abyssal circulation, on the assumption of a flat-bottomed ocean driven by uniform upwelling, predicts that flow is poleward and eastward throughout the interior ocean. However, in both the Pacific and Atlantic, the westward-extending tongues have the proper characteristics for CPW, which thus spreads equatorward from the Southern Ocean in the eastern South Pacific and South Atlantic. Thus, if the Stommel and Arons model were appropriate, the westward flow we observe would have to lie above the maximum in deep upwelling (*16*). The equatorial tongues, which suggest eastward flow, have been well-documented in the Atlantic (*12–15*); Böning and Schott's recent model (*17*) suggests that a kelvin wave mechanism can produce such a current and its slight southward shift relative to the equator. A second model that has been suggested for the South Pacific warm tongue is hydrothermal forcing from the East Pacific Rise (*7, 9*). Hydrothermal heating probably increases the temperature of waters in the eastern Pacific, although vertical diffusion also may be effective in spreading heat and salt downward in the tropics. The stronger signature of the warm tongue in the South Pacific relative to the North Pacific may be the result of asymmetric thermal forcing from the ridge crests. However, hydrothermal forcing is not likely the sole forcing mechanism for these deep low-latitude flows because there is no hydrothermal signature in the Atlantic.

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Extension of Life-Span by Overexpression of Superoxide Dismutase and Catalase in *Drosophila melanogaster*

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The hypothesis that oxygen free radicals are causally involved in the aging process was tested by a study of the effects of simultaneous overexpression of copper-zinc superoxide dismutase and catalase. As compared to diploid controls, transgenic flies carrying three copies of each of these genes exhibited as much as a one-third extension of life-span, a longer mortality rate doubling time, a lower amount of protein oxidative damage, and a delayed loss in physical performance. Results provide direct support for the free radical hypothesis of aging.

Although numerous hypotheses have been advanced, the nature of the causal mechanisms underlying the aging process is poorly understood and is a subject of intense debate. One hypothesis suggests that oxygen free radicals and hydroperoxides, collectively termed reactive oxygen species (ROS), are causal factors in aging (*1*). Reactive oxygen species are initially produced by the univalent reduction of dioxygen to generate sequentially superoxide anion radical and hydrogen peroxide. The latter, if not eliminated, generates the highly reactive hydroxyl free radical, which is widely believed to be the main agent of oxidative damage (*2*). The main assumption of the free radical hypothesis of aging is that the normal level of antioxidant defenses is not fully efficient, so that a fraction of ROS escape elimination. These ROS inflict molecular damage, some of which is irreparable and accumulates with age, thereby causing functional attrition associated with aging. Although this hypothesis is intuitively appealing, as a result of the ubiquitous generation of the potentially deleterious ROS, a direct causal link between ROS and aging has not been established.

If ROS are indeed a causal factor in aging, the enhancement of the defenses against ROS should reduce oxidative stress, decrease the rate of aging, and extend life-span. The present study tests the predictions of the free radical hypothesis by the examination in *Drosophila melanogaster* of the effect of the overexpression of Cu-Zn superoxide dismutase (SOD) and catalase genes, which, acting in tandem, provide the primary enzymatic antioxidant defenses. Superoxide dismutase converts superoxide anion radical to H_2O_2, and catalase breaks down H_2O_2 into water and oxygen, thus eliminating the possibility of the production of the highly reactive hydroxyl free radical. Because glutathione peroxidase, another enzyme involved in H_2O_2 removal, is absent in insects (*3*), SOD and catalase constitute the first coordinated unit of defense against ROS. The simultaneous overexpression of Cu-Zn SOD and catalase in an isogenic background was found to extend the life-span and slow down various age-related biochemical and functional alterations in *Drosophila melanogaster*.

Before the construction of transgenic lines overexpressing both Cu-Zn SOD and catalase, *Drosophila* lines bearing extra copies of SOD alone and catalase alone were created in an isogenic background (*4*). To generate flies with one extra copy of the SOD gene and one extra copy of the catalase gene, as well as control flies with two vector-only inserts, transgenic lines were first made heterozygous for dominantly marked balancer chromosomes (*5*). Appropriate heterozygotes were allowed to mate

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Table 1. Comparison of SOD and catalase activities, and mortality pattern between transgenic and control flies. Mortality data are based on initial samples of 100 flies in each group. Enzymatic activities were measured in whole-body homogenates of the flies according to the procedures in (*7*). Enzyme activity values are mean ± SEM of three to six determinations. MRDT was measured as ln 2/*G*, where *G* is the slope of the Gompertz plot. All experiments were repeated one or more times with essentially similar results.

