

Mitochondria, Cellular Stress Resistance, Somatic Cell Depletion and Lifespan

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Abstract: The causes of aging and determinants of maximum lifespan in animal species are multifaceted and complex. However, a wealth of experimental data suggests that mitochondria are involved both in the aging process and in regulating lifespan. Here we outline a somatic cell depletion (SCD) model to account for correlations between: (1) mitochondrial reactive oxygen species and lifespan; (2) mitochondrial antioxidant enzymes and lifespan; (3) mitochondrial DNA mutation and lifespan and (4) cellular stress resistance and lifespan. We examine the available data from within the framework of the SCD model, in which mitochondrial dysfunction leading to cell death and gradual loss of essential somatic cells eventually contributes to the decline in physiological performance that limits lifespan. This model is useful in explaining many of the mitochondrial manipulations that alter maximum lifespan in a variety of animal species; however, there are a number of caveats and critical experiments outstanding, and these are outlined in this review.

Keywords: Mitochondria, lifespan, reactive oxygen species, superoxide dismutase, glutathione peroxidase, mtDNA, base excision repair, mutation.

INTRODUCTION

Amongst animal species, a vast range in maximum lifespans (MLS) is observed, from several weeks to greater than 100 years. The determinants of animal lifespans and mechanisms of aging have been the focus of recent research and substantial progress has been made toward their understanding. While it is clear that multiple mechanisms contribute to aging and therefore limit lifespan, a common theme that has emerged from numerous studies is the involvement of mitochondrial oxidative metabolism. The exact nature of this involvement remains elusive, perhaps in part because it is cell type and organism specific. Nonetheless, a mitochondrial theory of aging based on the production of oxygen radicals continues to provide a cornerstone for much research in the field. In this review, we critically examine aspects of mitochondrial biology that may influence the MLS potential of animal species. We begin by examining some of the implicit assumptions regarding how aging occurs that have informed experimental design and interpretation. We examine the hypothesis of somatic cell depletion (SCD) that has been implicit in many studies of aging, and use this hypothesis to reconcile disparate observations regarding the relationships between animal size, cellular stress resistance, and lifespan. We then explore specific aspects of mitochondrial biology that are known to affect cell viability. We conclude by summarizing the evidence for mitochondrial-mediated SCD. In developing our basic approach, we omit to include the potential role of cell replacement by resident stem cell populations. While such progenitor cell populations exist within most tissues (for example, see [2] for a review in heart), it seems that their

capacity for replacing lost cells in adult tissues is rather limited under normal circumstances.

If we confine our focus to a single class, Mammalia, a strong correlation can be identified between species size and maximum lifespan (Fig. 1). It is interesting in this context to note that a particular animal species becomes larger by proliferating more cells during development, and not by simply having larger cells. Horse hepatocytes are approximately the same size as those of mice [1]. This is true also for cardiomyocytes [2] and probably most cell types, despite vast differences between species in organ size. Therefore, within a particular tissue or organ, cell number is typically greater for larger and longer-lived animals. In many tissues, physiological decline associated with aging can be related to a reduction of cell number due to the apparent loss of irreplaceable (or unreplaced) cells (egs. [3-6]) (see also Table 1). In particular cases, such as in the substantia nigra of the brain (see below), there are clear thresholds of cell loss that can be tolerated before tissue function is compromised. This being so, it is logical to hypothesize that having more cells might provide a greater time until that threshold is reached and fatal failures occur, therefore increasing MLS. This aspect of organism size may be one important determinant of species MLS.

However, in many cases species of similar size (and therefore presumably similar cell numbers for a given tissue or organ) have greatly disparate lifespans. For example, MLS of the naked mole rat (*Heterocephalus glaber*), or the little brown bat (*Myotis lucifugus*), are almost 10-fold greater than that of similarly sized mice (*Mus musculus*) [7,8]. Even within an individual species (e.g. *M. musculus*), longevity is a malleable trait, influenced by diet and genetics [9]. This indicates that, within a given animal size (and therefore constant cell number), there are mechanisms for extending lifespan. A common theme that has emerged from studies within-species, or between species of similar body mass, is that cellular resistance to stress correlates well with longevity. Cellular stress resistance is typically measured as resistance to death

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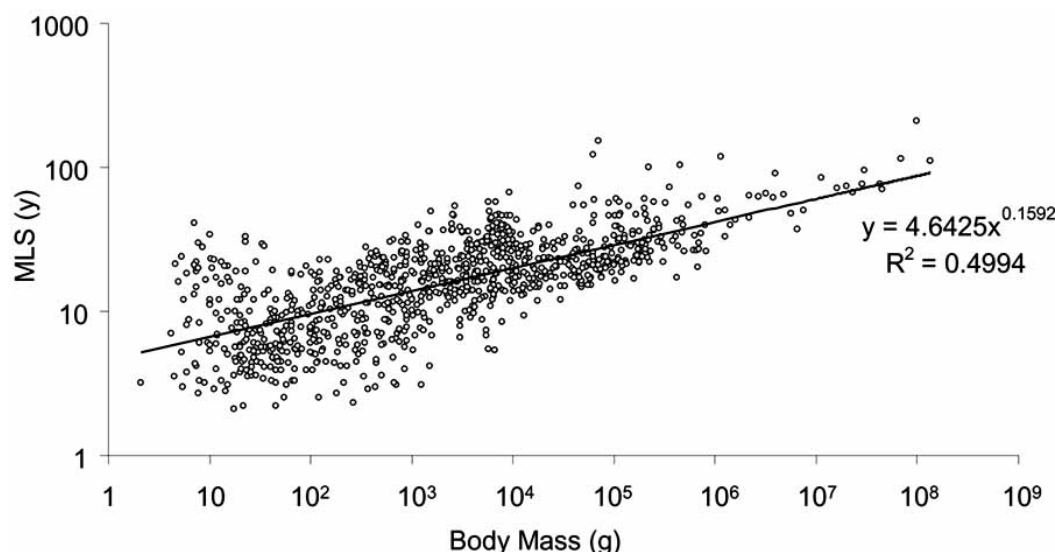


Fig. (1). Positive correlation between species MLS and average adult body mass in mammalian. Data plotted are for approximately one thousand mammalian species taken from AnAge build 10 [186].

induced by exogenous toxins. The correlation between this property and lifespan can therefore be understood from within the paradigm of cell loss (SCD) and tissue functional thresholds, as resistance to cell death may postpone the time to reach critical thresholds of cell loss in specific tissues. And, given the central role of mitochondria in apoptotic cell death, this provides a potential link between mitochondrial function and longevity. Below, we begin by reviewing the literature on stress resistance and lifespan, and then relate this to specific aspects of mitochondrial function.

Table 1. Somatic Cell Depletion and Aging in Mammals

Tissue/Cell Type	Observation	Ref.
Cardiomyocyte	Cell loss in left ventricle or rats from 11 to 20 months	[173]
Cardiomyocyte	Cell loss in both ventricles throughout rat lifespan	[164]
Cardiomyocyte	Cell loss in aging human hearts	[3]
Cardiomyocyte	Cell death in aging human hearts	[165]
Skeletal muscle motor neurons	Cell loss in aging rats	[169]
Retinal ganglion cells	Cell loss in aging mice	[5]
Substantia nigra dopaminergic neurons	Cell loss in aging humans (review; see ref therein)	[170]
Pre-optic hypothalamus cells	Cell loss in aging mice; reduced by caloric restriction	[171]
Urethral rhabdosphincter myocyte	Age dependent loss of cells in humans	[166]
Skeletal muscle myocyte	Age dependent loss of cells in various muscles	[167]
Skeletal muscle myocyte	Age-related cell losses (review; see refs therein)	[168]

Animal Stress Resistance and Lifespan

Data collected from a variety of experimental models suggest that extended animal longevity occurs concurrently with enhanced stress resistance (summarized in Table 2). This was first noted in experiments with *Drosophila melanogaster* and *Caenorhabditis elegans*, in which selective breeding of individuals resistant to stressors including desiccation, starvation and heat exposure produced populations with increased mean and maximum lifespans [10,11]. These experiments indicated a genetic control of lifespan. More recently, intracellular signaling pathways regulating gene expression to confer the long-lived phenotype have been identified [9].

Prolonged reduction in signaling through the insulin/insulin-like growth factor-1 (IGF-1) pathway is associated with increased longevity in worms, flies and mammals. The mechanistic steps in this pathway have been elucidated in a number of experimental models and for the purposes of this review will not be discussed further (see [12] for review). Manipulation of insulin/IGF-1 signaling as a means of promoting stress resistance and extending lifespan has been studied extensively in *C. elegans*. A loss of function mutation in *daf-2*, which encodes an IGF-1 receptor orthologue [13,14], doubles lifespan in *C. elegans* [13]. Similar lifespan extension is observed following a loss of function mutation in the *age-1* gene of *C. elegans*, which is also involved in this pathway [15]. These long-lived mutants display an enhanced resistance to a broad range of exogenous stressors, including the chemical superoxide generator paraquat [16], extreme heat [17] and heavy metal toxicity [18]. Disruption of the insulin/IGF-1 signaling pathway has also been shown to have an effect on the lifespan of *D. melanogaster*, in which a loss of function mutation in an insulin receptor homologue, InR, extends lifespan by up to 80% [19].

Similar studies of stress resistance in mammals are limited; however, research conducted using *M. musculus* suggests that

Table 2. Whole Organism and Cellular Stress Resistance in Animal Models of Longevity

Comparison	Source of Data	Change in Stress Resistance		Reference
		Increase	Decrease	
<i>C. elegans age-1</i> mutant	Whole animal	Paraquat, H ₂ O ₂ , cadmium	Unknown	[16,15,18]
Calorie restricted <i>Drosophila melanogaster</i>	Whole animal	Unknown	Paraquat	[30]
IGF-1 ^{-/-} mice	Whole animal	Paraquat	Unknown	[21]
p66 ^{shc-/-} mice	Whole animal	Paraquat	Unknown	[23,24]
Snell dwarf vs. wild type mice	Whole animal	Unknown	Acetaminophen	[48]
Snell dwarf vs. wild type mice	Dermal fibroblasts	Paraquat, H ₂ O ₂ , heat, UV light, cadmium, methyl methanesulfonate, rotenone, low glucose	Tunicamycin, thapsigargin	[37,42,45-48]
Ames dwarf mice	Dermal fibroblasts	Paraquat, H ₂ O ₂ , UV light	Cadmium	[45]
Growth hormone null mice	Dermal fibroblasts	Paraquat, H ₂ O ₂ , UV light	Cadmium	[45]
Comparative study between 8 mammalian species	Dermal fibroblasts	Paraquat, H ₂ O ₂ , tert-butyl hydrogen peroxide, sodium arsenite, alkaline pH	Unknown	[36]
Comparative study between 8 rodents, one species of bat and laboratory mice	Dermal fibroblasts	Heat, cadmium, H ₂ O ₂ , rotenone, low glucose	UV light, paraquat, methyl methanesulfonate	[47]
Naked mole rat vs. mouse	Dermal fibroblasts	Paraquat, heat, cadmium, methyl methanesulfonate	H ₂ O ₂ , UV exposure, tunicamycin, thapsigargin	[39]
	Cultured arteries	H ₂ O ₂ , heat	Unknown	[40]
Calorie restricted mice	Liver	Clofibrate	Unknown	[172]
	Lens epithelial cells	H ₂ O ₂	Unknown	[173]
5 adenylyl cyclase null mice	Cardiac myocytes	H ₂ O ₂ , UV light	Unknown	[69]
	Embryonic fibroblasts	H ₂ O ₂ , UV light	Unknown	[69]
	Dermal fibroblasts	H ₂ O ₂ , UV light	unknown	[69]

the connection between stress resistance and lifespan observed in *C. elegans* and *D. melanogaster* is also present in mammals. In mice, complete deletion of the IGF-1 receptor gene is lethal [20], but heterozygous null mice live up to 26% longer than their wildtype littermates [21]. They are also more resistant to oxidative stress induced by intraperitoneal injection of paraquat [21]. Interestingly, deletion of the insulin receptor exclusively in white adipose tissue similarly extends the lifespan of mice, although the ability of this mutation to enhance systemic stress resistance is unknown [22]. Deletion of the p66^{shc} gene in mice, which encodes a critical insulin adapter protein, also extends lifespan, and similarly imparts enhanced resistance to apoptosis and organism death induced by paraquat injection [23,24]. Thus, these data indicate that mutations rendering mice longer-lived also appear to impart resistance to stress-induced organism death.

