

## Oxidative Damage and Aging: Spotlight on Mitochondria

Nancy J. Linford,<sup>1</sup> Samuel E. Schriener,<sup>2</sup> and Peter S. Rabinovitch<sup>1</sup>

<sup>1</sup>Department of Pathology, University of Washington, Seattle, Washington and <sup>2</sup>Center for Molecular and Mitochondrial Medicine and Genetics, Departments of Biological Chemistry and Ecology and Evolutionary Biology, University of California, Irvine, Irvine California

### Abstract

**Whereas free radical damage has been proposed as a key component in the tissue degeneration associated with aging, there has been little evidence that free radical damage limits life span in mammals. The current research shows that overexpression of the antioxidant enzyme catalase in mitochondria can extend mouse life span. These results highlight the importance of mitochondrial damage in aging and suggest that when targeted appropriately, boosting antioxidant defenses can increase mammalian life span.** (Cancer Res 2006; 66(5): 2497-9)

### Background

The free radical theory of aging, originally proposed a half century ago, asserts that buildup of macromolecular damage from oxygen free radicals leads to the functional decline associated with aging in multicellular organisms (1). According to the current theory, leakage of electrons from the mitochondrial electron transport chain leads to production of superoxide, which is converted to hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) by superoxide dismutase (SOD). H<sub>2</sub>O<sub>2</sub> production was originally estimated at up to 2% of total resting electron flux (2) but current studies suggest this value is closer to 1/10 of the original estimate or less (3). The highly reactive hydroxyl radical is then generated by the Fenton reaction in the presence of reduced metal atoms unless H<sub>2</sub>O<sub>2</sub> is reduced by a variety of scavenging enzymes including mitochondrial glutathione peroxidase, mitochondrial peroxiredoxin, and catalase. The latter is principally located within peroxisomes in mammals. If these antioxidants are not effective, then oxidation of intracellular macromolecules will occur and build up over time, particularly in differentiated tissues. Support for this theory comes from evidence of the accumulation of oxidatively damaged proteins, lipids, and DNA with age and from increased expression of antioxidant proteins with aging (4), indicating a response to oxidative stress. Additionally, accumulation of oxidatively damaged proteins is reduced in long-lived mouse models (4), establishing a correlation between longevity and reduced oxidative damage. Attempts to directly test the relationship between oxidative damage and life span have met with mixed results. In *Drosophila*, overexpression of catalase and SOD or SOD2 alone has led to life span extension in some studies (5, 6), but not in others (7, 8), a result at least partially attributable to the background strain chosen. Use of antioxidant enzyme mimetics in *C. elegans* has similarly met with mixed results and may be dependent on husbandry conditions (9, 10). In mice, the overexpression of SOD1 had no effect on life span (11) although increased thioredoxin was reported to increase life span in short-

lived mice (12). One possible explanation for the lack of response to antioxidant overexpression in some models is that whereas considerable oxidative damage builds up in the cell, the localization of the antioxidant protein is crucial for an effect on life span. In our studies, the goal was to overexpress antioxidant enzymes in different intracellular compartments and examine the effect on mouse life span.

In mice, catalase is primarily expressed in liver, kidney, and erythrocytes. Normally, catalase functions in the peroxisome to break down the H<sub>2</sub>O<sub>2</sub> generated by  $\beta$ -oxidation of long-chain fatty acids but it can also break down H<sub>2</sub>O<sub>2</sub> diffusing in from other sources if intracellular H<sub>2</sub>O<sub>2</sub> reaches sufficiently high levels. However, the nucleus is left virtually unprotected although damage to DNA, if left unrepaired, can lead to mutations, genomic instability, cancer, and other phenotypes of aging. Furthermore, the mitochondrion is only partially protected although it is both a source of H<sub>2</sub>O<sub>2</sub> through leakage of the electron transport chain and a major target for damage that would lead to a reduction in metabolic function (Fig. 1A). Mitochondrial H<sub>2</sub>O<sub>2</sub> is reduced by mitochondrial antioxidant enzymes, such as glutathione peroxidase and mitochondrial peroxiredoxin, but is dependent on the presence of reduced cofactors. With the potential targets of free radical damage in mind, catalase was overexpressed in mice with a targeting signal for the peroxisome (endogenous sequence, PCAT), nucleus (NCAT), or mitochondrion (MCAT), under the control of a  $\beta$  actin promoter, which achieved high levels of expression, particularly in heart and muscle. Nuclear localization of NCAT (13) and mitochondrial localization of MCAT (14) was confirmed by immunofluorescence. In the MCAT animals, expression of mitochondrial catalase was robust but mosaic in nature (Fig. 1C) in all tissues examined.

