

Relationship Between Catalase and Life Span in Recombinant Inbred Strains of *Caenorhabditis elegans*

PHIL HARTMAN,¹ PATRICK BELMONT,¹ SYLVIA ZUBER,¹ NAOAKI ISHII,² AND JULIE ANDERSON³

Abstract: Johnson and Wood constructed recombinant inbred strains of *Caenorhabditis elegans* with life spans ranging from 10 to 31 days. Using these strains, we have demonstrated previously that hyperoxia and methyl viologen inhibited development at rates inversely correlated with life span. The growth rates of the short-lived recombinant inbred strains were more profoundly inhibited by oxidative stress than were those of the long-lived strains. Here we report a positive correlation between life span and catalase levels in these same strains. Specifically, when compared to short-lived strains at 10 days after fertilization, the long-lived strains possessed higher levels of total enzymatic catalase. Northern blots indicated a similar relationship between life span and *ctl-1*mRNA (the cytosolic catalase). This suggests that at least some of the polygenes that influence life span are also responsible for regulating gene expression of catalase, an important defense component against oxidative stress.

Key words: aging, catalase, *Caenorhabditis elegans*, life span, oxidative stress, recombinant inbreds.

There is perhaps no biological process for which genetics and the environment are so entwined as aging. *Caenorhabditis elegans* has proven particularly useful and popular for unraveling the relative contributions of “nature” and “nurture” to the dynamics and mechanisms of organismal aging. This is numerically evidenced by the proliferation of review articles, currently numbering more than 60, that encompass all or various elements of aging and life-span determination in the free-living nematode (e.g., Finch and Ruvkun, 2001; Guarente and Kenyon, 2000; Johnson et al., 2001).

One of the most striking relationships to have emerged from these investigations is that between life span and resistance to oxidative stress. Specifically, long-lived strains usually show heightened resistance to various presentations of oxidative stress, whereas some short-lived strains are hypersensitive to oxidative stress. For example, loss-of-function mutations in *mev-1* and *gas-1* shorten life span and render animals hypersensitive to oxidative stress (Hartman et al., 2001; Ishii et al., 1998). Both encode subunits of complexes of the electron transport system, and mutations in *mev-1* directly increase superoxide anion production (Senoo-Matsuda et al., 2001). Long-lived mutants show increased resistance rather than sensitivity to various presentations of oxidative stress (reviewed in Johnson et al., 2001). This is consistent with the theory that aging is a deleterious response to oxidative stress, a popular notion originally espoused by Harman (1956). It is clear that the mechanisms regulating superoxide anion and catalase levels are central to life-span determination. For example, *age-1* mutations that extend life span result in elevated superoxide dismutase and catalase levels in

geriatric worms (Larsen, 1993; Vanfleteren, 1993). In addition, dauer larvae can survive for months longer than other larval stages and have been recently shown to possess elevated levels of superoxide dismutase and catalase (Henthoofd and Vanfleteren, pers. comm.).

Most efforts have focused on investigating single-gene mutations that influence life span. However, the polygenic nature of aging in *C. elegans* has also received some attention (Ebert et al., 1996; Hartman et al., 1995; Johnson and Wood, 1982). Such analyses began by crossing different wild-type animals to generate recombinant inbred (RI) strains. Owing to independent assortment and genetic recombination, each RI will contain a more or less random mixture of the two parental genomes. This approach was first employed to create, in the absence of any selection, RIs with mean life spans from 10 to 31 days (Johnson and Wood, 1982). The mean life spans of the two parents were about 18 days. The analysis of RIs has, among other things, illuminated the role of oxidative stress in life-span determination. Ebert et al. (1996) exposed a heterogeneous population consisting of many different RI strains to an otherwise toxic level of hydrogen peroxide and identified among the survivors a specific parental chromosomal region that was over-represented, suggesting it conferred resistance to reactive oxygen species (ROS). Hartman et al. (1995) determined that there was a negative correlation between RI life span and the growth-rate inhibition imposed by either hyperoxia or methyl viologen. Thus, at least some of the same polygenes that contribute to life span also control oxidative stress responses. In this report, we extend this second approach by examining the expression of catalase in the RI strains. While there are currently more than 80 RI strains available in the Johnson laboratory alone, we choose to examine the same strains that we had employed previously (Hartman et al., 1995). Our results indicate that catalase levels correlate positively with life span, providing the first molecular linkage between the polygenetic basis of life-span determination and oxidative stress resistance in *C. elegans*.