Caloric restriction (also termed dietary restriction), a reduction in calorie intake without malnutrition, is a well known intervention that has been demonstrated to increase lifespan in a variety of animals ([25]; also see [26]). Consistent with the hypothesis that models of extended longevity display enhanced resistance to stress, calorie restricted organisms are more resistant to both oxidative and non-oxidative stressors than their fully fed counterparts. Dietary restricted *C. elegans* are both longer-lived and more stress resistant than fully fed worms

[27,28]. Similar effects are observed in *D. melanogaster* [29,30] and mice [31-33]. In many organisms, caloric restriction induces a quiescent state, such as torpor, concurrent with an increase in lifespan and stress resistance [34,35].

Cellular Stress Resistance and MLS

There is considerable evidence that the superior stress resistance of longer-lived animals can be traced to cellular properties that are altered by genetic or dietary manipulations. Indeed, even within the paradigm of correlated lifespan and body mass, cellular stress resistance is seen to correlate positively with lifespan. Much of the experimental data examining relationships between lifespan and cellular stress resistance in mammals has employed cultured fibroblasts. Kapahi *et al.* [36] revealed a positive correlation between maximum species lifespan and resistance to stressors including paraquat, hydrogen peroxide, *tert*-butyl hydroperoxide, sodium arsenite and alkaline pH, in cultured primary dermal fibroblasts derived from eight mammalian species. Similarly, primary dermal fibroblasts isolated from eight rodents, the little brown bat and a laboratory mouse strain, and exposed to a series of six different stressors revealed a positive correlation between lifespan and resistance to cadmium and hydrogen peroxide that remained significant following regression analysis for traits such as body size and phylogeny [37]. Within rodents, however, there was no significant correlation between lifespan and resistance to UV irradiation or paraquat in the examined species [37].

Data gathered from models of extreme longevity are of particular interest for their potential insight into the mechanisms responsible for limiting lifespan. The naked mole rat is similar in size to a mouse, but has a maximum recorded lifespan of 28.3 years - the longest reported in a rodent (see [38] for review). Dermal fibroblasts and vascular endothelial cell cultures established from the naked mole rat appear more resistant than mouse cells to some stressors [39,40]. Dermal fibroblasts of the naked mole rat are more resistant to cell death induced by paraquat, cadmium, heat and DNA methylating agents than mice; however, they show a heightened sensitivity to hydrogen peroxide stress and UV irradiation [39]. In contrast, vascular tissue of the naked mole rat is more resistant than mouse vascular tissue to apoptotic cell death induced by hydrogen peroxide [40]. The discrepancy in stress resistance between these two different cell types is interesting, and may suggest tissue-specific variations in the mechanisms which impart animal stress resistance.

Cross species comparisons of longevity inevitably suffer the same shortcomings, in that correlation of a multifaceted response, such as cellular stress resistance to lifespan, will always be confounded by extraneous factors that are not controlled for. Cellular mechanisms relating to animal longevity have been explored by comparing dermal fibroblasts from the long lived Snell and Ames dwarf mice to those of their normal littermates, eliminating some of these concerns. In Snell dwarf mice, a mutation in the gene encoding the transcription factor Pit-1 results in an increase in lifespan accompanied by a reduction in body size and altered hormonal levels [41]. Dermal fibroblast cultures derived from dwarf mice have been used extensively to demonstrate the relationship between cellular stress resistance *in vitro* and organism lifespan. Increased resistance to cellular stressors in fibroblasts of dwarf mice is apparent from cells taken from adult mice and evidence of enhanced stress resistance is stable for several population doublings. Dermal fibroblasts from Snell dwarf mice are resistant to a variety of disparate stressors including paraquat, hydrogen peroxide, UV irradiation and cadmium [42]. In addition to resistance to acute stressors, it appears that fibroblasts derived from Snell dwarf mice are also more resistant than fibroblasts of their normal littermates to the chronic negative effects of atmospheric oxygen exposure in culture. At atmospheric oxygen (20%), mouse embryonic fibroblasts (MEFs) undergo replicative senescence more readily than when cultured at more physiological conditions of 3% oxygen [43]. The increase in senescence at 20% oxygen is believed to be related to a state of oxidative stress in these cells. Fibroblasts from Snell dwarf mice are resistant to growth arrest observed at 20% oxygen, suggesting that they are more resistant to the damaging effects of chronic oxygen exposure [44]. Increased stress resistance is not unique to fibroblasts of the Snell dwarf mice, and is also observed in dermal fibroblasts derived from other mouse models of longevity. Dermal fibroblasts of Ames dwarf mice and mice null for growth hormone receptor are resistant to stress induced by hydrogen peroxide, UV irradiation and paraquat, but not to cadmium [45].

An interesting observation is that fibroblasts of some long-lived organisms are also resistant to metabolic

stressors. Dermal fibroblasts from Snell dwarf mice are more resistant than cells of their wildtype littermates to glucose-deprivation stress, and the effects of rotenone [46]. Lifespan in eight rodent species, the little brown bat and a laboratory strain of mice also correlates positively with resistance to rotenone [47]. Resistance to stressors targeted to disrupt oxidative phosphorylation may reflect a change in the metabolic profile of these cell types. However, we have observed no differences in the apparent aerobic or anaerobic metabolic capacity of Snell dwarf mouse fibroblasts, relative to those of their normal littermates [unpublished observations].

One criticism of the above studies is their heavy reliance on dermal fibroblasts. As mentioned above, in the naked mole rat differences in stress-specific resistance are observed between cell types, which cautions against the use of any single cell type in developing an understanding of the relationship between cellular stress resistance and longevity. For example, Snell dwarf mice, although longer lived, are more sensitive than their wild type littermates to the harmful effects of the liver toxin acetaminophen [48]. A second caution is that within interspecies comparisons there is limited commonality in the specific stressors long-lived animals are resistant to. This may imply that the factors imparting cellular stress resistance are unique amongst different species, and this should be considered in the search for specific cellular mechanisms capable of increasing stress resistance and extending lifespan. Thirdly, data derived from cell culture experiments must account for possible artifacts attributed to conditions employed in standard cell culture protocols. For example, standard culture conditions employ atmospheric oxygen conditions of 20%, which result in a hyperoxic growth medium [49]. Hyperoxic conditions may be sufficient to induce an oxidative stress on the cells, and this may change the cellular response to subsequent stress [50]. In the context of the SCD theory, it is perhaps most important to extend the observations made with fibroblasts to other cell types, in particular post-mitotic cells such as in the heart and brain, as it is loss of these cell populations that will determine the functional decline of the soma.

Nonetheless, if we accept that, with the above caveats, stress resistance at the level of the whole organism and individual cells appears to correlate with animal longevity, it becomes interesting to investigate molecular mechanisms conferring this property. The relationship between resistance to cell death and longevity provides a framework to understand the potential role of mitochondria in longevity, because this organelle is a direct mediator of cell death. A common correlate of aging is the accumulation of oxidative damage within critical macromolecules. With this in mind, longevity has been related both to differences in the production of oxidants and to differences in the ability to withstand the adverse effects of oxidants. Below, we will examine both properties.

Mitochondrial Reactive Oxygen Species Production and MLS

It is estimated that 0.1-0.5% of all molecular oxygen within the respiratory chain prematurely reacts with electrons that leak from complexes I and III of the electron transport chain, producing superoxide anions (reviewed in [51]). Superoxide anions react readily with nitric oxide to produce the highly

reactive species peroxynitrite. Superoxide anions are also spontaneously or catalytically converted to hydrogen peroxide which can be further converted to the extremely reactive hydroxyl radical causing damage to DNA, proteins and lipids.

Comparative studies have typically reported that mitochondrial reactive oxygen species (ROS) generation is inversely related to MLS in mammals [52-56] (see Table 3 for a summary of relevant studies). Recently many of these studies have been criticized on the grounds of limitations inherent to the number and independence of data points (species) and statistical analyses employed [57]. However, in some instances these confounding factors have been appropriately controlled for (e.g. [56]), and the originally reported inverse correlation between lifespan and mitochondrial ROS production has been confirmed. This trend has been shown for a variety of tissues: isolated liver sub-mitochondrial particles [52], and isolated mitochondria from liver, heart, and kidney [53-56]. However, there are also exceptions to this trend. For example, while hydrogen peroxide production from isolated heart, brain and kidney mitochondria was found to be significantly lower in the

long-lived (MLS = 25y) little brown bat (*M. lucifugus*) compared to the short-lived (MLS = 2y) short-tailed shrew (*Blarina brevicauda*), it was lower still in the white-footed mouse (*Peromyscus leucopus*), which has an intermediate lifespan (MLS = 8y) [8]. Also, Lambert *et al.* [56] found that the rate of ROS production of isolated heart mitochondria from the long-lived naked mole rat (*H. glaber*) is similar to that of a mouse.

With respect to the above studies, it is important to note the following limitations inherent in the methods used. Firstly, the measurements are made in mitochondria respiring in atmosphere saturated medium (~21% O₂), which is at least an order of magnitude greater than these organelles encounter within cells *in vivo* [49,58,59]. Secondly, the rather vigorous process of tissue homogenization used to isolate mitochondria can introduce artifactual oxidative damage. Silva *et al.* [60] have shown that differences in mitochondrial antioxidant enzyme activities can make mitochondria more or less susceptible to oxidative damage *in vitro*. Thirdly, conditions of mitochondrial respiration *in vitro* are likely far removed from those *in vivo*, particularly with respect to rate of ADP phosphorylation and saturation of respiratory substrates. These

