Extension of mouse lifespan by overexpression of catalase

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Received: 1 September 2005 / Accepted: 1 December 2005
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Abstract The free radical theory of aging was originally proposed 50 years ago, and is arguably the most popular mechanism explaining the aging process. According to this theory, aging results from the progressive decline in organ function due to the damage generated by reactive oxygen species (ROS). These chemical species are a normal part of metabolism, and a group of enzymes exists to protect cells against their toxic effects. One of these species is hydrogen peroxide (H_2O_2), which can be degraded by catalase. To determine the role of hydrogen peroxide in aging and its importance in different subcellular compartments, transgenic mice were developed with increased catalase activities localized to the peroxisome (PCAT), nucleus (NCAT), or mitochondrion (MCAT). The largest effect on lifespan was found

in MCAT animals, with a 20% increase in median lifespan and a 10% increase in the maximum lifespan. A more modest effect was seen in PCAT animals, and no significant change was found in NCAT animals. Upon further examination of the MCAT mice, it was found that H_2O_2 production and H_2O_2 -induced aconitase inactivation were attenuated, oxidative damage and the development of mitochondrial deletions were reduced, and cardiac pathology and cataract development were delayed. These results are consistent with a role of H_2O_2 in the development of pathology and in the limitation of mouse lifespan. They also demonstrate the importance of mitochondria as a source, and possible target, of ROS.

Key words aconitase · aging · catalase · hydrogen peroxide · lifespan · mitochondria · mitochondrial DNA · reactive oxygen species · superoxide dismutase

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Abbreviations

FRTA	free radical theory of aging
GPX	glutathione peroxidase
MCAT	mitochondrial-targeted catalase
mtDNA	mitochondrial DNA
NCAT	nuclear-targeted catalase
PCAT	peroxisomal (wild-type) catalase
ROS	reactive oxygen species
SOD	superoxide dismutase

The free radical theory of aging

The free radical theory of aging (FRTA) was originally proposed by Harman (1956) 50 years ago (Beckman and Ames 1998). A simplified view of his idea is that antioxidant defenses are suboptimal in an animal, which allows oxidative damage from endogenous reactive oxygen species (ROS) to accumulate, resulting in cell loss, disease, organ failure and, ultimately, death. The major players of this theory, and their relationships to each other, are diagrammed in Figure 1. In addition to these, there are many other enzymes and small molecules involved in free radical biology which are not shown.

A principal function of mitochondria is to convert the energy in the food we eat into a form that our cells can use. This results in the generation of low energy electrons that are normally disposed of by the reduction of O_2 to H_2O . On rare occasions, electrons “escape” this process to form superoxide ($O_2^{\cdot-}$), which is a chemically reactive compound. A group of enzymes, called the superoxide dismutases (SOD), have evolved to convert $O_2^{\cdot-}$ to a less active compound, hydrogen peroxide (H_2O_2). However, in the presence of reduced iron (Fe^{2+}), H_2O_2 can be reduced to the

Figure 1 Simplified pathway showing the major reactive species and detoxifying enzymes, and their relationships pertinent to the free radical theory of aging. Catalase, but not GPX, generates O_2 from H_2O_2 . MT, mitochondria; SOD, superoxide dismutase; GPX, glutathione peroxidase

highly reactive hydroxyl ($\cdot OH$) radical. This radical attacks any and all molecules in the cell. It is here that catalase, the glutathione peroxidases (GPX), and others mediate their effects by degrading H_2O_2 and avoiding the generation of $\cdot OH$.

To date, numerous experiments have been conducted in animals to evaluate the FRTA. Up until the last 10 to 15 years, most of these experiments were dietary in nature and were conducted by feeding animals small antioxidant molecules, which frequently resulted in increases in average lifespan without corresponding increases in maximum lifespan (Harman 1968; Miquel and Economos 1979; Enesco and Verdone-Smith 1980). This result may be interpreted as rescuing a nutritional deficiency or a particular pathology, while not actually attenuating the aging process. Alternatively, it is possible that oral administration did not allow the antioxidant molecules to reach crucial sub-cellular compartments such as the mitochondrion. More recent studies of the FRTA have resulted in increased maximum lifespans. These include the overexpression of SOD and catalase (Orr and Sohal 1994) in the fruit fly, the treatment of worms with small molecules which have SOD/catalase activities (Melov et al. 2000), and thioredoxin (Mitsui et al. 2002) in the mouse. Several other life extension studies also support the FRTA in an indirect manner by suggesting that reduced ROS levels are a significant contributor to the life extension phenotype in these models (Larsen 1993; Lin et al. 1998; Migliaccio et al. 1999). The finding that the overexpression of catalase (Schriner et al. 2005) extends mouse lifespan also lends support to a role of ROS in the limitation of lifespan.