Group	Strain	SOD activity (units)	Catalase activity (units)	Mortality (days)			MRDT (days)
				Median	90%	100%	
Control	Control	13.1 ± 0.6	19.2 ± 0.8	54.5	64	71	8.4
A	cat1.5 sod1.4	16.5 ± 0.3	33.2 ± 0.9	63	78	95	10.1
B	cat1.1 sod1.4	17.3 ± 0.1	27.5 ± 0.4	58	74	81	9.9
C	cat1.1 sod1.2	16.8 ± 0.5	31.6 ± 0.7	72.5	83	93	11.5

and, in the next generation, w^+ male progeny lacking dominant markers were selected. Initially we examined 15 different SOD and catalase overexpressor lines for survivorship. Of these, eight showed a positive effect on life-span relative to control strains, six showed no effect, and one showed a negative impact. Such results were not entirely unexpected given the possibility of position effect on the insert as a result of variability in the location of P-element construct insertion. Thus, the modulation of antioxidant gene expression by *cis* regulatory elements near the site of insertion or impact of insertion on other nearby genes that affect viability could counteract the positive effects of overexpression. Three of the strains exhibiting extended life-span were selected and were the object of the detailed analysis presented in this study. Four different control groups, containing single P-element inserts in the second and third chromosome, were generated. One of these groups had low viability, most probably due to position effects, and was discarded. The remaining three control groups were analyzed for life-span and for activities of superoxide dismutase and catalase and were found to be remarkably similar. There were no statistically significant differences between the groups (6), and SEM values ranged between 2 and 4.8%. One of these groups was then used as a control for the experimental flies.

The SOD and catalase activities in the transgenics used in this study are listed in Table 1 (*7*). The effect of SOD and catalase overexpression on the mortality of the flies is presented (Fig. 1A and Table 1). The onset of median, 90%, and 100% mortality (maximum life-span) in the transgenic lines was delayed as compared to the controls. Relative to the controls, the percent increase in the transgenic lines ranged from 14 to 34% in the maximum life-span, 16 to 30% in the onset of 90% mortality, and 6 to 33% in the median life-span. A nonparametric analysis of survival data indicates that all three transgenic lines had a significantly longer life-span than that of the controls (6).

Age-specific mortality rates were plotted on a semi-log scale (ln) against age to obtain the exponential or Gompertz mortality rate coefficients, which are widely used to compare the aging rates of populations (8). The slope of the Gompertz plot was significantly steeper in the control group when compared to any of the three transgenic groups (9). Mortality rate doubling time (MRDT) of the transgenic lines, calculated from the slopes of the Gompertz plots (8), were prolonged by 20 to 37% as compared to the control (Fig. 1B and Table 1).

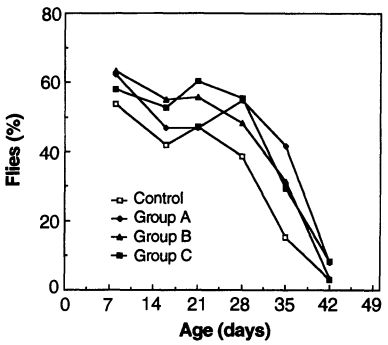

Fig. 3. Walking movement of flies at different ages. Negative geotaxis is a behavioral characteristic of flies to walk against gravity. Groups of 25 flies were gently tapped to the floor of a 50-ml glass cylinder. The percentage of flies walking 15 cm in 15 s was recorded. To avoid bias in sampling, testing was discontinued after 42 days of age because of the onset of mortality and the dramatic slowdown of the flies. Each point represents the average of 10 tests. The experiment was repeated twice with essentially similar trends.

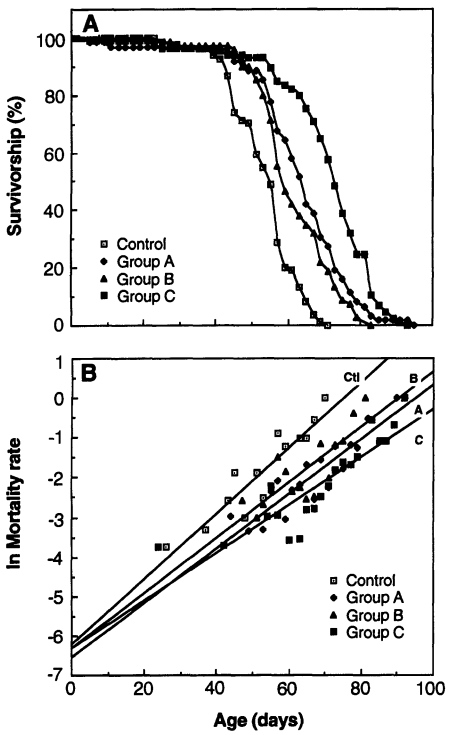

Fig. 1. Survivorship curves and mortality rates of adult male transgenic *Drosophila*. (**A**) Survivorship curves are based on an initial sample size of 100 in each group aliquoted into shell vials in batches of 25 flies each and are representative of two separate experiments. Flies were kept at 25°C in constant light and transferred daily. (**B**) Mortality rates of these flies are graphed on a semilogarithmic scale (Gompertz plots).