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¹ Department of Biology, Texas Christian University, Fort Worth, TX 76129.

² Department of Molecular Biology, School of Medicine, Tokai University, Isehara, Kanagawa 259-11, Japan.

³ Department of Biology, University of Wisconsin-Eau Claire, Eau Claire, WI 54701.

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E-mail: p.hartman@tcu.edu

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MATERIALS AND METHODS

Strains and general conditions: The RI strains of *C. elegans* were obtained from T. Johnson (University of Colorado) in 1986 and were frozen in liquid nitrogen at that time. The strains were then resuscitated, first in 1991 for the growth experiments (Hartman et al., 1995) and again in 1999 for the catalase experiments. The strains and their mean life spans given in parentheses as determined by Johnson (1987; http://ibgwww.colorado.edu/tj-lab/frame_worm1.html) were as follows: TJ215 (10.9 days), TJ299 (12.7 days), TJ148 (13.2 days), TJ135 (16.9 days), TJ142 (25.5 days), and TJ143 (28.5 days). The standard deviations for each of these RI strains was less than 1 day (Johnson, 1987; http://ibgwww.colorado.edu/tj-lab/frame_worm1.html). TJ299 was not included in the catalase enzyme determinations, as this strain could not be resuscitated from our liquid nitrogen stock collection at Texas Christian University. It was subsequently obtained from the Caenorhabditis Genetics Center (University of Minnesota) for the northern blot experiments. Stock cultures were maintained on Nematode Growth Medium (NGM) covered with confluent *Escherichia coli* OP50 following the methods of Brenner (1974). All cultures were maintained at 20 °C.

Catalase assays: Large, asynchronous nematode populations were first generated by growing animals on NGM enriched with 1% peptone. Synchronous populations were obtained by treating with alkaline bleach as previously described (Hartman and Herman, 1982). The resultant embryos were inoculated to peptone-enriched medium and grown for 2 days on *E. coli*. Animals were then transferred to NGM supplemented with 0.25 g/L fluorouracil that were previously seeded with a 50× concentrate of *E. coli*. Fluorouracil resulted in near-complete sterility without affecting life spans (Mitchell et al., 1979), such that preparations were not contaminated with embryos, larvae, and adults of subsequent generations. Animals were transferred periodically to fresh cultures to prevent starvation. At the appropriate times, animals were collected and concentrated by centrifugation and frozen at -20 °C. Shortly before the enzyme assay, animals were thawed, lysed by sonication (40 intervals of 10 s with 20 s intermissions using a Heat Systems-Ultrasonics, Inc. sonicator, model W 185 F [Plainview, NY], with the microtip at maximum power). Crude lysates were clarified by centrifugation at 12,000g for 1 minute. Total protein was determined using Bradford assays as described by the vendor (Sigma Chemical Co., St. Louis, MO). Catalase activity was measured by its ability to decompose hydrogen peroxide into water and oxygen, with one unit defined as the amount necessary to convert 1 μmol of H₂O₂ per milligram of protein per minute at 25 °C. Hydrogen peroxide was quantitated spectrophotometrically via its binding to titanium tetrachloride (Wolfe, 1962). Assays

were conducted in 3-ml volumes containing 10 mM H₂O₂, with 200 to 300 μg of total nematode protein added.

