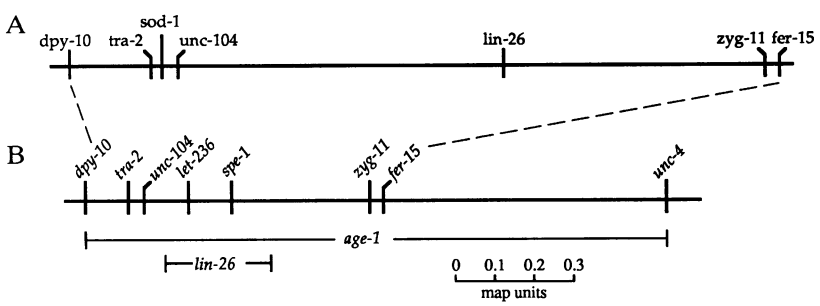

FIG. 4. Position of *sod-1* on physical and genetic maps. (*A*) Physical map of the *sod-1* region of chromosome II. (*B*) Corresponding region of the genetic map. The dark line represents the chromosome. Genes listed below the line have not been ordered genetically with respect to their nearest neighbors on the line. Approximately 1.5 map units separate *dpy-10* and *unc-4* (40).

would be that it acts on individual genes or gene products via transcription, translation, or determination of the half-life of mRNAs or proteins. Understanding SOD regulation with respect to advancing age is of particular interest because mutations in the human Cu/Zn SOD gene have been associated with the late-onset disease familial amyotrophic lateral sclerosis (46).

Indirect effects of *age-1* on SOD and catalase expression are also plausible. For example, dietary restriction increases life-span in many species, including *C. elegans* (12). Catalase and SOD activities are increased in liver from diet-restricted rats in comparison with the ad libitum-fed control rats (47). Dauer larvae do not feed, so dietary restriction may play a role in the nonaging of dauer larvae. If the *age-1* mutation results in a change analogous to diet restriction, then the increase in SOD activity in dauer larvae would be predicted to be independent of *age-1*, as observed. In the absence of feeding, no additional dietary restriction by *age-1* would be possible. It has been suggested that the *age-1* mutation does not induce dietary restriction (48), although it remains conceivable that inefficient food utilization or altered metabolism might generate the *age-1*-increased life-span. Regardless of the mechanism by which the *age-1* gene product acts, the expression of genes other than SOD and catalase may be altered, and some may also contribute to extension of life-span.

The genetically based biochemical alterations presented here support the hypothesis that oxidative damage is one of the proximal causes of aging in *C. elegans*. However, the *age-1* animals are not immortal; they still senesce and die. Thus, multiple cellular mechanisms of aging may act simultaneously, and some of them may not be regulated by the *age-1* gene product. One challenge is to determine the proportion of the aging phenotype that is a result of mechanisms associated with free radical damage. The relevant enzymes in *C. elegans* exist in all organisms, so this knowledge derived from a genetically and biochemically amenable invertebrate should apply to other species as well. Clearly, the relative impact of increased protection from oxidative damage in each species will vary according to their initial ability to defend and repair such damage. Accordingly, the greatest delay of onset of aging or increase in life-span would be realized by species for whom the oxidative defense and repair system is the most limiting.

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