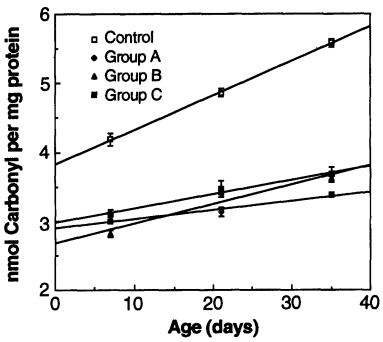

Fig. 2. Protein carbonyl content of flies at different ages. Protein carbonyls were measured in the whole-body homogenates by the method of Levine and co-workers with the use of 2,4-dinitrophenylhydrazine (*14*) as described in (*15*). Results are average ± SEM of four determinations.

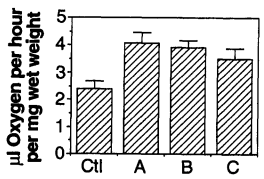

Fig. 4. Rate of oxygen consumption of flies at 40 days of age. Oxygen consumption was measured with a differential Gilson respirometer. For each determination, 50 flies were weighed and placed in the main chamber of the Warburg respirometer flask and allowed to equilibrate for 15 min with open stopcock, followed by 15 min of shut stopcock. Oxygen uptake was measured in the next 3-hour period. Results are based on average ± SD of seven to nine determinations. Ctl, control.

To determine if the overexpression of SOD and catalase genes lowered the in vivo oxidative damage to the tissues, a comparison of protein carbonyl content was made between the control and transgenic flies at different ages (Fig. 2). Cellular proteins undergo extensive oxidative modifications, manifested as carbonyl derivatives, as a consequence of aging in several model systems (*10*). At all ages, the protein carbonyl content of transgenic flies was notably lower than that of the controls. Furthermore, the age-related increase in carbonyl content was significantly slower in the transgenic lines as compared to the control flies, according to a test of the equality of regression lines, indicating that the latter suffer relatively greater age-associated oxidative damage to the proteins.

To examine further whether the transgenic and control flies have a different rate of aging, a test of physical fitness, which compares walking ability, was performed at different ages (*11*). The walking movement of the transgenic flies was superior to the controls at virtually all ages until 35 days of age (Fig. 3) (*12*). Until this age, a higher percentage of transgenic flies than of controls retained the specified speed of walking (1 cm s^{-1}). Relatively few flies from any group retained this speed of walking at 42 days of age. However, the test used for comparing walking capability only determines the percentage of flies with the ability to walk faster than 1 cm s^{-1}; it does not distinguish differences at lower speeds. Indeed, it was also empirically observed during fly husbandry that the transgenic flies tended to be relatively more active than the controls, especially at older ages. At 40 days of age, the rate of oxygen consumption of the control flies was 58 to 65% of the transgenic fly rate ($P < 0.001$); no significant differences were noted at 10, 20, or 30 days of age (Fig. 4).

Considered together, results of this study indicate that the overexpression of SOD and catalase has the effect of slowing the aging process in *Drosophila*. The lengthening of the MRDT and the extension of the life-span in the transgenics by up to one third compared to the controls suggest that there is an effect on the underlying rate of aging. This inference is further supported by the findings that transgenic flies exhibit delayed loss of motor ability, a lower level of protein oxidative damage, and a higher metabolic rate at older age than that of control flies.

The prolongation of the chronological length of life alone is not a sufficient criterion for the identification of factors affecting the aging process, especially in poikilothermic animals. For example, life-spans of poikilotherms can be considerably extended by regimes, such as ambient temperature or physical activity, that lower the metabolic rate (*13*). However, the metabolic potential or the total amount of oxygen consumed during life is not altered by these regimes. Obviously, increased life-span without a corresponding increase in metabolic potential would not be a meaningful gain from the perspective of the aging process. In the present study, the average metabolic rate of the transgenic flies was similar to that of the control flies at younger ages but was higher than that of the controls at older ages. The longer life-span of transgenics than that of the reference flies suggests that the metabolic potential of the transgenic flies is greater than that of the controls. Thus, the overexpression of antioxidant genes may also have important implications in the determination of the metabolic potential or the total amounts of oxygen consumed by the organisms during their lifetime.

Finally, the results of this study underscore the importance of an optimum balance between SOD and catalase. In our previous studies, the overexpression of Cu-Zn SOD alone or catalase alone had only a minor incremental effect (up to 10%) on the average life-span, but none on the maximum life-span of *Drosophila*. It would seem that co-overexpression of the two genes provides an efficient strategy for lowering the level of oxidative stress and extending life-span.

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