Northern blots: Synchronous populations were generated as described previously, except that fluorouracil was not employed. Instead, gravid hermaphrodites were suspended in water and allowed to settle. The supernatant was then aspirated and the process repeated. Embryos and larvae were eliminated with the supernatant, whereas the vast majority of adults were recovered. This process was repeated daily for as long as progeny were produced. Animals were concentrated by centrifugation. Aliquots of 0.1 ml packed worms and 0.4 ml water were made and either used immediately or stored at -75 °C. Frozen aliquots were thawed and 40 μl mix (1 M Tris, 0.5M EDTA, 2 M NaCl), 40 μl proteinase K (10 mg/ml), and 40 μl 10% sodium dodecyl sulfate were added and placed in a 55 °C water bath for 30 minutes to lyse the worms. Total RNA was isolated using TriReagent (Sigma Chemical Co., St. Louis, MO) following the protocol supplied by the vendor. Radioactive labeling, electrophoresis, transfer and immobilization of RNA, hybridization and washing, and autoradiography were accomplished using standard protocols (Sambrook et al., 1989). Densitometric analyses were performed using NIH Image (public domain version 1.62). Complete bands were measured in the lane whenever possible. However, bands in some lanes were distorted; therefore, half of each band was measured in these rare cases. The *ctl-1* data presented in Figure 1 were derived from two separate experiments. Because there were a number of procedural differences between the two experiments, the *ctl-1/gph* ratios were, on average, about 3.1× greater on the second experiment. Therefore, the data presented in Figure 2 were averaged after normalizing the second experiment to the first by multiplying by 3.1.

RESULTS

Five RI strains of *C. elegans* were tested for catalase activity, with extracts prepared from synchronous populations at 4, 7, and 10 days of growth (Fig. 2). There was a negative correlation between mean life span and catalase levels at 4 days of development; that is, the short-lived strains tended to have higher catalase activities than did the long-lived strains. Conversely, at 7 days of development, and even more so at 10 days, catalase levels tended to be higher in long-lived strains as compared with short-lived strains. These relationships are numerically evidenced by the three slopes, which were -4.1, 16.8, and 32.9, respectively. Catalase levels dropped slightly (ca. 4%) between 4 and 10 days for the three short-lived strains. Conversely, catalase activities in the two long-lived strains increased over 2-fold during the same time span. The correlation coefficient (R) was 0.45 between 10-day catalase levels and an extended

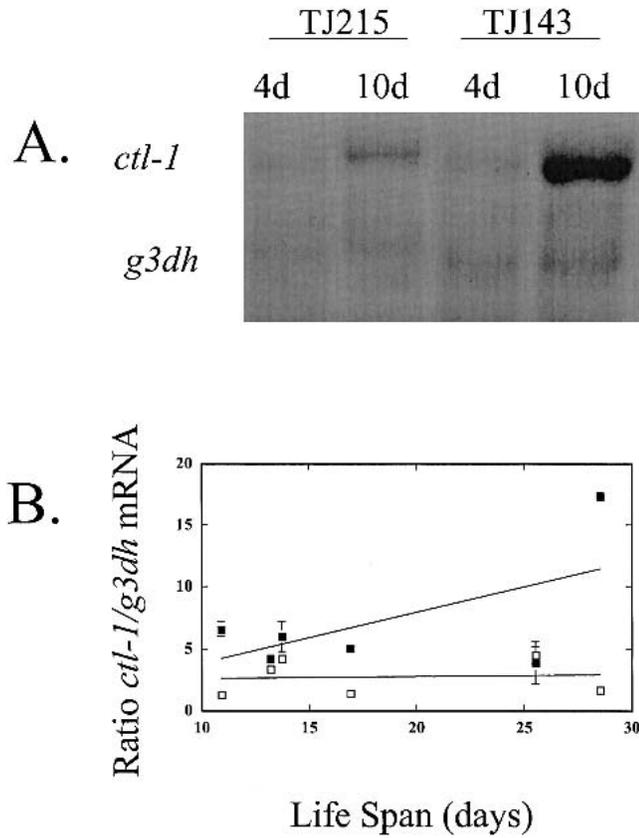


FIG. 1. A) Autoradiogram of a northern-blot hybridized against *ctl-1* and *g3pdh*. TJ215 and TJ143 are the shortest- (10.9 days) and longest- (28.5 days) lived strains examined. The lanes marked 4d and 10d correspond to RNA extracted from 4- and 10-day-old animals, respectively. B) Ratio of *ctl-1* to *gpd* mRNA for synchronous RI populations grown for 4 or 10 days. The means and standard deviations for two separate Northern blots are presented, based upon densitometric scans from autoradiograms such as depicted in A.

life span (Fig. 1), whereas a plot of the increase in catalase levels in the same strains from 4 days to 10 days yielded a correlation coefficient of 0.88 (data not shown).