Table 3. Mitochondrial and Cellular ROS Production in Long-Lived Animal Species

Comparison	Source of Material	ROS Measured	Relationship	Ref.
Pigeon vs. Rat	Heart mitochondria	H ₂ O ₂	Higher rates in shorter-lived species	[174]
Parakeet vs. Canary vs. Mouse	Heart mitochondria	H ₂ O ₂	Progressively lower rates in longer lived species (mouse> parakeet>canary)	[175]
Naked mole rat vs. Damara mole rat vs. Guinea pig vs. Mouse	Carotid arteries (endothelial cells and smooth muscle cells)	O ₂ ⁻ and H ₂ O ₂	No relationship with lifespan	[40]
Little brown bat vs. white-footed mouse vs. short-tailed shrew	Brain, heart and kidney whole tissue homogenates	H ₂ O ₂	No relationship with lifespan	[8]
White-footed mouse vs. House mouse	Aortas	O ₂ ⁻	Higher rates in shorter-lived species	[181]
	Heart mitochondria	H ₂ O ₂		
White-footed mouse vs. House mouse	Sub-mitochondrial particles from brain and heart	O ₂ ⁻	Higher rates in shorter-lived species	[182]
	Brain and heart mitochondria	H ₂ O ₂		
Correlative study (5 Mammalian species, 1 Insect)	Sub-mitochondrial particles from liver and flight muscles (Insecta)	O ₂ ⁻	Highest rates in shorter-lived species	[52]
Correlative study (6 Mammalian species, 2 Insect species)	Liver mitochondria or Flight muscle mitochondria	H ₂ O ₂	Highest rates in shorter-lived species	[53]
Correlative study (6 Mammalian species)	Sub-mitochondrial particles from heart and kidney	O ₂ ⁻ and H ₂ O ₂	Higher rates in shorter-lived species	[54]
Correlative study (12 Mammalian species, 2 Avian species)	Heart mitochondria	H ₂ O ₂	Higher rates in shorter-lived species	[56]
Correlative study (5 Insect species)	Flight muscle mitochondria	O ₂ ⁻ and H ₂ O ₂	Higher rates in shorter-lived species	[183]
CR rats vs. <i>ad libitum</i> fed rats	Heart mitochondria	H ₂ O ₂	Lower rates in CR rats fed rats	[179]
CR rats vs. <i>ad libitum</i> fed rats	Isolated hepatocytes	H ₂ O ₂	No difference detected	[185]
8.5% CR, 25% CR rats vs. <i>ad libitum</i> fed rats	Liver mitochondria	H ₂ O ₂	No difference detected	[180]
Methionine restricted rats vs. <i>ad libitum</i> fed rats	Heart mitochondria	H ₂ O ₂	Lower rates in methionine restricted rats	[178]
	Liver mitochondria	H ₂ O ₂	Lower rates in methionine restricted rats	
40% and 80% Methionine restricted rats vs. <i>ad libitum</i> fed rats	Liver mitochondria	H ₂ O ₂	Lower rates in methionine restricted rats	[184]
Cells cultured in serum from <i>ad libitum</i> fed vs. CR rats	HeLa cells	O ₂ ⁻ and H ₂ O ₂	Lower rates in cells cultured in CR serum	[63]

caveats have been addressed by studies using intact isolated or cultured cells to make similar measurements of mitochondrial ROS production.

ROS production from isolated hepatocytes appears to be greater in older mammals [61,62]. However, Lambert and Merry measured ROS production in hepatocytes isolated from calorie restricted (55% of control diet) rats and fully fed controls, and found no difference. In contrast, Lopez-Lluch *et al.* [63] showed that when rat hepatocytes, HeLa cells or FaO cells were grown in serum from calorie restricted or fully fed rats, ROS production was greater in the latter. It is difficult to reconcile these observations given the differences in experimental approaches used. The apparent contradictions between these studies and those described above suggest the need for experiments with isolated intact cells from a variety of tissues, to determine whether lifespan-related differences in mitochondrial ROS metabolism are confined to particular cell types or more globally manifested.

Genetically manipulated mice also offer insights into the relationship between mitochondrial ROS production and lifespan. $p66^{hc}$ is a splice variant of $p52^{shc}/p46^{shc}$, which functions in signal transduction [23]. $p66^{shc/-}$ mice are resistant to oxidative stressors (e.g. paraquat) and live up to 30% longer than wildtype mice. $p66^{shc/-}$ mice appear to provide a link between cellular oxidative stress and the mitochondrial apoptotic pathway, and this may relate directly to its role as a determinant of lifespan [24]. MEFs from $p66^{shc/-}$ are resistant to oxidant-induced cell death because they do not as readily undergo mitochondrial permeability transition and membrane depolarization. Thus, the results from studies of $p66^{shc/-}$ mice are consistent with the hypothesis that mitochondria modulate lifespan via their role in cell death and subsequent SCD.

If this hypothesis is correct, it becomes important to understand how differences in the ability of mitochondria to directly detoxify ROS might relate to species longevity. Differences in the expression of mitochondrial antioxidant enzymes could explain between-species differences in ROS production that have been determined using isolated mitochondria. If elevated mitochondrial antioxidant levels protect the organelles from oxidative damage during isolation, this may affect subsequent measurements of respiratory ROS production *in vitro*. Also, increased mitochondrial antioxidant capacity could alter the rates of ROS production measured in intact isolated cells, if ROS are immediately detoxified within mitochondria. Thirdly, increased mitochondrial antioxidant capacity in cells cultured from longer-lived animals might explain their apparent increased resistance to apoptotic cell death.

Mitochondrial Antioxidant Capacity and MLS

In most animal tissues, mitochondria contain several antioxidant enzymes that detoxify ROS produced as a byproduct of respiration. Manganese-dependent superoxide dismutase (MnSOD) is the only superoxide dismutase (SOD) in the mitochondrial matrix. A portion of MnSOD is associated with the inner membrane [64], which is interesting given that this is the site of virtually all respiratory superoxide production. CuZnSOD is primarily

a cytosolic enzyme. However, almost 3% of this enzyme can be found in the mitochondrial inter-membrane space (IMS) in rat liver [64]. Despite the low overall proportion of total CuZnSOD that is mitochondrial, the enzyme is likely highly concentrated within the IMS, given the small volume of this compartment relative to cytosol. Mitochondria generally lack the ability to detoxify H_2O_2 via catalase. Though this enzyme has been reported in the organelles isolated from heart tissue [65], other studies have failed to demonstrate this [66]. At least two isoforms of glutathione peroxidase (GPx1 and GPx4) localize to mitochondria (reviewed in [67]) where they can also detoxify H_2O_2 . In addition GPx4 can neutralize phospholipid hydroperoxides and a variety of other lipid hydroperoxide substrates. Thus, mitochondrial detoxification of ROS produced either within the matrix or in the IMS during respiration relies primarily on MnSOD, and GPx, and probably to a lesser extent on CuZnSOD.

MnSOD positively correlates with lifespan in some animal models of longevity. For example, it is elevated in long-lived *daf-2* mutant *C. elegans* [68], and type 5 adenylyl cyclase knockout mice [69]. MnSOD is the only antioxidant enzyme activity that is appreciably higher in liver tissue of the naked mole rat [70]; it is approximately twice that of size-matched mice. In cultured dermal fibroblasts from a range of mammalian species, MnSOD levels are positively correlated with MLS [71]. Transgenic overexpression of this enzyme in mice prevents mitochondrial membrane permeability transition and cell death [60] and generally confers increased cellular stress resistance (reviewed in [72,73]). Resveratrol, which has been associated with lifespan extension, upregulates MnSOD activity in human skin fibroblasts and in mice [74,75]. In contrast, MnSOD ablation is lethal and knockdown reduces lifespan in most animal species [e.g. 76].

There is, however, a number of exceptions and caveats related to these observations, and representative results from *C. elegans*, *D. melanogaster* and *M. musculus* will be discussed in this context below. For example, while Honda and Honda [77], Murphy *et al.* [78] and Yang *et al.* [68] identified mitochondrial SOD as a downstream target of the longevity-conferring transcription factor *daf-16* in *C. elegans*, Yang *et al.* [68] observed no effect of RNAi mediated SOD-2 knockdown on *daf-2* lifespan. Unfortunately, none of the data regarding MnSOD mRNA or protein levels have been corrected for mitochondrial abundance, though in several animal models of increased longevity, significant mitochondrial proliferation is observed. Thus, increased levels of exclusively mitochondrial enzymes such as SOD-2 may simply reflect a greater abundance of the organelle in long-lived strains. In *C. elegans*, interpretation of the results regarding mitochondrial SOD may also be confounded by the unusual existence of two mitochondrial SOD isoforms [79], SOD-2 and SOD-3, that share 88% sequence identity [80]. The two isoforms clearly elicit different effects on both stress resistance and lifespan in *C. elegans*. Whereas abolishing SOD-2 significantly reduced stress resistance and the lifespan extension phenotype of *daf-2* mutants, *sod-3* mutants showed no effect on stress resistance or increased lifespan [81]. Finally, in the wildtype genetic background, double knockout of the two mitochondrial SOD isoforms significantly reduced resistance to paraquat, but an apparent 13% reduction in MLS was not significant. Thus, the role of mitochondrial SOD activity in regulating longevity of *C. elegans* remains unclear.

In this organism, with only ~1000 somatic cells, it would seem that a strong correlation between an anti-apoptotic function and increased longevity should be evident if the SCD theory is correct.

One of the major problems in interpreting the effect of transgenic MnSOD overexpression is the potential for interfering with redox modulated signaling pathways, particularly during development. Sun *et al.* [82] overexpressed MnSOD conditionally in adult *D. melanogaster*. In this study transgenic MnSOD overexpression, over a range of MnSOD levels, correlated well with lifespans [82]. CuZnSOD expression in adult *D. melanogaster* can provide a similar outcome of lifespan extension [83], though the extent to which this is caused by IMS CuZnSOD is not known and apparently has not been considered. CuZnSOD is also found within the IMS of yeast (*Saccharomyces cerevisiae*, [84]) and in various mammalian tissues [85], suggesting that this may be a common or even ubiquitous characteristic of its intracellular distribution in eukaryotes. In any case, despite the ability of SODs to extend lifespan in *D. melanogaster*, there is apparently no evidence that long-lived mutants or dietarily restricted flies have elevated levels of either enzyme [86]. However, these measurements employed spectrophotometric assays of crude homogenates, which can be problematic [87,88].

In mice, MnSOD is critical for survival, and *Sod2*^{-/-} mice live only 10-18 days [74,89]. Surprisingly, *Sod2*^{+/-} mice have normal lifespans [90], despite reduced mitochondrial respiratory control ratios (RCR) and increased susceptibility of isolated mitochondria to permeability transition [91,92]. Apoptotic cell death is elevated in cardiomyocytes from *Sod2*^{+/-} mice compared to wildtype, but only in old mice and those treated with oxidants. The *Sod2*^{+/-} mice are also more sensitive to an intraperitoneal injection of paraquat [90]. These observations are difficult to reconcile with the SCD theory of aging, but a more complete study of cell loss with age in these mice might be informative.

The results summarized above provide evidence (though somewhat equivocal) that dismutation of superoxide in the matrix or IMS may promote increased longevity, possibly via amelioration of SCD. Greater SOD activities would be expected to reduce steady state levels of superoxide. This in turn will affect redox signaling pathways as well as potentially nitric oxide (NO) signaling, particularly in tissues where this is an important pathway. Mathematical models predict that elevated mitochondrial SOD activities will increase H₂O₂ flux when the superoxide source is the respiratory chain [93]. H₂O₂ also participates in a number of reactions, including those capable of inducing apoptotic cell death. Therefore, concomitant detoxification of this molecule may be important.

A mechanism for the protective effect of mitochondrial GPx isoforms has been presented by Imai and Nakagawa [67]. These authors suggest that it is the specific protection of the unique inner membrane phospholipid cardiolipin from peroxidation that is responsible for resistance to cell death. Cardiolipin maintains an important electrostatic interaction with cytochrome c in the IMS that is disrupted

by peroxidation of the phospholipid. This leads to cytochrome c release, providing a second condition, formation of an outer membrane pore, is also met. Thus, GPx1/4, and perhaps other mitochondrial antioxidants, may enhance cellular stress resistance by preventing cytochrome c release and subsequent apoptotic cell death.