The merits of catalase

Catalase is a heme-containing tetrameric protein composed of 60 kD monomers. Its activities are highest in the liver, kidney, and red blood cells and relatively low in the brain, heart, and skeletal muscle. The majority of catalase is localized to the peroxisome, where it is thought to decompose H_2O_2 generated from enzymes involved in the

oxidation of fatty acids (Percy 1984; Halliwell and Gutteridge 1999). It may also be present to some extent in heart mitochondria (Radi et al. 1991). While there is only a single structural gene in mammals, catalase activity may be regulated by as many as 19 other loci (Schisler and Singh 1991). The enzyme has two catalytic activities. At relatively high H_2O_2 levels, it converts two H_2O_2 molecules to water and oxygen (Figure 2). At lower levels, when a second H_2O_2 may be in limited supply, it can act as a peroxidase and oxidize a variety of substrates including alcohols, formic acid, nitrite, mercury, and β adrenergic hormones (Percy 1984; Halliwell and Gutteridge 1999). Mice deficient in catalase have been recently developed with surprisingly limited physiological consequences (Ho et al. 2004).

In comparison to catalase, GPX is part of a more complex enzyme system. In order to degrade H_2O_2 , these proteins require another compound, reduced glutathione, which participates in its own reactions independent of GPX (Townsend et al. 2003). Two additional enzymes are important for maintaining GPX function. Glutathione reductase restores reduced glutathione levels while γ -glutamylcysteine synthetase catalyses the synthesis of glutathione. There are at least five GPX proteins in the mouse with varying expression patterns and enzyme activities. In addition to H_2O_2 , GPX proteins can also degrade organic peroxides, and Gpx4 is noted for its ability to degrade lipid peroxides (Imai and Nakagawa 2003).

When compared to GPX, catalase is a relatively simple enzyme system, with its singular structural protein and predominant function of H_2O_2 degradation. This argument also applies when one considers two other important antioxidant systems, the thioredoxin and peroxiredoxin families. Thus, the intent in overexpressing catalase was to ask a simple question: what happens

during aging in a mouse when H_2O_2 levels are lowered?

Catalase extends mouse lifespan

Our original interest when this project was initiated was to understand the role of oxidative nuclear DNA damage during aging. This led to the decision to target catalase to the nucleus (NCAT), in addition to overexpressing the wild-type peroxisomal catalase (PCAT), with the intent to specifically protect the nucleus from H_2O_2 -induced damage. The development of a mitochondrial-targeted catalase (MCAT) mouse was brought about by two arguments. As the majority of H_2O_2 is believed to be generated in mitochondrion, a mitochondrial catalase should afford the maximum level of cellular protection against H_2O_2 -induced damage, and a maximum benefit on lifespan and health span. The second advantage is that measuring ROS levels in vivo is notoriously difficult. The mitochondrial localization of catalase would allow us a relatively straightforward method for estimating the effects of additional cellular catalase on H_2O_2 levels. Functional mitochondria can be isolated and the amount of H_2O_2 diffusing out of the mitochondrion could then be measured.

To determine the relevance of H_2O_2 levels in lifespan and health span, we allowed cohorts of our PCAT, NCAT and MCAT animals to age until death. Of the three constructs, the MCAT enhanced lifespan the greatest (Table 1; Schriener et al. 2005), the PCAT a modest amount, and the NCAT apparently not at all. All three of our models utilized the same promoter and enhancer element which resulted in broadly similar, though not identical, patterns of expression and activities (Table 2). These results are consistent with the expectation that MCAT would afford the greatest advantage. While it is tempting to suggest that mitochondria are the primary target of H_2O_2 , there are also likely cytosolic targets as well, as evidenced by the life extension in the mice expressing the PCAT vector. The absence of a life extension phenotype in the NCAT animals does argue against a significant role of H_2O_2 in modulating lifespan through nuclear DNA damage.

Figure 2 Catalase enzyme activities. (A) Catalatic activity resulting in the degradation of H_2O_2 and production of O_2 . (B) Peroxidatic activity with the oxidation of a target molecule RH_2

Table 1 Catalase activities in catalase transgenic tissues relative to control tissues.