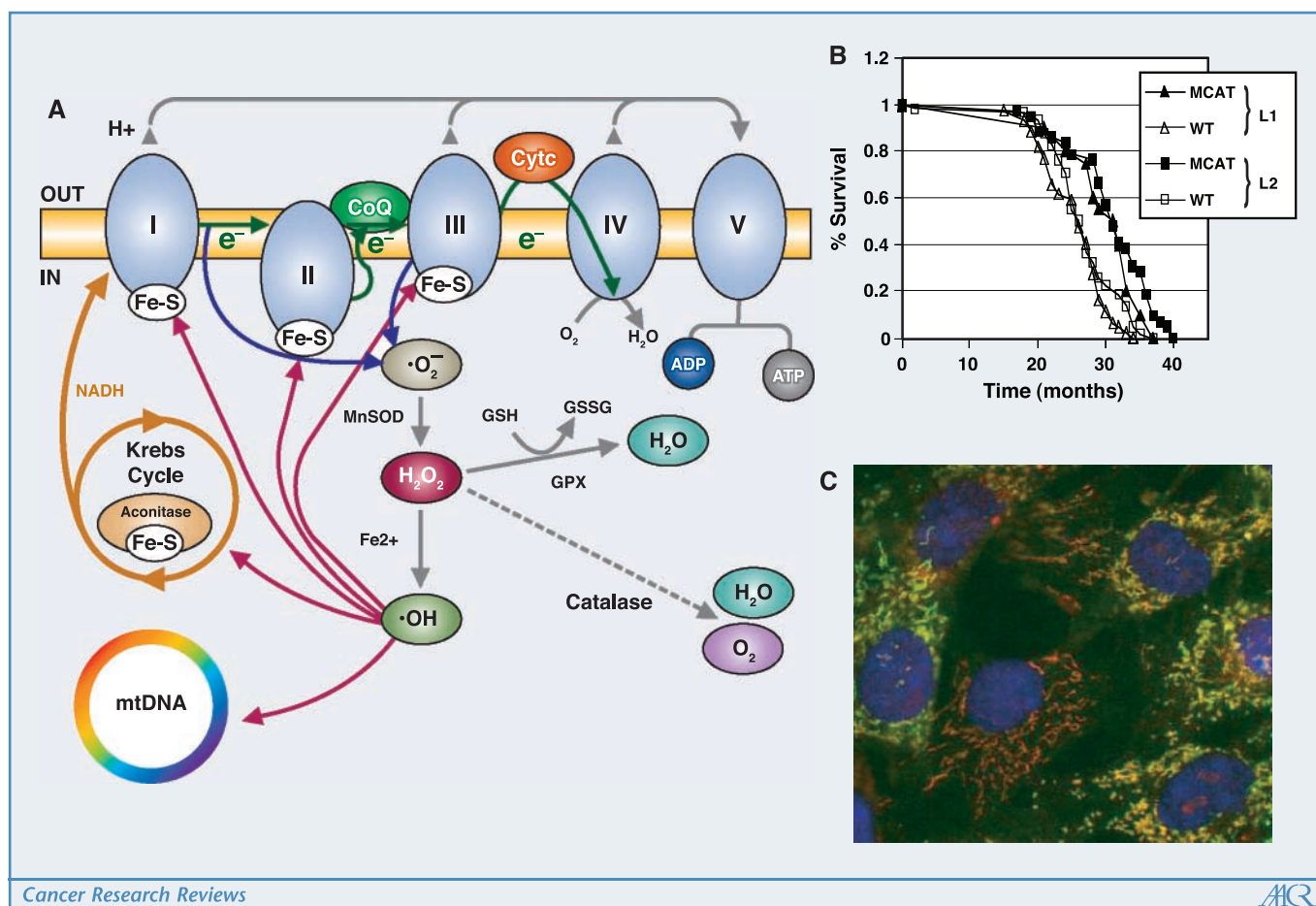
### Key Finding

Life spans of cohorts from two founder lines for each of PCAT, NCAT, and MCAT were compared with littermate controls (Fig. 1B). There was a small increase in median life span for NCAT and PCAT but the MCAT lines showed an approximated 20% increase in both median and maximal life span (14). The PCAT line was also crossed to a line of mice overexpressing SOD1, which had previously been shown to not affect life span (11). Overexpression of both genes led to substantial increase in median life span, indicating that SOD1 overexpression can enhance the effects of other antioxidants. Because increase in maximal life span is believed to be indicative of a change in the underlying aging processes, the MCAT mice, with an increase in both mean and maximal life span, and robust mitochondrial expression, primarily heart and skeletal muscle, were investigated further (14).

Reduction in H<sub>2</sub>O<sub>2</sub> release from isolated cardiac mitochondria, decreased accumulation of oxidized DNA, and decreased susceptibility of mitochondrial aconitase to H<sub>2</sub>O<sub>2</sub>-induced damage were all observed in MCAT mice, all consistent with elevated antioxidant

**Requests for reprints:** Nancy J. Linford, Department of Pathology, University of Washington, Box 357705, 1959 NE Pacific Avenue, HSB K081, Seattle, WA 98195. Phone: 206-616-8201; Fax: 206-616-8271; E-mail: nantzee@u.washington.edu.