Recently, transgenic mice overexpressing the mitochondrial isoform of the enzyme GPx (GPx4) have been produced, and these provide insight into the role of GPx4 specifically in this organelle. The GPx4 overexpressors are protected from loss of mitochondrial and tissue function due to ischemia-reperfusion injury [94]. The effect of this global GPx4 overexpression on lifespan has not yet been assessed in these mice. However, Ran *et al.* [95] reported that GPx4 knockout heterozygous mice, with reduced GPx4 levels in all major tissues, did not have reduced lifespan. This contrasts with a report from [96], in which transgenic mice expressing a mitochondrial-targeted catalase did live significantly longer. It is difficult to understand the significance of mitochondrial H₂O₂ from these studies. While they demonstrate that mitochondrial H₂O₂ detoxification is clearly protective from the toxic and apoptotic effects of oxidants, and the potential for attenuation of SCD is clear, more empirical evidence of an effect on lifespan is required.

mtDNA Damage/Mutation and MLS

While ROS can react with all cellular constituents, causing damage to lipids, proteins and nucleic acids, accumulation of mtDNA damage may be particularly harmful, due to its potentially permanent interference with transcription and replication. ROS mediated damage occurs in both nuclear and mitochondrial genomes, however, mtDNA is believed to be particularly susceptible to damage, in part due simply to its proximity. One element of a mitochondrial theory of aging suggests that accumulated damage and mutations in mtDNA cause an aberrant ROS production that further damages the organelle. This has been termed the "vicious cycle of mitochondrial ROS production" [97]. In this scenario, the accumulation of mutations in mtDNA decreases oxidative phosphorylation efficiency, which in turn increases ROS production and damage leading to cellular ATP crisis and ultimately cell death [98]. The order and prevalence of these events remain controversial, and are yet to be demonstrated experimentally, while recent evidence refuting the theory has accumulated. In any case, validation of the existence of the vicious cycle of mitochondrial ROS production requires that mitochondrial dysfunction in cells with high levels of mtDNA mutation be concurrent with increased rates of ROS production. This idea has been the premise of some excellent recent studies and these are discussed below. However, we first briefly review the relevant background on mtDNA damage, repair and mutation.

Mitochondria possess a genome of approximately 16k base pairs, which in mammals encodes 13 protein subunits of the electron transport chain as well as 22 tRNAs and 2 rRNAs necessary for their production. Despite the relatively few proteins encoded in mtDNA, these are absolutely necessary for oxidative phosphorylation and mitochondria lacking DNA are not capable of synthesizing ATP [99]. mtDNA is thought to be susceptible to damage and mutation for a number of reasons, including: (1) its close proximity to the electron transport

chain, the primary site of ROS generation in many cells. (2) the absence of protective histones [100]. Mitochondrial single stranded DNA-binding protein and transcriptional factor A have been observed to co-localize with mtDNA, the latter in sufficient amounts to possibly coat the genome, but the protective abilities of these structures has not been fully explored [101]. (3) the absence of introns, which increases the probability that damage will affect protein coding regions of the genome. (4) multiple copies of mtDNA exist within every mitochondrion, and replicate even in post-mitotic cells. Continual degradation and resynthesis of mtDNA may act as a mechanism to repair damage, but has the potential to increase mutation rate, especially in highly metabolic tissues where mtDNA is quickly synthesized and degraded [100]. For example, the average lifetime of a mtDNA molecule in a rat brain cell is estimated to be approximately 2-4 weeks [102], and this high rate of turnover is important to understanding how mtDNA mutations can expand with age.

Oxidative DNA damage has been well characterized. Oxidative stress may result in oxidative lesions, abasic sites, strand breaks and base modifications [see 103 and references therein]. However, the oxidative adduct 8-oxodeoxyguanine (8-oxodG) is commonly measured as an indicator of overall oxidative damage in mtDNA. The *syn* conformation of 8-oxodG has the potential to induce GC-TA transversion mutations, as well as the misincorporation of 8-oxodGMP opposite adenine [104]. Oxidative damage to mtDNA (8-oxodG) appears to correlate positively with age, and negatively with lifespan [105, 106]. Unfortunately, however, methodologies for measuring the presence of 8-oxodG vary greatly between research groups, and substantial differences between measurements question the validity of this technique [107].

mtDNA accumulates high levels of oxidative lesions under normal physiological conditions [108] and mitochondria were once believed to lack the ability to repair damaged DNA [109]. The absence of mitochondrial DNA repair was subsequently disproved by demonstration that mitochondria are able to conduct base excision repair (BER), the pathway responsible for the removal of most types of oxidative damage [110-112]. A long list of DNA repair enzymes have now been observed in isolated mammalian mitochondria. These include, but are not limited to, DNA glycosylases [112-114], thymine glycol glycosylase (*nth1*) [115] and a homologue of *E. coli* MutY glycosylase (MYH) [116]. The BER pathway is responsible for the removal of base adducts and abasic sites in DNA and is thus an important defense against oxidative damage. Generally, BER involves four sequential steps: (1) the altered base is removed by a damage-specific glycosylase (2) the sugar backbone remaining at the abasic site is cleaved by an endonuclease to produce a free 3'-hydroxyl group (3) the subsequent nucleotide gap is filled by polymerase γ and (4) the synthesized strand is annealed by a DNA ligase [111]. The importance of BER in removal of oxidative DNA lesions in mitochondria has been investigated within several mutants in whom the genes encoding the enzymes that catalyze the first two steps of this pathway have been deleted or overexpressed. Below, we will discuss mammalian models, which

evaluate the physiological effects of mtDNA repair, damage and mutation.

It is generally hypothesized that extensive oxidative damage to mtDNA will result in mitochondrial dysfunction, increased ROS production and oxidative stress. 8-oxoguanine DNA glycosylase (OGG1) is a nuclear encoded enzyme responsible for the majority of 8-oxodG removal in mitochondria. In an *in vitro* assay, mitochondrial homogenates from the OGG1 null mice are unable to incise a substrate containing 8-oxodG [117], suggesting the absence of back up activities. However, in contention with the idea that elevated levels of mtDNA damage lead to cellular ATP crisis, examination of respiratory function in mice null for OGG1 revealed that in spite of having levels of the 8-oxodG lesion at least 20-fold higher than that observed in wildtype mice [117], these mice display a normal phenotype [118]. Respiratory complex activities and respiratory properties of isolated mitochondria are not different from wildtype mice [119]. It may be that alternative pathways for 8-oxodG repair do exist *in vivo*, and that the ability to incise 8-oxodG by these enzymes was not addressed under the experimental conditions utilized. A second possibility is that the accumulation of this oxidative lesion and subsequent mutation is insufficient to affect mitochondrial bioenergetic function. In any event, the above studies suggest that OGG1 does not play a critical role in defense of mtDNA integrity.

Uracil that arises from the deamination of cytosine in mtDNA is efficiently removed by uracil-DNA glycosylase (UNG) [120]. In addition, UNG removes several forms of damage caused by uracil oxidation and is upregulated in response to oxidative stress [121,122]. As with OGG1, protein extracts from mitochondria lacking UNG are unable to incise uracil from oligonucleotides, indicating the absence of backup mechanisms for uracil removal [122,123]. However, similar to observations made in the absence of OGG1, deficient removal of uracil does not appear to have a negative effect on mitochondrial function in several experimental models. Inhibition of uracil removal in mtDNA does not lead to increased mutation or mitochondrial dysfunction in immortalized human breast epithelial cells [124]. Activities of mitochondrial respiratory complexes I and IV in whole tissue homogenates of liver, heart and brain from mice lacking the ability to remove uracil are not different from activities in wildtype mice. This lack of difference is evident even when mice are maintained on a folate deficient diet (M.M. Page *et al.*, unpublished data), which promotes uracil incorporation and mtDNA mutation [125]. This suggests that the activity of uracil-DNA glycosylase may not be required to maintain mitochondrial bioenergetic function in mammalian cells.

It is interesting that although the activities of individual glycosylase enzymes can be inactivated without apparent consequence to mitochondrial function, overexpression of these enzymes has been shown to confer cellular stress resistance. Transfection of HeLa cells with a vector containing the sequence of human OGG1 with a mitochondrial targeting sequence effectively increases OGG1 activity in mitochondria of these cells. Concurrent with the increase in OGG1 activity, these cells become more resistant to cell death than controls when exposed to the redox cycling drug menadione [126]. Targeting human OGG1 to mitochondria in HeLa cells also increases their resistance to the chemical apoptosis inducer 2-

methoxyestradiol, supposedly by reducing mtDNA damage [127]. Similar observations are made when human OGG1 is targeted to oligodendrocytes [128,129]. Alternatively, there is evidence to suggest that overexpression of DNA glycosylases may lead to increased sensitivity to stressors [130,131]. These contradictory observations may be related to differences in the intracellular concentration of the transgenic protein and the mechanisms of expression in each experiment. As in many biological systems, balance between the components of the BER pathway appears to be important to its functioning.

AP endonuclease (APE1) is responsible for the second step in the BER pathway and is implicated in the repair of oxidized damage, abasic sites as well as in the repair of some single strand breaks [132]. Removal of this enzyme has severe consequences on animal and cell viability. In addition to its DNA repair activity, APE1 also acts as a red/ox regulator of transcription factor activities in mammalian cells [for review, see ref. 133]. APE1 is present in both the nucleus and mitochondria in fairly high concentration [134,135]. This enzyme is critical to a number of repair systems, and may represent a bottle neck for many DNA repair substrates. Furthermore, it seems that there are no alternative enzymes able to compensate for a loss of AP endonuclease [132]. Deletion of the *ape1* gene in mice is embryonic lethal [136], and both activities of the enzyme are required for life in mammals [137]. Due to the lethality of the APE1 gene deletion, characterization of oxidative stress and mitochondrial function in mammalian mutants lacking APE is impossible. A transgenic mouse has been created that is heterozygous for the gene encoding AP endonuclease [138]. Embryonic fibroblasts derived from this mutant are highly sensitive to oxidative stress induced by chemicals such as paraquat. Markers of oxidative stress, such as lipid peroxidation and isoprostanes are also elevated in the mice, suggesting that reduced APE1 activity makes them more sensitive to oxidative stress [138]. However, it is important to note that in this study, the participation of APE1's two distinct activities in response to oxidative stress was not explored. It may be that a decrease in mtDNA repair enhances sensitivity to stress induced by chemical oxidants; however, it is also possible that decreased red/ox regulatory function has a negative impact on other important intracellular pathways mediating cell death. The contribution of each of APE1's activities in cellular stress resistance requires further exploration.

Elevated APE1 activity in cultured cells increases the ability of Chinese hamster cells to resist the negative effects of DNA alkylating agents [139], and to protect neuronal cells from exogenous oxidative stress [140]. In contrast, overexpression of APE1 has also been shown to have a negative effect on cell survival in breast cancer cells treated with a chemical stressor [141]. A recent study demonstrated that targeting human APE1 to mitochondria in cultured human umbilical vein endothelial cells showed an increased resistance to oxidative stress and reduced apoptosis in the transgenic cells [142]. Increased stress resistance was observed when the transgenic APE1 was targeted to the mitochondria, possibly suggesting mtDNA damage is critical to apoptosis in response to oxidative stress. It will be important to develop approaches to isolate

the mtDNA repair function of APE1 so that the importance of this activity with respect to mitochondrial function and lifespan can be ascertained.

Enhanced expression of enzymes which participate in the BER pathway seems to protect cultured cells from acute oxidative stress. However, the impact of overexpressing DNA repair enzymes on whole animal physiology has not yet been determined. It is critical to determine if increased expression of these enzymes provides protection from endogenous levels of stress, and if this manipulation is of any benefit to the whole animal. With this in mind, the absence of pathological phenotypes in mice deficient in BER but producing physiological concentrations of ROS is extremely interesting, as it questions the importance of mtDNA damage repair as a determinant of lifespan.