Northern blots were performed to determine if steady-state levels of mRNA varied in RI strains. Total RNA was isolated from synchronous RI strains and, after electrophoresis and transfer, was hybridized to *ctl-1* and *g3pdh* probes (Fig. 1A). The *ctl-1* probe hybridizes to the three genes that encode the cytosolic (and abundant) catalase of *C. elegans*. Since *g3pdh* was assumed to be constant from strain to strain, the ratio of *ctl-1* to *g3pdh* was taken as an index of relative *ctl-1* mRNA levels in the various RI strains. As with enzyme levels, *ctl-1* mRNA concentrations were positively correlated with mean life span in preparations from animals harvested at 10 days (Fig. 2B).

While both enzyme (Fig. 2) and mRNA (Fig. 1B) levels correlated with life span, there was considerable variation from strain to strain. To determine if apparent correlations were due to experimental error, enzyme levels were directly compared with mRNA levels

on a strain-by-strain basis (Fig. 3). There was a striking correlation for the 10-day samples; for example, the same strains that demonstrated high enzyme activities also harbored more *ctl-1* mRNA. Given that these experiments derived from different sample preparations and were conducted by different individuals, this relationship strongly suggests that the observed strain-to-strain variation was due to genetic differences between strains rather than experimental error.

Similar northern-blot analyses were performed to assess *sod-3* and *hsp-16-48* mRNA levels in these RI strains. *sod-3* encodes one of the superoxide dismutase genes in *C. elegans* and was shown to be elevated in the long-lived *daf-2* mutant (Honda and Honda, 1999). *hsp-16-48* en-