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**Figure 1.** A, proposed mechanism of free radical generation in mitochondria and protection by catalase. Leakage of free electrons from the electron transport chain (blue arrows) leads to production of superoxide; the latter is converted to hydrogen peroxide, which must be reduced if damage to cellular machinery is to be avoided. When catalase is targeted to mitochondria, the ability to reduce locally generated hydrogen peroxide is increased. B, mitochondrial overexpression of catalase leads to extension of mean and maximum life span when compared with littermate controls. L1 and L2, independently generated transgenic founder lines. C, catalase (green) overexpression is targeted to mitochondria (red) in mouse embryonic fibroblast cells isolated from MCAT mice with a mosaic pattern of expression.

defenses in mitochondria of these animals. Aconitase inactivation by H<sub>2</sub>O<sub>2</sub> was attenuated in young, middle-aged, and old animals. Additionally, the presence of mitochondrial DNA deletion products, proposed to be associated with oxidative damage (15), was reduced in the aged MCAT animals. Reduction in the appearance of cardiac pathology (mineralization, arteriosclerosis, and myopathy) and cataracts was observed with age in a cross-sectional study of MCAT mice, showing a link between increased mitochondrial antioxidant defenses and alteration in the pathologies of aging. These results together suggest that not only do the MCAT mice display increased life span, they also have increased resistance to oxidative stress at sites of potential damage, reduced evidence of oxidative damage with age, and reduced incidence of aging pathologies associated with oxidative damage.

### Meaning and Implications

The current results highlight the importance of mitochondrial damage in aging and suggest that when targeted appropriately, increasing antioxidant defenses can increase mammalian life span. It is notable that these mice appear indistinguishable from

the controls in size, morphology, and eating habits. This sets the current mouse model apart from other models of extended life span in which there are major size differences compared with controls and extensive disruption of endocrine pathways.

Whereas the current result is somewhat unexpected in light of mixed results achieved in attempts to reduce oxidative stress in other organisms, it highlights the possibility that the effects of modulation of hydrogen peroxide production may depend very strongly on the location (both intracellular and tissue-specific), timing, and level of expression as well as the particular cause of death associated with different strains and organisms. Alleviating oxidative damage will only be effective in extending life span if the “weakest link” for a particular organism is caused by oxidative damage. The current result indicates that increased mitochondria-specific antioxidant defenses can have an effect on the pathologies of aging and life span and that refinement of this strategy may increase the life span extension even further.

The most likely explanation for the extension of life span by mitochondrial catalase overexpression is by preventing the damage resulting from slow leakage of electrons from the electron transport chain. This oxidative damage is presumed to damage the DNA, lipids, and proteins, particularly in the mitochondria

(Fig. 1A). However, rapid diffusion of H<sub>2</sub>O<sub>2</sub> could lead to damage in other parts of the cell. Mitochondrial DNA damage has been associated with accelerated aging in animal models (16), a finding consistent with the present work. Whereas glutathione peroxidase and mitochondrial peroxiredoxin can reduce H<sub>2</sub>O<sub>2</sub> in the mitochondrion, catalase would reduce H<sub>2</sub>O<sub>2</sub> in a manner that is independent of the availability of electron donors and prevent a potential "vicious cycle" associated with oxidative damage, leading to the production of even more oxidative damage. This type of damage buildup would accumulate over the life span, particularly in differentiated tissues such as heart and skeletal muscle.

However, whereas prevention of oxidative damage is the likely explanation for the current result, there is also a sizeable literature showing the usefulness of H<sub>2</sub>O<sub>2</sub> as an intracellular signal transducer and mitogen (17). Therefore, alternative explanations, such as reduction in the mitogenic potential of H<sub>2</sub>O<sub>2</sub> within

cancerous cells, would contribute to life span extension in the mouse in which tumors are found in the majority of end-of-life cases. An additional possible explanation would be that reduction in H<sub>2</sub>O<sub>2</sub> signaling could lead to a mild stress that would cause a life span-extending hormetic response and potentially explain the mosaic expression pattern. Furthermore, possible beneficial effects of mitochondrial catalase expression include potential prevention of reactive oxygen species-induced apoptosis in postmitotic tissues and suppression of H<sub>2</sub>O<sub>2</sub> signaling in immune response.

A second generation of MCAT mice is being developed to address some of these questions, particularly whether the catalase overexpression is necessary early or late in life and the effects of tissue-specific expression. Additionally, the extension of these results to other strains and other species will further determine the conditions under which prevention of mitochondrial oxidative damage is beneficial.

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