The physiological relevance of mtDNA mutations has been addressed in a series of experiments using transgenic mice expressing a mutant form of polymerase γ (polg) (mutator mice), the only known polymerase identified in mammalian mitochondria [143]. The engineered polg possesses a substitution mutation that eliminates its ability to proofread DNA. Polg transgenic mice therefore accumulate mtDNA point mutations and deletions with increasing age, first estimated to be 3- to 11-fold greater than observed in wildtype mice [144,145]. Although the transgenic mice are apparently healthy at birth, with increasing age they demonstrate severe symptoms similar to those associated with advanced aging in humans and their lifespan is only ~60% that of wildtype controls [144,145]. Mitochondrial respiratory complex activities are substantially reduced in polg mutant mice, presumably due to mutations in regions of mtDNA encoding critical subunits [144,145]. Interestingly, although these mice display elevated levels of mtDNA mutation, and a near 90% reduction in oxygen consumption in some tissues, no signs of oxidative stress are observed. Mitochondrial protein carbonylation, isoprostane levels and accumulation of 8-oxodG were unchanged between the polg mutant mice and wildtype controls [144,145]. The activity of aconitase, a citric acid cycle enzyme frequently used as a proxy of matrix oxidative stress, was also unchanged in the mutant mice [146]. Therefore, high levels of mtDNA point mutations do not increase oxidative stress. Direct measurement of ROS production in mutator mouse cells similarly reveals no increase in the rate of ROS production. Generation of superoxide from polg mutant embryonic fibroblasts is not different from the controls [146] and measurements of mitochondrial hydrogen peroxide production from a variety of tissues are also similar to those observed in the wildtype control [145]. Finally, embryonic fibroblasts from polg mutant mice do not display a decrease in resistance to hydrogen peroxide, suggesting that although they have a substantial accumulation of mtDNA mutation, their ability to withstand an oxidative stress is unaltered. A compensatory response to increased ROS was also tentatively ruled out by Trifunovic and colleagues, as mRNA levels of antioxidant enzymes were unchanged between mutator and control mice [146]. Taken together, these results offer no support for the 'vicious cycle' theory of mitochondrial involvement in aging and lifespan.

In the mutator mice, accelerated aging was not attributed to oxidative stress, but rather to an elevated level of cell death. The mtDNA mutator mouse shows a significant increase in

cleaved caspase 3, a marker of apoptosis, in skeletal muscle at 9 months of age when compared to controls [145], suggesting that increased mtDNA mutation load is associated with increased death in some cell types. The impact of increased mutations in specific tissues was further addressed in a transgenic mouse expressing an error prone polg specifically in heart tissue [147]. Experiments with these transgenic mice revealed that the error prone polg increases cardiac cell apoptosis, associated with cardiomyopathy [148]. Cardiovascular disease is frequently associated with increasing age, and it is therefore very interesting that the accumulation of mtDNA mutation has the potential to increase apoptosis and lead to organ dysfunction under conditions of normal physiological stress. These studies provide a link between mtDNA mutation and aging, but also suggest that this link is not ROS production. The precise mechanism(s) whereby mtDNA mutation appears to promote cell death and curtail lifespan remains to be determined.

The mutation load in polg mutant mice was initially estimated to be approximately 3- to 11-fold that observed in wildtype mice. However, a reappraisal of this value using the random mutation capture method [for a description of this method see ref 149], found that the polg mutant mice harbor a point mutation load approximately 2500-fold greater than wildtype mice. In addition, in spite of this substantial increase in mutation load, the mice were healthy at the time of the measurement, suggesting that the endogenous mtDNA point mutation load does not contribute to aging phenotypes [150]. Mice that are heterozygous for the error-prone polymerase harbor considerably fewer mtDNA point mutations (500-fold wildtype values) and experience no decrease in lifespan [150]. It is therefore unlikely that wildtype mice would accumulate such dramatic levels of mtDNA point mutations with age as to limit lifespan. In polg mutator mice the negative effects of accumulating mutations throughout development are unknown, and the systemic accumulation of mtDNA mutations may not be representative of the normal pattern of mutation accumulation with age. It is also possible that other types of mutation may be a basis for mitochondrial dysfunction in aging.

Interestingly, mtDNA mutations in the form of deletions are commonly observed in the elderly, those suffering Parkinsonism and patients with chronic mitochondrial disorders [151]. The mechanisms that contribute to the formation of these deletions may be related to mutations in nuclear genes that encode enzymes involved in mtDNA replication and maintenance but can also arise spontaneously. mtDNA deletions have been proposed to occur during repair, when double strand breaks in the genome occur leaving the single strands able to anneal with other regions of the genome, and this may explain their prevalence in tissues with high mtDNA turnover [for review see ref 152]. Deletions may occur in protein-coding genes of the mitochondria, or within the tRNA and rRNA genes, which pose a substantial threat as mutations in these regions have the potential to severely affect all mtDNA encoded genes. mtDNA mutations accumulate differentially with age across tissue types, and are commonly found in skeletal muscle [153-155]. The

physiological threat posed by systemic accumulation of large scale deletions was investigated using transgenic mice harboring a mutant form of the *twinkle* gene, which encodes a pivotal mitochondrial helicase. Although these mice accumulate substantial levels of mtDNA mutations, they display only mild respiratory deficiencies and do not suffer symptoms of severe accelerated aging [156]. The systemic accumulation of mtDNA mutation in these mice may not be sufficient to lead to mitochondrial dysfunction and pathological phenotype.

mtDNA deletions have been characterized in brain tissue of aged humans [157]. In the context of Parkinson's disease and Parkinsonism, which are characterized by the progressive loss of dopaminergic neurons of the substantia nigra, the mechanisms behind neuronal cell death are of extreme importance in understanding the molecular basis and progression of the disease. The role of mitochondrial dysfunction in the acquisition of Parkinsonism has been addressed in a transgenic model termed the MitoPark mouse. In these mice, disruption of mitochondrial transcription factor A specifically in dopaminergic neurons greatly reduces the efficiency of oxidative phosphorylation [158]. This mutation leads to an age-dependent onset of Parkinson's symptoms, and is strong evidence for deficiency in oxidative phosphorylation having a primary role in the death of dopaminergic neurons [158]. mtDNA is hypothesized to be responsible for the decline in respiratory function observed in Parkinsonism.

The types of mtDNA mutation responsible for respiratory chain deficiencies that occur in Parkinsonism have been investigated. Two groups independently examined mtDNA deletions in individual neurons of the substantia nigra, and found a high percentage of large scale deletions within these cells [159,160]. Neurons that contain mtDNA deletions of over 60% show a dramatic reduction in cytochrome c oxidase activity, a critical enzyme in oxidative phosphorylation with mtDNA encoded subunits, suggesting that high levels of mtDNA deletions have a negative affect on energy production *via* oxidative phosphorylation [159,160]. Examination of mtDNA mutation in other neurons of the brain does not yield similar patterns of mutation accumulation, suggesting that this mutation rate is unique to neurons in the substantia nigra [159,160]. Although the experiments by Bender *et al.* [159], and Kraysberg *et al.* [160] represent an important finding in Parkinson's research, their studies contained a limited number of subjects and expansion of this in the future would be of use in confirming their results.

The precise reason why mitochondria of the substantia nigra are particularly susceptible to accumulating mtDNA deletion mutations is unknown. These neurons are present in a highly oxidative environment that results from dopamine metabolism, and the increased oxidative stress may have an important role in the accumulation of these mutations. It may be that the turnover of mtDNA is very fast as a result of the oxidizing conditions surrounding these neurons, and this high rate of turnover allows for rapid expansion of the mutated mtDNA molecules [151], followed by ATP crisis and cell death. If this is the case, antioxidants targeting specifically to this region of the brain may be of significant use in the treatment of Parkinson's disease. In this context, the ability of resveratrol to elevate MnSOD levels in brain tissue [75] is interesting.

Recognition of the prevalence of large scale deletions within mitochondria of the substantia nigra was due to laser capture technique, a method in which individual neurons can be examined. This method of examining individual cells represents an important technological advance and has revealed interesting observations about the relationship between individual mitochondrion and the overall functioning of a cell. Multiple copies of mtDNA exist within every mitochondrion, and multiple mitochondria within every cell. Mutated mtDNA are therefore able to coexist with wildtype mtDNA, termed heteroplasmy. Wild type mtDNA are able to compensate for the defects in mutated mtDNA, and the resulting complementation between genomes prevents respiratory deficiency and ATP crisis [161]. The ratio of wildtype and mutated mtDNA is critical to determining if a defect in mitochondrial activity will be observed. Respiratory deficiency is generally observed when the percentage of mitochondria containing mtDNA deletions surpasses 60% [162]. Many investigations probing for changes in mitochondrial function use whole tissue homogenates or suspensions of many cells to perform their measurements. This has the potential to obscure their measurements by portraying a homogeneous pattern of mitochondrial mutations, when in reality there is a mosaic pattern of respiratory deficient cells within every tissue. This may also overlook localized areas of energy deficiency in regions of critical tissues as brain and heart.

Thus, from the data summarized above, it appears that mtDNA mutations, in particular deletions, can negatively impact mitochondrial function to the extent that cell death and SCD ensue. Many of the observations made with respect to mtDNA deletion mirror those made during aging, suggesting that accumulation of this type of mutation could limit lifespan, or at least impair tissue functions, under normal physiological conditions. Mechanisms of its formation and repair in mitochondria are unclear, and it would be of great interest to determine if interventions that could decrease the occurrence of mtDNA deletions specifically would have a positive effect on MLS. Repair of oxidative damage to mtDNA is capable of protecting against acute cellular stress *in vitro*, and it will be important to develop models to investigate the ability of enhanced mtDNA repair to protect against cell death and prolong lifespan *in vivo*. However, data from the mutator mice refutes the “vicious cycle of mitochondrial ROS production”, as mitochondria containing a high mutation burden show no evidence of increased oxidative stress, nor do they produce high levels of ROS. It may be, however, that mitochondrial dysfunction ultimately culminates not in wide spread oxidative stress, but rather the death of individual cells, and this may not be obvious in measurements of heterogeneous tissue samples. Improving our understanding of cell specific events related to aging should lead to a better understanding of the determinants of MLS.

CONCLUSIONS

Mitochondria are centrally positioned in the life and death of animal cells, and a mitochondrial theory of aging remains viable as a partial explanation for the effects of

aging in some animal tissues. Recently, our understanding of the role of mitochondrial ROS as a mediator of aging and thus a regulator of lifespan has been refined. ROS can damage mtDNA, but conclusive experimental evidence linking oxidative mtDNA damage to aging and lifespan is still awaited. The ‘vicious cycle’ theory, wherein mtDNA damage/mutation and ROS production interact in both directions, has not been supported experimentally, at least at the level of whole tissue function and organism aging. mtDNA oxidative damage and subsequent mutation is likely involved in some cell loss and tissue degeneration. However, this may be a one-way street, with oxidative stress damaging mtDNA leading to deletions, energetic failure, cell death and SCD, but no increase in ROS production due to accumulation of deletions. Oxidative damage to mitochondrial membranes is important in initiating apoptosis via disruption of interactions between cardiolipin and cytochrome c, prompting its release into the cytosol. Cells that are resistant to this oxidant-induced apoptosis are generally stress resistant. Reduced endogenous mitochondrial ROS production and elevated antioxidant enzyme activities probably all confer stress resistance via this mechanism. In any event, cell death via oxidative stress or other mechanisms will contribute to the loss of irreplaceable (or very slowly replaced) cells in critical tissues, ultimately leading to the physiological declines and organ failures that characterize aging and limit lifespan. The SCD hypothesis connecting mitochondrial dysfunction with aging and lifespan via their role in cell death provides a good framework for understanding one way in which these organelles influence lifespan. This hypothesis is not exclusive of other determinants of MLS; for example, the role of cancer is not accounted for, nor is it excluded. Similarly, it is unclear to what extent lost cells are replaceable by tissue resident progenitor cells. It will be interesting to investigate the possible contribution of mitochondrial-mediated cell death in populations of progenitor cells to animal aging and lifespan.

