A causative role for reactive oxygen species (ROS) in aging processes, referred to as the free radical theory of aging (1), proposes that ROS in biological systems attack molecules and cause the functional decline of organ systems that eventually leads to death. Accumulation of this damage over time is thought to result in pathologies associated with aging, including arteriosclerosis, neoplasia, and cataracts (2). ROS are generated, in large part, from single electrons escaping the mitochondrial respiratory chain and reducing molecular oxygen to form the superoxide anion (O$_2^-$). Superoxide dismutase (SOD) converts O$_2^-$ to hydrogen peroxide (H$_2$O$_2$) that then produces a highly reactive hydroxyl radical (OH) in the presence of reduced metal atoms unless H$_2$O$_2$-induced aconitase inactivation was attenuated, and the development of mitochondrial deletions was reduced. These results support the free radical theory of aging and reinforce the importance of mitochondria as a source of these radicals.

To determine the role of reactive oxygen species in mammalian longevity, we generated transgenic mice that overexpress human catalase localized to the peroxisome, the nucleus, or mitochondria (MCAT). Median and maximum life spans were maximally increased (averages of 5 months and 5.5 months, respectively) in MCAT animals. Cardiac pathology and cataract development were delayed, oxidative damage was reduced, H$_2$O$_2$ production and H$_2$O$_2$-induced aconitase inactivation were attenuated, and the development of mitochondrial deletions was reduced. These results support the free radical theory of aging and reinforce the importance of mitochondria as a source of these radicals.

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To determine whether the expression of PCAT, NCAT, or MCAT could modulate life span, we maintained transgenic animals and wild-type littermates until death. PCAT animals showed a slight extension of median life span of 3 months (10%) and 3.5 months (13%) in the two founder lines compared with controls (Fig. 3A); this was significant only for the 2088 line (P = 0.02). Differences in maximal life span were not statistically significant. NCAT mice showed only 1-month (4%) and 3-month (11%) increases in median life span in the two founders; neither was significant (Fig. 3B). Targeting catalase to the mitochondria, however, afforded 4.5-month (17%, P < 0.0001) and 5.5-month (21%, P = 0.0002) increases in median life spans of founders 4403 and 4033, respectively (Fig. 3C). There was a similar extension of maximal life span: The 10% longest-lived MCAT animals showed a 4.5-month longer median life span than wild-type littermates (both founders combined, P = 0.001). Increased life span was evident in both males (P < 0.0001) and females (P = 0.0003) without any statistically significant sex differences (fig. S2). The MCAT longevity data fit a Gompertz distribution (exponential increase in mortality rate with age) with parallel log mortality rates for MCAT and wild-type littermates (fig. S3), a result often interpreted as a delay in onset of aging. None of the transgenic lines showed a difference in weight or food consumption when compared to littermate controls (table S1), and there were no gross physical abnormalities.

Young (9 to 11 months) and older (20 to 25 months) MCAT and wild-type littermates were examined by histopathology. Little ab-
normality was seen in either group at 9 to 11 months of age. In older animals, there was a trend toward reduced splenomegally and splenic lymphoid neoplasia in MCAT (1 of 21) compared with wild-type (4 of 24) mice, but this effect was not statistically significant. Cardiac pathology (subendocardial interstitial fibrosis, hyaline cytoplasmic change, vacuolization of cytoplasm, variable myocyte fiber size, hypercellularity, collapse of sarcomeres, mineralization, and arteriolosclerosis) was the most consistent difference between 20- to 25-month MCAT and wild-type mice. These changes are also commonly observed in elderly human hearts, often in association with congestive heart failure (14); the latter has also been associated with functional abnormalities of mitochondria (15). The severity of pathology was graded on a score of 0 to 4 for a cross-sectional cohort of 21 MCAT and 20 wild-type mice age 20 to 25 months from both founder lines. The severity of arteriosclerosis was 1.29 on average for MCAT and 1.85 for wild-type (P = 0.04). The severity of cardiomyopathy was 1.19 for MCAT and 2.00 for wild-type (P = 0.004; P = 0.002 when combined with arteriosclerosis). This demonstrates the potential of the MCAT protein to protect the heart and suggests that these mice experience a prolonged health span as well as life span. The severity of cataracts, quantitated on a four-point scale by slit-lamp examination, was reduced in 17-month-old founder 4033 MCAT mice compared with age-matched wild-type mice (1.5 ± 0.13 and 1.95 ± 0.13, respectively, P = 0.003) but not in founder 4403 compared with wild-type. However, this trend became of borderline significance at 27 months (P = 0.06), and by the age of 30 months both groups had similar cataract scores of ~2.5.

Fig. 2. Mitochondrial localization of human catalase. MCAT (A) and WT (B) mouse cardiac tissue (9 months old) stained for human catalase (green) and the mitochondrial marker cytochrome c (red) with a 4',6'-diamidino-2-phenylindole (DAPI) nuclear counterstain (blue). MCAT (C) and WT (D) mouse embryonic fibroblast cultures stained for human catalase (green) and the mitochondrial marker prohibitin (red) with a sytox green nuclear counterstain (blue). Scale bars indicate 20 μm.

Mitochondrial deletions associated with oxidative damage were measured as low molecular weight products by long-extension polymerase chain reaction (LX-PCR). These increased with age in both wild-type heart and skeletal muscle (16); however, a statis-
tically significant decrease in the number of deletion products was noted in 21-month-old MCAT skeletal muscle (Fig. 4F). A decrease was also detected in 30- to 33-month-old MCAT skeletal muscle and 21-month-old MCAT heart, but neither reached statistical significance.

To examine the possibility that combined enhanced antioxidant defenses might provide further extension of life span in mammals, we bred hemizygous PCAT-overexpressing animals to hemizygous SOD1-overexpressing animals (17). The double transgenic mice had an 18.5% extension of median life span compared with wild-type ($P < 0.0001$) and a 7% extension compared with PCAT littermates ($P = 0.036$), but without extension of maximum life span (Fig. 3D). There were no apparent deleterious phenotypic changes in these animals. It seems likely that SOD1 mRNA life span extension phenotype. Mosaicism may result from selection against cells expressing high catalase activity during early development because ROS may be an important mitogen (20). In addition, silencing of the CAG promoter-enhancer and/or the progressive loss of transgene expression as the founder C3H alleles from the B6 (B6C3F1) hybrid embryos were diluted out through successive B6 back crosses may have reduced or modified MCAT expression (21). As a result, the observed MCAT protection against mitochondrial $H_2O_2$ toxicity, oxidative DNA damage, and mitochondrial DNA deletion accumulation might have been much higher in the aging cohort mice than in the mice that were subsequently tested in biochemical assays. Aging cohort and cardiac pathology studies were performed on mice two to four generations after establishing the transgenic lines. Biochemical tests were done at generation 9 or later, when the genetic background was >99% B6 and the mice had been moved to a new facility, and the life span extension phenotype appears to be diminished. Nonetheless, these results support the conclusion that mitochondrial ROS can be an important limiting factor in determining mammalian longevity and provide impetus for studies of new and combined antioxidant mouse models.

**References and Notes**

12. Materials and methods are available as supporting material on *Science* Online.
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**Supporting Online Material**

www.sciencemag.org/cgi/content/full/1106653/DC1

Materials and Methods

Figs. S1 to S3

Table S1

References

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