0, no change; + <2-fold; ++, 2–5-fold; +++ >5-fold.

Tissue	PCAT1	NCAT1	NCAT2	MCAT1	MCAT2
Liver	0	0	0	0	0
Kidney	0	0	0	0	0
Heart	+++	+++	+++	+++	+++
Muscle	+++	+++	++	+++	+++
Brain	+	+++	++	++	0

In addition to lifespan, the health of the MCAT animals was also improved. Compared to their littermate controls, MCAT animals were found to have a reduced prevalence of cardiac pathology including arteriosclerosis. This probably resulted from a combination of the high amount of MCAT expression in this tissue, its high concentration of mitochondria, and high rate of oxidative metabolism. The lens, a tissue also sensitive to oxidative damage, benefited from MCAT expression as well, as cataract formation was attenuated in these animals.

The mechanism of MCAT action on cell survival

The most direct explanation for the benefits of MCAT is simply that of lowered H_2O_2 levels.

Table 2 Percent lifespan extension in catalase and SOD1 transgenic mice relative to controls.

Line	Median	Maximum
PCAT1 ^a	13*	NS
PCAT2	NS ^b	NS
NCAT1	NS	NS
NCAT2	NS	NS
MCAT1	17**	10 ^{c**}
MCAT2	21**	10 ^{c**}
PCAT1 ^a	7*	NS
SOD1	NS	NS
PCAT+SOD1	19**	NS

^a Two independent aging cohorts of the same founder line.

^b There was a 10% increase in this line, but significance was likely not reached because of an exceptionally small sample size (n = 8).

^c P-values were calculated from a combined sample of both founders.

*P < 0.05; **P < 0.0005; NS, no significance.

MCAT may mediate this in two ways. The first is to lower the normative H_2O_2 levels resulting in reduced $\cdot OH$ formation, and decreased oxidative damage. The second would be to only protect the cell during a pathological burst of ROS formation, and not to degrade H_2O_2 at normal physiological levels. Since the FRTA argues that aging results from the accumulation of oxidative damage, we would predict that decreased ROS levels would result in lower levels of oxidative damage and a longer lifespan. Our results are consistent with this prediction. In the heart, where MCAT expression and activity were the greatest, we found that H_2O_2 levels produced in isolated mitochondria were lowered. In skeletal muscle, we also found that the levels of two markers of oxidative damage, 8-hydroxydeoxyguanosine, and mitochondrial DNA (mtDNA) deletions, were decreased. These results suggest that MCAT may function by decreasing H_2O_2 levels, and in turn, decreasing mtDNA damage resulting in an extended lifespan.

A second mode of action is that H_2O_2 levels may be reduced to such an extent as to impair a signaling pathway that would normally limit lifespan in some way. While we anticipate that the action of catalase would be limited to relatively higher levels of H_2O_2 resulting primarily from pathological oxidative stress, it may interact with the lower levels of H_2O_2 used in cell signaling pathways through its peroxidatic activity. Numerous signaling pathways may utilize H_2O_2 as a messenger. For example, it has been shown to act as a growth factor (Maulik and Das 2002), to signal apoptosis (Simon et al. 2000) and act as an inflammatory agent and activate B cells (Reth 2002). The potential for MCAT to block apoptosis may be especially relevant, and it is possible that attenuated cell death may be responsible for the extended lifespan. This is an intriguing possibility, as another age-retarded model also appears to be

anti-apoptotic (Migliaccio et al. 1999), while some age-accelerated models appear to mediate their phenotypes through increased apoptosis (Trifunovic et al. 2004; Tyner et al. 2002).

It should be mentioned that there is no difference in size between the MCAT animals and their controls, demonstrating that the MCAT does not significantly interfere with signaling pathways affecting development. This may in part be due to its mosaic expression pattern, but also suggests that MCAT may not impair endogenous signaling pathways which utilize H_2O_2 .

A third possible explanation is that MCAT extends lifespan through the process of hormesis. This is a phenomenon in which a mild initial stress can result in an increased stress resistance later on, likely through changes in gene expression (Rattan 2004). Catalase may do this by functioning as a pro-oxidant at the lower normative H_2O_2 levels. Under these conditions, it would bind a single H_2O_2 molecule and act as a peroxidase when a second molecule cannot be found. Through this action it can oxidize a number of physiological substrates. It also could generate oxidative stress by altering available iron concentrations in the cell, as it requires iron at its active site. Finally, as catalase produces O_2 as a result of H_2O_2 degradation, it may increase oxidative stress by increasing O_2 levels in anoxic parts of the cell. Increased O_2 or available iron levels may explain the ability of catalase overexpression to sensitize cells in culture to bleomycin and paraquat (Schriner et al. 2000).