REFERENCES

- [1] Porter RK and Brand MD. Cellular oxygen consumption depends on body mass. *Am J Physiol* 269: R226-R228 (1995).
- [2] Buja LM and Vela D. Cardiomyocyte death and renewal in the normal and diseased heart. *Cardiovasc Pathol* In press (2008).
- [3] Olivetti G, Melissari M, Capasso JM and Anversa P. Cardiomyopathy of the aging human heart: myocyte loss and reactive cellular hypertrophy. *Circ Res* 68: 1560-1568 (1991).
- [4] Ferrari AU, Radaelli A and Centola M. Aging and the cardiovascular system. *J Appl Physiol* 95: 2591-2597 (2003).
- [5] Danias J, Lee KC, Zamora M-F, Chen B, Shen F, Filippopoulos T, et al. Quantitative analysis of retinal ganglion cell (RGC) loss in aging DBA/2NNia glaucomatous mice: comparison with RGC loss in aging C57/BL6 mice. *Invest Ophthalmol Visual Sci* 44: 5151-5162 (2003).
- [6] Pollack M, Phaneuf S, Dirks A and Leeuwenburgh C. The role of apoptosis in the normal aging brain, skeletal muscle and heart. *Ann NY Acad Sci* 959: 93-107 (2006).
- [7] Buffenstein R. The naked mole-rat: a new long-living model for human aging research. *J Gerontol A Biol Sci Med Sci* 60: 1369-1377 (2005).
- [8] Brunet-Rossini AK. Reduced free-radical production and extreme longevity in the little brown bat (*Myotis lucifugus*) versus two non-flying mammals. *Mech Ageing Dev* 125: 11-20 (2004).
- [9] Murakami S. Stress resistance in long-lived mouse models. *Exp Gerontol* 10: 1014-1019 (2006).
- [10] Rose MR, Vu LN, Park SU and Graves JL Jr. Selection on stress resistance increases longevity in *Drosophila melanogaster*. *Exp Gerontol* 27: 241-250 (1992).

- [11] Munoz MJ and Riddle DL. Positive selection of *Caenorhabditis elegans* mutants with increased stress resistance and longevity. *Genetics* 163: 171-180 (2003).
- [12] Holzenberger M. The GH/IGF-axis and longevity. *Eur J Endocrinol* 151: s23-s27 (2004).
- [13] Kenyon C, Chang J, Gensch E, Rudner A and Tabtiang R. A *C. elegans* mutant that lives twice as long as wild type. *Nature* 366: 461-464 (1993).
- [14] Friedman DB and Johnson TE. A mutation in the age-1 gene in *Caenorhabditis elegans* lengthens life and reduces hermaphrodite fertility. *Genetics* 118: 75-86 (1988).
- [15] Larsen, PL. Aging and resistance to oxidative damage in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* 90: 8905-8909 (1993).
- [16] Vanfleteren JR. Oxidative stress and ageing in *Caenorhabditis elegans*. *Biochem J* 292: 605-608 (1993).
- [17] Lithgow GJ, White TM, Hinerfeld DA, Johnson TE. Thermotolerance of a long-lived mutant of *Caenorhabditis elegans*. *J Gerontol* 49: 270-276 (1994).
- [18] Barsyte D, Lovejoy DA and Lithgow GJ. Longevity and heavy metal resistance in daf-2 and age-1 long-lived mutants of *Caenorhabditis elegans*. *FASEB J* 15: 627-634 (2001).
- [19] Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM and Garofalo RS. A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292: 107-110 (2001).
- [20] Liu P, Baker J, Perkins AS, Robertson EJ and Efstratiadis A. Mice carrying null mutations of the genes encoding insulin-like growth factor 1 (Igf-1) and type 1 IGF receptor (igf1r). *Cell* 75: 59-72 (1993).
- [21] Holzenberger M, Dupont J, Ducos B, Leneuve P, Géloën A, Even PC, *et al.* IGF-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature* 421: 182-187 (2003).
- [22] Blüher M, Kahn B and Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299: 572-574 (2003).
- [23] Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi T, Pandolfi PP, *et al.* The p66shc adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309-313 (1999).
- [24] Pinton P, Rimessi A, Marchi S, Orsini F, Migliaccio E, Giorgio M, *et al.* Protein kinase C beta and prolyl isomerase 1 regulate mitochondrial effects of the life-span determinant p66Shc. *Science* 315: 659-663 (2007).
- [25] Bishop NA and Guarente L. Genetic links between diet and lifespan: shared mechanisms from yeast to humans. *Nat Rev Genet* 8: 835-844 (2007).
- [26] Harper JM, Leathers CW and Austad SN. Does caloric restriction extend life in wild mice? *Aging Cell* 5: 441-449 (2006).
- [27] Lee GD, Wilson MA, Zhu M, Wolkow CA, de Cabo R, Ingram DK, *et al.* Dietary deprivation extends lifespan in *Caenorhabditis elegans*. *Aging Cell* 5: 515-524 (2006).
- [28] Houthoofd K and Vanfleteren JR. The longevity effect of dietary restriction in *Caenorhabditis elegans*. *Exp Gerontol* 41: 1026-1031 (2006).
- [29] Partridge L, Piper MD and Mair W. Dietary restriction in drosophila. *Mech Ageing Dev* 126: 938-950 (2005).
- [30] Burger JM, Hwangbo DS, Corby-Harris V and Promislow DE. The functional costs and benefits of dietary restriction in *Drosophila*. *Aging Cell* 6: 63-71 (2007).
- [31] Taylor A, Lipman RD, Jahngen-Hodge J, Palmer V, Smith D, Padhye N, *et al.* Dietary calorie restriction in the Emory mouse: effects on lifespan, eye lens cataract prevalence and progression, levels of ascorbate, glutathione, glucose, and glycohemoglobin, tail collagen breaktime, DNA and RNA oxidation, skin integrity, fecundity and cancer. *Mech Ageing Dev* 79: 33-57 (1995).
- [32] Apte UM, Limaye PB, Desai D, Bucci TJ, Warbritton A and Mechendale HM. Mechanisms of increased liver tissue repair and survival in diet-restricted rats treated with equitoxic doses of thioacetamide. *Toxicol Sci* 72: 272-282 (2003).
- [33] Ferguson M, Rebrin I, Forster MG and Sohal RS. Comparison of metabolic rate and oxidative stress between two different strains of mice with varying response to caloric restriction. *Exp Gerontol* In press (2008).
- [34] Koizumi A, Tsukada M, Wada Y, Masuda H and Weindruch R. Mitotic activity in mice is suppressed by energy-restriction induced torpor. *J Nutr* 122: 1446-1453 (1992).
- [35] Heldmaier G, Ortmann S and Elvert R. Natural hypometabolism during hibernation and daily torpor in mammals. *Resp Physiol Neurobiol* 141: 317-329 (2004).
- [36] Kapahi P, Boulton ME, Kirkwood TB. Positive correlation between mammalian life span and cellular resistance to stress. *Free Radic Biol Med* 26: 495-500 (1999).
- [37] Harper JM, Salmon AB, Leiser SF, Galecki AT and Miller RA. Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. *Aging Cell* 6: 1-13 (2007).
- [38] Buffenstein R. The naked mole-rat: a new long-living model for human aging research. *J Gerontol A Biol Sci Med Sci* 60: 1369-1377 (2005).
- [39] Salmon AB, Sadighi Akha AA, Buffenstein R, Miller RA. Fibroblasts from naked mole-rats are resistant to multiple forms of cell injury, but sensitive to peroxide, ultraviolet light and endoplasmic reticulum stress. *J Gerontol A Biol Sci Med Sci* 63: 232-241 (2008).
- [40] Labinskyy N, Csiszar A, Orosz Z, Smith K, Rivera A, Buffenstein R, *et al.* Comparison of endothelial function, O₂⁻ and H₂O₂ production, and vascular oxidative stress resistance between the longest-living rodent, the naked mole rat, and mice. *Am J Physiol Heart Circ Physiol* 291: 2698-2704 (2006).
- [41] Bartke A and Brown-Borg H. Life extension in the dwarf mouse. *Curr Top Dev Biol* 63: 189-225 (2004).
- [42] Murakami S, Salmon A and Miller RA. Multiplex stress resistance in cells from long-lived dwarf mice. *FASEB J* 17: 1565-1566 (2003).
- [43] Parrinello S, Samper E, Krtolica A, Goldstein J, Melov S and Campisi J. Oxygen sensitivity severely limits the replicative lifespan of murine fibroblasts. *Nat Cell Biol* 5: 741-747 (2003).
- [44] Maynard SP and Miller RA. Fibroblasts from long-lived Snell dwarf mice are resistant to oxygen-induced *in vitro* growth arrest. *Aging Cell* 5: 89-96 (2006).
- [45] Salmon AB, Murakami S, Bartke A, Kopchick J, Yasumura K and Miller RA. Fibroblast cell lines from young adult mice of long-lived mutant strains are resistant to multiple forms of stress. *Am J Physiol Endocrinol Metab* 289: 23-29 (2005).
- [46] Leiser SF, Salmon AB and Miller RA. Correlated resistance to glucose deprivation and cytotoxic agents in fibroblast cell lines from long-lived pituitary dwarf mice. *Mech Ageing Dev* 127: 821-829 (2006).
- [47] Harper JM, Salmon AB, Leiser SF, Galecki AT and Miller RA. Skin-derived fibroblasts from long-lived species are resistant to some, but not all, lethal stresses and to the mitochondrial inhibitor rotenone. *Aging Cell* 6: 1-13 (2007).
- [48] Harper JM, Salmon AB, Chang Y, Bonkowski M, Bartke A and Miller RA. Stress resistance and aging: influence of genes and nutrition. *Mech Ageing Dev* 127: 687-694 (2006).
- [49] Brown MF, Gratton TP and Stuart JA. Metabolic rate does not scale with body mass in cultured mammalian cells. *Am J Physiol Regul Integr Comp Physiol* 292: 2115-2121 (2007).
- [50] Halliwell B. Oxidative stress in cell culture: an under-appreciated problem? *FEBS Lett* 540: 3-6 (2003).
- [51] Brand MD, Affourtit C, Esteves TC, Green K, Lambert AJ, Miwa S, *et al.* Mitochondrial superoxide: production, biological effects, and activation of uncoupling proteins. *Free Radic Biol Med* 37: 755-767 (2004).
- [52] Sohal RS, Svensson I, Sohal BH and Brunk UT. Superoxide anion radical production in different animal species. *Mech Ageing Dev* 49: 129-135 (1989).
- [53] Sohal RS, Svensson I and Brunk UT. Hydrogen peroxide production by liver mitochondria in different species. *Mech Ageing Dev* 53: 209-215 (1990).
- [54] Ku H-H, Brunk UT and Sohal RS. Relationship between mitochondrial superoxide and hydrogen peroxide production and longevity of mammalian species. *Free Radic Biol Med* 15: 621-627 (1993).
- [55] Barja G. Rate of generation of oxidative stress-related damage and animal longevity. *Free Radic Biol Med* 33: 1167-1172 (2002).
- [56] Lambert AJ, Boysen HM, Buckingham JA, Yang T, Podlutzky A, Austad SN, *et al.* Low rates of hydrogen peroxide production by

- isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell* 6: 607-618 (2007).
- [57] Speakman JR. Correlations between physiology and lifespan-two widely ignored problems with comparative studies. *Aging Cell* 4: 167-175 (2005).
- [58] Balin AK, Pratt L. Oxygen modulates the growth of skin fibroblasts. *In Vitro Cell Dev Biol Anim* 38: 305-310 (2002).
- [59] Gnaiger E, Lassnig B, Kuznetsov A, Rieger G, Margreiter R. Mitochondrial oxygen affinity, respiratory flux control and excess capacity of cytochrome c oxidase. *J Exp Biol* 201: 1129-1139, (1998).
- [60] Silva JP, Shabalina IG, Dufour E, Petrovic N, Backlund EC, Hultenby K, et al. SOD2 overexpression: enhanced mitochondrial tolerance but absence of effect on UCP activity. *EMBO J* 24: 4061-4070 (2005).
- [61] Hagen TM, Yowe DL, Bartholomew JC, Wehr CM, Do KL, Park JY, et al. Mitochondrial decay in hepatocytes from old rats: membrane potential declines, heterogeneity and oxidants increase. *Proc Natl Acad Sci USA* 94: 3064-3069 (1997).
- [62] Cavazzoni M, Barogi S, Baracca A, Castelli GP and Lenaz G. The effect of aging and an oxidative stress on peroxide levels and the mitochondrial membrane potential in isolated rat hepatocytes. *FEBS Lett* 449: 53-56 (1999).
- [63] Lopez-Lluch G, Hunt N, Jones B, Zhu M, Jamieson H, Hilmer S, et al. Calorie restriction induces mitochondrial biogenesis and bioenergetic efficiency. *Proc Natl Acad Sci USA* 103: 1768-1773 (2006).
- [64] Okado-Matsumoto A and Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver. *J Biol Chem* 276: 38388-38393 (2001).
- [65] Nohl H, Jordan W. The metabolic fate of mitochondrial hydrogen peroxide. *Eur J Biochem* 111: 203-210 (1980).
- [66] Zhou Z, Kang YJ. Cellular and subcellular localization of catalase in the heart of transgenic mice. *J Histochem Cytochem* 48: 585-594 (2000).
- [67] Imai H and Nakagawa Y. Biological significance of phospholipid hydroperoxide glutathione peroxidase (PHGPx, GPx4) in mammalian cells. *Free Radic Biol Med* 34: 145-169 (2003).
- [68] Yang W, Li J and Hekimi S. A measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of *Caenorhabditis elegans*. *Genetics* 177: 2063-2074 (2007).
- [69] Yan L, Vatner DE, O'Connor JP, Ivessa A, Chen W, Hirotsani S, et al. Type 5 adenylyl cyclase disruption increases longevity and protects against stress. *Cell* 130: 247-258 (2007).
- [70] Andziak B, O'Connor TP and Buffenstein R. Antioxidants do not explain the disparate longevity between mice and the longest-living rodent, the naked mole-rat. *Mech Ageing Dev* 126: 1206-1212 (2005).
- [71] Brown MF and Stuart JA. Correlation of mitochondrial superoxide dismutase and DNA polymerase beta in mammalian dermal fibroblasts with species maximal lifespan. *Mech Ageing Dev* 128: 696-705 (2007).
- [72] Warner DS, Sheng H and Batinic-Haberle I. Oxidants, antioxidants and the ischemic brain. *J Exp Biol* 207: 3221-3231 (2004).
- [73] Solaini G and Harris DA. Biochemical dysfunction in heart mitochondria exposed to ischaemia and reperfusion. *Biochem J* 390: 377-394 (2005).
- [74] Robb EL, Page MM, Wiens BE, Stuart JA. Molecular mechanisms of oxidative stress resistance induced by resveratrol: Specific and progressive induction of MnSOD. *Biochem Biophys Res Commun* 367: 406-412 (2008).
- [75] Robb EL, Winkelmolen L, Visanji N, Brochie J, Stuart JA. Dietary resveratrol administration increases MnSOD expression and activity in mouse brain. *Biochem Biophys Res Commun* 372: 254-259 (2008).
- [76] Lebovitz RM, Zhang H, Vogel H, Cartwright Jr J, Dionne L, Lu N, et al. Neurodegeneration, myocardial injury, and perinatal death in mitochondrial superoxide dismutase-deficient mice. *Proc Natl Acad Sci USA* 93: 9782-9787 (1996).
- [77] Honda Y and Honda S. The daf-2 gene network for longevity regulates oxidative stress resistance and Mn-superoxide dismutase gene expression in *Caenorhabditis elegans*. *FASEB J* 13: 1385-1393 (1999).
- [78] Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, et al. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424: 277-283 (2003).
- [79] Hunter T, Bannister WH and Hunter GJ. Cloning, expression, and characterization of two manganese superoxide dismutases from *Caenorhabditis elegans*. *J Biol Chem* 272: 28652-28659 (1997).
- [80] Suzuki N, Inokuma TK, Yasuda K and Ishii N. Cloning, sequencing and mapping of a manganese superoxide dismutase gene of the nematode *Caenorhabditis elegans*. *DNA Res* 3: 171-174 (1996).
- [81] Honda Y, Tanaka M and Honda S. Modulation of longevity and diapause by redox regulation mechanisms under the insulin-like control in *Caenorhabditis elegans*. *Exp Gerontol* 43: 520-529 (2008).
- [82] Sun J, Folk D, Bradley TJ and Tower J. Induced overexpression of mitochondrial Mn-superoxide dismutase extends the lifespan of adult *Drosophila melanogaster*. *Genetics* 161: 661-672 (2002).
- [83] Sun J and Tower J. FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult *Drosophila melanogaster* flies. *Mol Cell Biol* 19: 216-228, (1999).
- [84] Sturtz LA, Diekert K, Jensen LT, Lill R and Culotta VC. A fraction of yeast CuZn-superoxide dismutase and its metallochaperone, CCS, localize to the intermembrane space of mitochondria. *J Biol Chem* 276: 38084-38089 (2001).
- [85] Kira Y, Sato EF and Inoue M. Association of CuZn-type superoxide dismutase with mitochondria and peroxisomes. *Arch Biochem Biophys* 399: 96-102 (2002).
- [86] Kabil H, Partridge L, Harshman LG. Superoxide dismutase activities in long-lived *Drosophila melanogaster* females: chico1 genotypes and dietary dilution. *Biogerontology* 8: 201-208 (2007).
- [87] Beyer WF Jr. and Fridovich I. Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. *Anal Biochem* 161: 559-566 (1987).
- [88] Spitz DR and Oberley LW. An assay for superoxide dismutase activity in mammalian tissue homogenates. *Anal Biochem* 179: 8-18 (1989).
- [89] Li Y, Huang T-T, Carlson EJ, Melov S, Ursell PC, Olson JL, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet* 11: 376-381 (1995).
- [90] Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics* 16: 29-37 (2003).
- [91] Williams MD, Van Remmen H, Conrad CC, Huang T-T, Epstein CJ and Richardson A. Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. *J Biol Chem* 273: 28510-28515 (1998).
- [92] Van Remmen H, Williams MD, Guo Z, Estlack L, Yang H, Carlson EJ, et al. Knockout mice heterozygous for *Sod2* show alterations in cardiac mitochondrial function and apoptosis. *Am J Physiol Heart Circ Physiol* 281: H1422-H1432 (2001).
- [93] Buettner GR, Ng CF, Wang M, Rodgers VG, Schafer FQ. A new paradigm: manganese superoxide dismutase influences the production of H₂O₂ in cells and thereby their biological state. *Free Radic Biol Med* 8: 1338-1350 (2006).
- [94] Dabkowski ER, Williamson CL, Hollander JM. Mitochondria-specific transgenic overexpression of phospholipid hydroperoxide glutathione peroxidase (GPx4) attenuates ischemia/reperfusion-associated cardiac dysfunction. *Free Radic Biol Med In press* (2008).
- [95] Ran Q, Liang H, Ikeno Y, Qi W, Prolla TA, Roberts LJ 2nd, et al. Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. *J Gerontol* 62: 932-942 (2007).
- [96] Schriener SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308: 1909-1911 (2005).
- [97] Lenaz G. Role of mitochondria in oxidative stress and ageing. *Biochim Biophys Acta* 1366: 53-67 (1998).
- [98] Miquel J. An integrated theory of aging as the result of mitochondrial-DNA mutation in differentiated cells. *Arch Gerontol Geriatr* 12: 99-117 (1991).
- [99] Shen J, Khan N, Lewis LD, Armand R, Grinberg O, Demidenko E, et al. Oxygen consumption rates and oxygen concentration in nolt-4