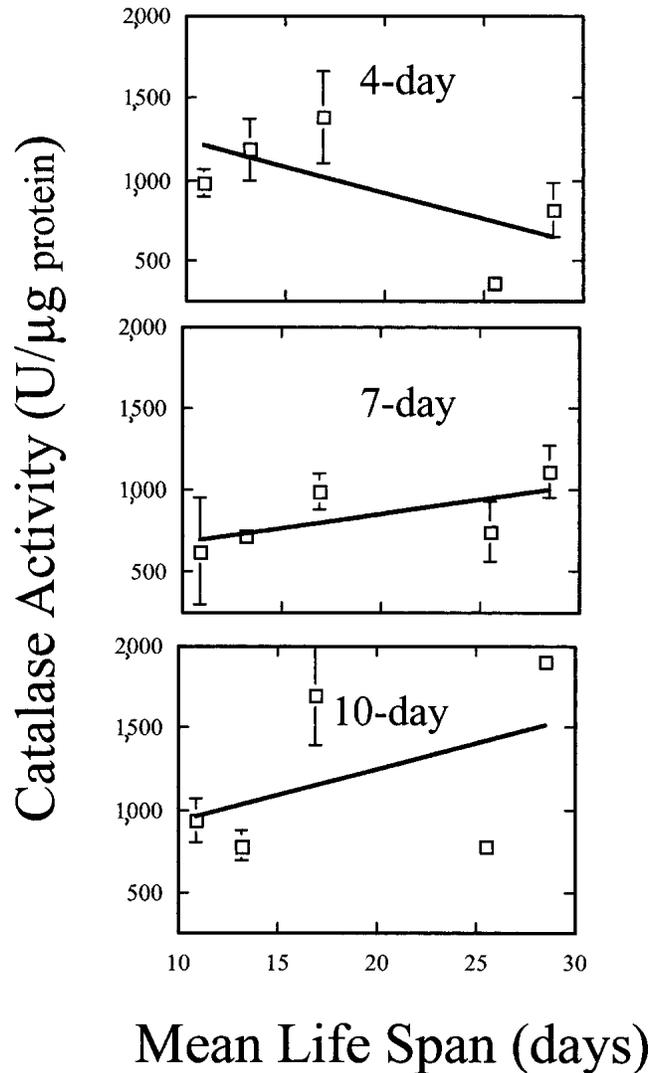


FIG. 2. Catalase levels as a function of life span. Staged populations of five RI strains with mean life spans of 10.9, 13.2, 16.9, 25.5, and 28.5 days were assayed for total catalase activity at 4, 7, and 10 days of development. Means and standard deviations for each datum point are compilations of a minimum of three determinations from independently grown populations. The correlation coefficient (R) was 0.45 between 10-day catalase levels and life span, whereas a plot of the increase in catalase levels in the same strains from 4 days to 10 days yielded a correlation coefficient of 0.88 (data not shown).

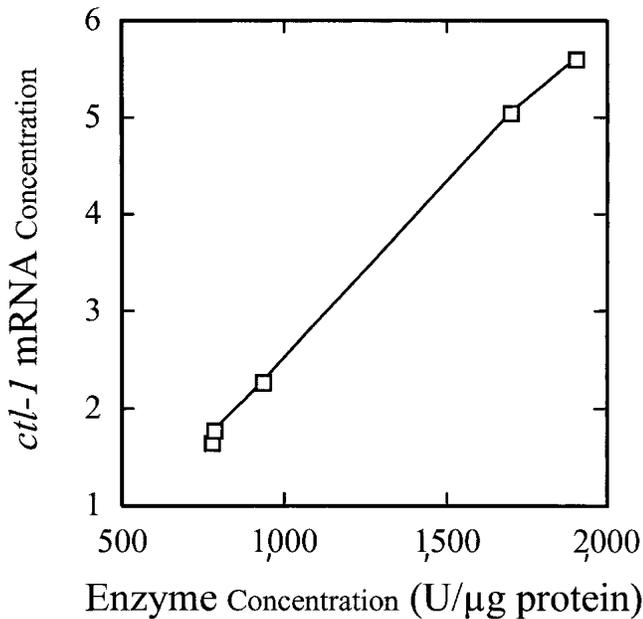


FIG. 3. The relationship between catalase activity and *ctI-1* mRNA levels of 10-day-old animals on a strain-by-strain basis. Values were taken from Figures 1 and 2B.

codes a heat shock protein and is overexpressed in an *age-1* genetic background (Walker et al., 2001). Although some strain-to-strain variation existed, neither of these two genes was over-expressed in 10-day-old animals. In addition, there was no correlation between life span and mRNA levels for these two genes (data not shown).

DISCUSSION

Since its conception (Harman, 1956), much effort and various experimental approaches have been employed to test the free radical theory of aging (reviewed in Beckman and Ames, 1998). These include, but are not limited to, experimental manipulations of oxygen tension or the rate of living, supplementation with dietary antioxidants, interspecies comparisons, studies of dietary restriction, use of transgenics, and epidemiological approaches. Studies also include the examination of the levels of various defensive enzymes (e.g., superoxide dismutase and catalase) throughout the life spans of different organisms. Interestingly, the data are contradictory; for example, superoxide dismutase and catalase levels have been recently reported to both decrease (Hall et al., 2001; Sandhu and Kaur, 2002; Tian et al., 1998) and increase (Guo et al., 2001) in a variety of tissues as rodents age. In a similar fashion, attempts to correlate the life spans of different mutants or strains with oxidative stress defenses have yielded disparate results. There is usually a positive correlation between enzyme levels and life span; for example, short-lived and long-lived mutant mice show changes in catalase levels during aging that are consistent with their relative life spans (Brown-Borg and Rakoczy, 2000). How-