While the reduced mtDNA damage may suggest a role in extending longevity, it may be only partly responsible, may be only correlative, or may independently result from the extended lifespan. To adequately determine the role of mtDNA damage in aging, one would need to develop a model with reduced levels of mtDNA damage without changes in ROS or antioxidant enzyme levels. A mouse expressing a mutant mtDNA repair enzyme, pol γ , was found to have increased mtDNA mutations, decreased mitochondrial function and several other phenotypes consistent with age acceleration including small body size, osteoporosis and cardiomyopathy (Trifunovic et al. 2004). A somewhat opposite treatment—increasing mtDNA damage—resulted in the op-

posite phenotype: a shortened lifespan. Thus, lifespan and mtDNA damage appear to be strongly correlated, and this is consistent with the action of MCAT through the protection of mtDNA.

It is becoming increasingly apparent that mitochondria are intimately involved in aging and disease. This is not surprising, as they generate the majority of cellular ATP, play a critical role in apoptosis and are involved in heat generation, ROS production, calcium buffering and multiple necessary biochemical pathways. Thus, it is easy to imagine that mitochondrial damage could result in pathology and aging. The poly mutant mice are the most visible example of this. However, in contrast to these animals, mice heterozygous for SOD2 have reduced enzyme activity and increased mitochondrial damage, yet no reduction in lifespan (Van Remmen et al. 2003). This is the strongest argument against both the FRTA and the importance of mitochondria in aging and disease. It is also noteworthy that mice heterozygous for a truncated p53 protein have a phenotype very similar to the poly mutant mice, though this is a large deletion affecting up to 24 genes, including at least one mitochondrial protein (Tyner et al. 2002; Gentry and Venkatachalam, 2005). These results raise a concern that any treatment that sensitizes cells to apoptosis may in fact generate an age-accelerated phenotype without actually being involved in the normal aging process. The resolution of the role of apoptosis and mitochondrial dysfunction in aging is a critical subject which warrants considerable further study.

Another issue which merits discussion regarding the action of MCAT is its mosaic expression pattern. One may question how it could benefit the animal when present in perhaps a minority of cells. One possibility is that those cells which do express MCAT have essentially no H_2O_2 , little or no DNA damage and severely retarded aging. Thus the animals are partly composed of a group of highly protected, non-aging cells and a group of normal cells that are vulnerable to all the regular physiological sources of damage. The alternate possibility is that MCAT acts in a non-cell-autonomous manner. This is reasonable, as H_2O_2 can cross lipid membranes. While MCAT expressing cells may be relatively more protected, they will also protect their neighbors to some

degree, resulting in a tissue with a reduced total H_2O_2 load.

A failure in flies

While it has been shown that overexpressing catalase and targeting it to the mitochondrion can extend the lifespan of the mouse, the identical experiment in *Drosophila* did not extend lifespan (Mockett et al. 2003), even though H_2O_2 levels were reduced (Kwong et al. 2000). One possibility is that flies are producing H_2O_2 levels that are much lower than mammals, and would not benefit from MCAT. Comparing our work and theirs, it appears that the rates of H_2O_2 production between fly and mouse mitochondria are roughly similar, of the order of 1 nmol/min/mg protein. However, these H_2O_2 measurements are usually conducted using isolated mitochondria in an artificial environment with much higher levels of O_2 and substrates than would be normally seen *in vivo*. Thus, these assays can only tell us if the MCAT protein has the potential to reduce H_2O_2 levels, and not that it actually does so *in vivo*. To answer this, one needs to look for changes in markers of oxidative damage and, unfortunately, this data has not yet been presented for the fly. The magnitude of the mitochondrial catalase enzyme activity may also be important. In the MCAT fruit fly, there were large increases in mitochondrial catalase activity (roughly 14-fold), and more modest increases in the total catalase activity (up to 2.5-fold). In our mice, we obtained somewhat greater increases in the levels of catalase activities. Activities in the heart mitochondrial fraction were increased 50-fold, and the total heart catalase activity was increased 50- to 100-fold.