ever, two groups of researchers have examined catalase levels in short- vs. long-lived strains of *Drosophila*. Catalase levels were found to be higher in the long-lived strain in one case (Arking, 2001) but, paradoxically, lower in the long-lived strain in the second study (Mockett et al., 2001). The data are more consistent in *C. elegans*, as both polygenic and single-gene approaches suggest that catalase regulation is pivotal in life-span determination. For example, it has been known for some time that catalase levels selectively increase as the long-lived *age-1* mutant ages (Larsen, 1993; Vanfleteren, 1993). These animals are also resistant to oxidative stress. We have now demonstrated that catalase mRNA (Fig. 1) and enzyme (Fig. 2) levels correlate with life span in the RI strains created by Johnson and Wood (1982). These data serve to logically extend the relationship between oxidative stress and life span in these same RI strains (Hartman et al., 1995). Interestingly, there was a much stronger correlation between the increase in catalase levels in the RI strains as they aged as compared with their absolute catalase levels. This is numerically evidenced by the fact that the correlation coefficient (*R*) was 0.45 between 10-day catalase levels and life span (Fig. 1), whereas a plot of the increase in catalase levels in the same strains from 4 days to 10 days yielded a correlation coefficient of 0.88 (data not shown).

Although an overall correlation exists between catalase levels and life span (Figs. 1, 2), there was considerable strain-to-strain variation between the two. This probably reflects true biological differences rather than experimental error. Such variation is to be expected, as many genes impact aging through many different mechanisms and in subtle ways. Some, but not all, of these involve oxidative stress and would include factors that regulate the production of free radicals, enzymatic and non-enzymatic scavenging systems, and repair pathways. The strain-to-strain variation is strikingly illustrated by comparing the two longest-lived strains (TJ142 and TJ143). TJ142 had a life span only several days shorter than that of TJ143 (25.5 vs. 28.5 days) but its catalase levels were significantly lower (Figs 1, 2). A similar relationship existed between life span and the responses of these two strains to methyl viologen (Fig. 3 from Hartman et al., 1995); that is, growth in TJ142 was more strongly inhibited by methyl viologen than was growth of TJ143. Thus, while oxidative stress resistance is paramount to the longevity of TJ143, other factors may confer the longevity of TJ142. However, it is also clear that catalase levels (and associated factors) are pivotal in determining resistance to oxidative stress. This is numerically evidenced by the fact that a plot of the increase in catalase levels from 4 to 10 days (Fig. 1) vs. the susceptibility to 0.3 mM methyl viologen (Hartman et al., 1995) yielded a correlation coefficient of 0.985. Thus, life span, catalase levels, and resistance to oxidative stress are intimately associated.

Particularly at the transcriptional level, it could be argued that most of the correlation between life span and *ctl-1* expression was driven by TJ143 (mean life span 28.5 days). While this may be true, it also can be stated that catalase activity increased with aging in the long-lived but not the short-lived strains; specifically, catalase levels remained essentially unchanged in the three short-lived strains but were significantly higher in the two long-lived strains at 10 days as opposed to 4 days (Fig. 2). In addition, the high correlation coefficient between increase in catalase levels and life span (0.88) indicates the relationship is not due simply to higher catalase levels in a single strain.

A growing number of studies provide evidence of some global regulation of the genetics of oxidative stress resistance; namely, there are likely *trans*-acting factors that orchestrate expression of the genes encoding enzymes such as catalase and superoxide dismutase. Indeed, it is generally the case that changes in expression of these two enzymes parallel one another (e.g., Brown-Borg and Rakoczy, 2000; Hall et al., 2001; Sandhu and Kaur, 2002). However, this is not universally the case, as evidenced in such disparate organisms as the bean weevil (Seslija et al., 1999) and a cephalopod (Zielinski and Portner, 2000). In both instances superoxide dismutase levels increased with age whereas catalase levels did not. Thus, there is likely an interplay between both *cis*- and *trans*-acting factors that facilitate this global regulation. It is clear that such global regulation occurs in *C. elegans*, as both differential display (Kushibiki et al., 1996; Tawe et al., 1998) and microarrays (Kim et al., 2001) have detected elevated expression of specific genes in response to oxidative stress.

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