Another confounding issue may be the expression pattern. The overexpression of SOD1 appeared to be ineffective with regard to lifespan when expressed in an entire animal, however this treatment did extend longevity when limited to the motor neurons (Parkes et al. 1998). The same phenomenon may affect the mitochondrial catalase, and it too may prove beneficial with a more restricted expression pattern. A similar situation in the MCAT mice may have been avoided by the

fortuitous expression of MCAT being predominantly limited to the heart and skeletal muscles. It also may have provided no benefit given a broader expression pattern.

Orr and Sohal's experiment in mice

In 1994, it was demonstrated that overexpressing catalase and SOD1 together could extend the lifespan of the fruit fly (Orr and Sohal 1994), despite the fact that each one alone could not (Orr and Sohal 1992, 1993). This indicated a synergistic action between these two antioxidant enzymes, and such a phenomenon is likely to occur in the mouse as well. This may be explained by the Fenton reaction if O_2^- were, in part, the species responsible for reducing iron, which would then generate $\cdot\text{OH}$ from H_2O_2 (Halliwell and Gutteridge 1999). In such a scenario, the overexpression of SOD would indeed result in a decrease in O_2^- levels, but this would be balanced by a corresponding increase in H_2O_2 , and unless O_2^- were completely removed, the impact on $\cdot\text{OH}$ production may be minimal. Overexpression of catalase by itself would not be expected to be beneficial unless there were an excess of H_2O_2 , a situation that may not normally occur without SOD overexpression. The fact that SOD alone extends lifespan when expressed in some tissues, or SOD2 in the mitochondria (Sun et al. 2002), suggests that these locations may already have an excess of catalase, or catalase-like activity, capable of degrading the additional H_2O_2 .

In order to determine if such a phenomenon could be replicated in mammals, mice overexpressing SOD1 from a genomic promoter were bred with the PCAT animals. While PCAT alone had a modest increase in mean lifespan, the addition of SOD increased it a further 8%. As in the fly, SOD1 alone did not increase lifespan in the mouse (Huang et al. 2000). Thus, a synergistic effect also appears to occur in the mammal. Interestingly, while there was an increase in median lifespan, no change in maximum lifespan was seen in either the PCAT alone or in combination with SOD. This is supportive of the view that the most vulnerable component of the cell may in fact be the mitochondrion.

Aconitase and aging

Aconitase is a TCA cycle enzyme which converts citrate to isocitrate. It contains an iron-sulfur center that is especially sensitive to oxidative stress and can be reversibly inactivated (Gardner and Fridovich 1992). Aconitase also directly binds mitochondrial DNA (Chen et al. 2005) and may regulate gene expression in response to energy demands and ROS production. In both flies and mouse brain, there has been shown to be an age-related loss of aconitase activity (Liang and Patel 2004; Yan et al. 1997). We also found this to be true in the mouse heart, which the MCAT protein was unable to attenuate in the absence of challenge (Figure 3A). However, the MCAT protein did protect aconitase in isolated mitochondria that were challenged with a bolus of H_2O_2 . This also supports the view that MCAT is not likely to lower the normative levels of H_2O_2 in the heart, but is available to act when H_2O_2 levels are elevated. Interestingly, in addition to the age-related decrease in absolute activity, there was an age-related increase in the sensitivity of aconitase to H_2O_2 challenge, which was attenuated by MCAT (Figure 3B). That is, as control animals aged, the ability of heart mitochondria to protect themselves from H_2O_2 decreased. These results argue that the antioxidant defenses of the heart mitochondria decrease as mice age, and that MCAT is able to bolster these defenses.

The loss of aconitase may be a potential explanation for the age-related decline in physical performance. However, Armstrong et al. (2004) take a view that the age-related loss of aconitase may actually be a defense against ROS production. They propose that aconitase acts as a sensor to ROS levels and, by being inactivated, it can directly reduce ROS formation by limiting electron flow into the respiratory chain. It is not too difficult to imagine that aconitase may then bind mtDNA to protect it from damage, and to also down-regulate proteins involved in oxidative phosphorylation, further reducing ROS formation. This may benefit the cell in the short run, but ultimately insufficient ATP will be generated, resulting in cell death. Even though we found no evidence that MCAT could stop the age-related decline in aconitase activity, mosaic expression

Figure 3 (A) Aconitase activities versus age in mouse heart mitochondria. (B) Proportion of aconitase activity remaining after H_2O_2 challenge

does not preclude this from happening in those cells with high MCAT activities.

MCAT and aging

Mice overexpressing a catalase localized to the mitochondrion were found to have an extended lifespan, but did they have a reduced rate of aging? Aging is very difficult to adequately define, but an essential parameter is an exponential increase in the probability of death per unit time. That is, as an animal ages its chance of dying increases.

The Gompertz model (Finch 1990) is the simplest mathematical model of lifespan in which the logarithm of mortality rate is plotted versus age,

resulting in an approximation of a straight line. In this model, lifespan can be minimally described by two parameters. One, the rate of aging, is the slope of line; the other, the Y-intercept, has been termed the “intrinsic vulnerability” or initial mortality rate. Mortality can be decreased by either slowing the rate of aging, resulting in a decreased slope (Figure 4A, line a), or by reducing the vulnerability of the animals, which would lower the Y-intercept (Figure 4A, line b), or both. When the MCAT life table data is plotted in this way, we see parallel mortality rate curves (Figure 4B), consistent with the latter scenario. The MCAT mice appear to have a reduced intrinsic vulnerability, that is, a lower mortality of similar magnitude at every point assayed in their lives, with no apparent slowing of the rate of aging. Thus, our mice are likely living longer not because they are aging more slowly, but because they are tougher. Because of the limited number of animals used in the lifespan studies, we cannot determine what the mortality rate curve looks like early in life. As a result, this could also be interpreted as delay in the onset of aging. Nonetheless, the rate of aging during most of the lifespan is not altered by MCAT. These results, along with the aconitase challenge experiments, are consistent with our original aim of using catalase: it would be largely benign except in the situation where the cell is under oxidative challenge. Thus MCAT may essentially act as a stress response protein, functional only when the animal is under stress.

The future

This work has demonstrated that reducing H_2O_2 in the mitochondrion can alter the lifespan of a mammal. Exactly how it does this has not been defined. While it appears that degradation of H_2O_2 under conditions of excessive oxidative stress with a concomitant increase in DNA damage is primarily responsible, several other possibilities, including modulation of cell signaling pathways and changes in the expression of other genes, cannot be ruled out.

In the current model, expression of MCAT is largely limited to a proportion of cells in post-

Figure 4 (A) Effects on mortality versus time when (a) the rate of aging is attenuated, or (b) the initial mortality rate is decreased. (B) Gompertz curve comparing MCAT transgenic and control mice. Slopes are not significantly different according to a linear regression analysis, ($P = 0.9$), but Y-intercepts are ($P < 0.0001$)

mitotic tissues. Therefore, one cannot say what would happen if MCAT were overexpressed in every cell in every tissue, or even in every cell of one particular tissue. Perhaps, like in the fly, expression in particularly vulnerable tissues will mediate the greatest effects. To express MCAT everywhere should result in a more dramatic phenotype, either an even longer-lived mouse or a dead one. One confounding factor in such an experiment is the potential function of H_2O_2 as a signaling molecule during development. It is possible that high levels of MCAT expression may lower H_2O_2 levels to such a degree as to impair development. To address these issues, we are actively developing new mouse models in which gene expression can be driven by a Cre/loxP inducible system. Global expression can be obtained in order to evaluate the effects of MCAT and H_2O_2 during development. Should embryonic lethality result, a tamoxifen inducible Cre can be

used to activate MCAT in adult mice. In addition, there are now numerous tissue-specific Cre recombinase mice in existence which will allow us to express MCAT in a particular tissue such as the heart or cerebral cortex, and to begin to define tissue-specific vulnerabilities to ROS.

Conclusion

The free radical theory of aging (FRTA) has been around for 50 years now and is arguably the most popular explanation for the mechanism of aging, both in scientific and popular circles. This work has attempted to evaluate the role of ROS, and one species in particular, in mammalian lifespan, by the overexpression of antioxidant enzymes targeted to different subcellular organelles. The largest effect was found when catalase was targeted to the mitochondrion, consistent with this organelle being a critical source of H_2O_2 production, as well as possibly being the primary site of attack. More sophisticated models are now being developed to further test the FRTA, including evaluating tissue-specific sensitivities to ROS. These models should allow us to more deeply understand the role of ROS in aging and disease, with the ultimate intent to improve the quality of human life.

Acknowledgments This work was supported by NIH grants AG001751 and AG13154. We kindly thank Pinar E. Coskun for her insightful comments.

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