- cells and their mtDNA depleted (rho0) mutants. *Biophys J* 84: 1291-1298 (2003).
- [100] Kang H and Hamaski N. Maintenance of mitochondrial DNA integrity: repair and degradation. *Curr Genet* 4: 311-322 (2002).
- [101] Garrido N, Griparic L, Jokitalo E, Wartiovaara J, van der Bliek AM and Spelbrink JN. Composition and dynamics of human mitochondrial nucleoids. *Mol Biol Cell* 244: 1552-1562 (2003).
- [102] Gross HJ, Getz GS and Rabinowitz M. Apparent turnover of mitochondrial deoxyribonucleic acid and mitochondrial phospholipids in the tissues of the rat. *J Biol Chem* 244: 1552-1562 (1969).
- [103] Mandavilli BS, Santos JH and Van Houten B. Mitochondrial DNA repair and aging. *Mutat Res* 509: 127-151 (2002).
- [104] Evans MD, Dizdaroglu M and Cooke MS. Oxidative DNA damage and diseases: induction, repair and significance. *Mutat Res* 567: 1-61 (2004).
- [105] Barja G and Herrero A. Oxidative damage to mitochondrial DNA is inversely related to maximum life span in the heart and brain of mammals. *FASEB J* 14: 312-318 (2000).
- [106] Hamilton ML, Van Remmen H, Drake JA, Yang H, Guo ZM, Kewitt K, *et al.* Does oxidative damage to DNA increase with age? *Proc Natl Acad Sci USA* 98: 10469-10474 (2001).
- [107] Collins AR, Cadet J, Möller L, Poulsen HE, Viña J. Are we sure we know how to measure 8-oxo-7,8-dihydroguanine in DNA from human cells? *Arch Biochem Biophys* 423: 57-65 (2004).
- [108] Richter C, Park JW and Ames BN. Normal oxidative damage to mitochondrial and nuclear DNA is extensive. *Proc Natl Acad Sci USA* 85: 6465-6467 (1988).
- [109] Clayton DA, Doda JN and Friedberg EC. The absence of pyrimidine dimer repair mechanism in mammalian mitochondria. *Proc Natl Acad Sci USA* 71: 2777-2778 (1974).
- [110] LeDoux SP, Driggers WJ, Hollenworth BS and Wilson GL. Repair of alkylation and oxidative damage in mitochondrial DNA. *Mutat Res* 434: 149-159 (1999).
- [111] Croteau DL, Stierum RH and Bohr VA. Mitochondrial DNA repair pathway. *Mutat Res* 434: 137-148 (1999).
- [112] Driggers WJ, LeDoux SP and Wilson GL. Repair of oxidative damage within the mitochondrial DNA of RINr 38 cells. *J Biol Chem* 268: 22042-22045 (1993).
- [113] Domena JD and Mosbaugh DW. Purification of nuclear and mitochondrial uracil-DNA glycosylase from rat liver. Identification of two distinct subcellular forms. *Biochemistry* 24: 7320-7328 (1985).
- [114] Kang D, Nishida J, Iyama A, Nakabeppu Y, Furuichi M, Fujiwara T, *et al.* Intracellular localization of 8-oxo-dGTPase in human cells, with special reference to the role of enzyme in mitochondria. *J Biol Chem* 270: 14659-14665 (1995).
- [115] Takao M, Kanno S, Shiromoto T, Hasegawa R, Ide H, Ikeda S, *et al.* Novel nuclear and mitochondrial glycosylases revealed by disruption of the mouse Nth1 gene encoding an endonuclease III homolog for repair of thymine glycols. *EMBO J* 21: 3486-3493 (2002).
- [116] Englander EW, Hu Z, Sharma A, Lee HM, Wu ZH and Greeley GH. Rat MYH, a glycosylase for repair of oxidative damaged DNA has brain-specific isoforms that localize to neuronal mitochondria. *J Neurochem* 83: 1471-1480 (2002).
- [117] de Souza-Pinto NC, Eide L, Hogue BA, Thybo T, Stevnsner T, Seeberg E, *et al.* Repair of 8-oxodeoxyguanosine lesions in mitochondrial DNA depends on the oxoguanine DNA glycosylase (OGG1) gene and 8-oxoguanine accumulates in the mitochondrial DNA of OGG-1 defective mice. *Cancer Res* 61: 5378-5381 (2001).
- [118] Klungland A, Rosewell I, Hollenbach S, Larsen E, Daly G, Epe B, *et al.* Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. *Proc Natl Acad Sci USA* 96: 13300-13305 (1999).
- [119] Stuart JA, Bourque BM, de Souza-Pinto NC and Bohr VA. No evidence of mitochondrial respiratory dysfunction in OGG1-null mice deficient in removal of 8-oxodeoxyguanine from mitochondrial DNA. *Free Radic Biol Med* 38: 737-745 (2005).
- [120] Stierum RH, Dianov GL and Bohr VA. Single-nucleotide patch base excision repair of uracil by mitochondrial protein extracts. *Nucleic Acids Res* 27: 3712-3719 (1999).
- [121] Endres M, Biniszkiwicz D, Sobol RW, Harms C, Ahmadi M, Lipski A, *et al.* Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase. *J Clin Invest* 113: 1711-1721 (2004).
- [122] Akbari M, Otterlei M, Pena-Diaz J and Krokan HE. Different organization of base excision repair of uracil in DNA in nuclei and mitochondria and selective upregulation of mitochondrial uracil-DNA glycosylase after oxidative stress. *Neuroscience* 145: 1201-1212 (2007).
- [123] Akbari M, Visnes T, Krokan HE and Otterlei M. Mitochondrial base excision repair of uracil and AP sites takes place by single-nucleotide insertion and long-patch DNA synthesis. *DNA Repair* 7: 605-616 (2008).
- [124] Kachhap S and Singh KK. Mitochondrial inhibition of uracil-DNA glycosylase is not mutagenic. *Mol Cancer* 1: 3-32 (2004).
- [125] Chang CM, Yu CC, Lu HT, Chou YF, Huang RF. Folate deprivation promotes mitochondrial oxidative decay: DNA large deletions, cytochrome c oxidase dysfunction, membrane depolarization and superoxide overproduction in rat liver. *Br J Nutr* 97: 855-863 (2007).
- [126] Dobson AW, Xu Y, Kelley MR, LeDoux SP and Wilson GL. Enhanced mitochondrial DNA repair and cellular survival after oxidative stress by targeting the human 8-oxoguanine glycosylase repair enzyme to mitochondria. *J Biol Chem* 275: 37518-37523 (2000).
- [127] Chatterjee A, Chang X, Nagpal JK, Chang S, Upadhyay S, Califano J, *et al.* Targeting human 8-oxoguanine DNA glycosylase to mitochondria protects cells from 2-methoxyestradiol-induced mitochondria-dependent apoptosis. *Oncogene* 27: 3710-3720 (2008).
- [128] Druzhyna NM, Hollenworth SB, Kelley MR, Wilson SP and LeDoux SP. Targeting human 8-oxoguanine glycosylase to mitochondria of oligodendrocytes protects against menadione-induced oxidative stress. *Glia* 42: 370-378 (2003).
- [129] Druzhyna NM, Musiyenko SL, Wilson GL and LeDoux SP. Cytokines induce nitric oxide-mediated mtDNA damage and apoptosis in oligodendrocytes. Protective role of targeting 8-oxoguanine glycosylase to mitochondria. *J Biol Chem* 280: 21673-21679 (2005).
- [130] Fishel ML, Seo YR, Smith ML and Kelley MR. Imbalancing the DNA base excision repair pathway in the mitochondria; targeting and overexpressing N-methylpurine DNA glycosylase in mitochondria leads to enhanced cell killing. *Cancer Res* 63: 608-615 (2003).
- [131] Zhang H, Mizumachi T, Carcel-Trullols J, Li L, Naito A, Spencer HJ, *et al.* Targeting human 8-oxoguanine DNA glycosylase (hOGG1) to mitochondria enhances cisplatin cytotoxicity in hepatoma cells. *Carcinogenesis* 28: 1629-1637 (2007).
- [132] Demple B and Sung J-K. Molecular and biological roles of Ape1 protein in mammalian base excision repair. *DNA Repair* 4: 1442-1449 (2005).
- [133] Evans AR, Limp-Foster M and Kelley MR. Going APE over ref-1. *Mutat Res* 461: 83-108 (2000).
- [134] Frossi B, Tell G, Spessotto P, Colomabtti A, Vitale G and Pucillo C. H(2)O(2) induces translocation of APE/Ref-1 to mitochondria in the Raji B-cell line. *J Cell Physiol* 193: 180-186 (2002).
- [135] Chattopadhyay R, Wiederhold L, Szczesny B, Boldogh I, Hazra TK, *et al.* Identification and characterization of mitochondrial abasic (AP)-endonuclease in mammalian cells. *Nucleic Acids Res* 34: 2060-2076 (2006).
- [136] Xanthoudakis S, Smeyne RJ, Wallace JD and Curran T. The redox/DNA repair protein, Ref-1, is essential for early embryonic development in mice. *Proc Natl Acad Sci USA* 93: 8919-8923 (1996).
- [137] Izumi T, Brown DB, Naidu CV, Bhakat KV, MacInnes MA, Saito H, *et al.* Two essential but distinct functions of the mammalian abasic endonuclease. *Proc Natl Acad Sci USA* 102: 5739-5743 (2005).
- [138] Miera LB, Devaraj S, Kisby GE, Burns DK, Daniel RL, Hammer RE, *et al.* Heterozygosity for the Mouse *Apex* Gene Results in Phenotypes Associated with Oxidative Stress. *Cancer Res* 61: 5553-5557 (2001).
- [139] Tomicici M, Eschbach E and Kaina B. Expression of yeast but not human apurinic/apyrimidinic endonuclease renders Chinese hamster cells more resistant to DNA damaging agents. *Mutat Res* 383: 155-165 (1997).
- [140] Ho R, Racheck LI, Xu Y, Kelley MR, LeDoux SP and Wilson GL. Yeast apurinic/apyrimidinic endonuclease Ape1 protects mammalian neuronal cell line from oxidative stress. *J Neurochem* 102: 13-24 (2007).

- [141] Shokolenko IN, Alexeyev MF, Robertson FM, Ledoux SP and Wilson GL. The expression of Exonuclease III from *E. Coli* in mitochondria of breaks cancer cells diminishes mitochondrial DNA repair capacity and cell survival after oxidative stress. *DNA Repair* 2: 471-482 (2003).
- [142] Li M-X, Wang D, Zhong Z-Y, Xiang D-B, Li Z-P, Xie J-Y, *et al.* Targeting truncated APE1 in mitochondria enhances cell survival after oxidative stress. *Free Radic Biol Med In press* (2008).
- [143] Graziewicz MA, Longley MJ and Copeland WC. DNA polymerase γ in mitochondrial DNA replication DNA replication and repair. *Chem Rev* 106: 383-405 (2006).
- [144] Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, *et al.* Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429: 417-423 (2004).
- [145] Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth S, *et al.* Mitochondrial DNA mutations, oxidative stress and apoptosis in mammalian aging. *Science* 309: 481-484 (2005).
- [146] Trifunovic A, Hansson A, Wredenberg A, Rovio AT, Dufour E, Khvorostov I, *et al.* Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci USA* 102: 17993-17998 (2005).
- [147] Zhang D, Mott JL, Chang SW, Denniger G, Feng Z and Zassenhaus HP. Construction of transgenic mice with tissue-specific acceleration of mitochondrial DNA mutagenesis. *Genomics* 69: 151-161 (2000).
- [148] Zhang D, Mott JL, Farrar P, Ryerse JS, Chang S-W, Stevens M, *et al.* Mitochondrial DNA mutations activate the mitochondrial apoptotic pathway and cause dilated cardiomyopathy. *Cardiovasc Res* 57: 147-157 (2003).
- [149] Bielas JH and Loeb LA. Quantification of random genetic mutations. *Nat Methods* 2: 285-290 (2005).
- [150] Vermulst M, Bielas JH, Kujoth GC, Ladiges WC, Rabinovitch PS, Prolla TA, *et al.* Mitochondrial point mutations do not limit the natural lifespan of mice. *Nature Genet* 39: 540-542 (2007).
- [151] Reeve AK, Krishnan KJ, Elson JL, Morris CM, Bender A, Lightowlers RN, *et al.* Nature of mitochondrial DNA deletions in substantia nigra neurons. *Am J Hum Genet* 82: 228-235 (2008).
- [152] Taylor RW and Turnbull DW. Mitochondrial DNA mutations in human disease. *Nat Rev Genet* 6: 389-401 (2005).
- [153] Liu VW, Zhang C and Nagley P. Mutations in mitochondrial DNA accumulate differentially in three different tissues during aging. *Nucleic Acids Res* 26: 1268-1275 (1998).
- [154] Lee HC, Pang CY, Hsu HS and Wei YH. Differential accumulations of 4,977bp deletion in mitochondrial DNA of various tissues in human ageing. *Biochim Biophys Acta* 1226: 37-43 (1994).
- [155] Pesce V, Cormio A, Fracasso F, Vecchiet J, Felzani G, Lezza AM, *et al.* Age-related mitochondrial genotypic and phenotypic alterations in human skeletal muscle. *Free Radic Biol Med* 30: 1223-1233 (2001).
- [156] Tynjismaa H, Mjosund KP, Wanrooij S, Lappalainen I, Ylikallio E, Jalank A, *et al.* Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice. *Proc Natl Acad Sci USA* 102: 17687-17692 (2005).
- [157] Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF and Wallace DC. Mitochondrial DNA deletions in human brain: regional viability and increase with advanced age. *Nat Genet* 2: 324-329 (1992).
- [158] Ekstrand MI, Terzioglu M, Galter D, Zhu S, Hofstetter C, Lindqvist E, *et al.* Progressive parkinsonism in mice with respiratory-chain-deficient dopamine neurons. *Proc Natl Acad Sci USA* 104: 1325-1330 (2007).
- [159] Bender A, Krishnan KJ, Morris CM, Taylor GA, Reeve AK, Perry RH, *et al.* High levels of mitochondrial DNA deletions in substantia nigra neurons in aging and Parkinson disease. *Nat Genet* 38: 515-517 (2006).
- [160] Kraysberg Y, Kudryavtseva E, McKee AC, Geula C, Kowall NW and Khrapko K. Mitochondrial DNA deletions are abundant and cause functional impairment in aged human substantia nigra neurons. *Nat Genet* 38: 518-520 (2006).
- [161] McFarland R, Chinnery PF, Blakley EL, Schaefer AM, Morris AA, Foster SM, *et al.* Homoplasmy, heteroplasmy, and mitochondrial dystonia. *Neurology* 69: 911-916 (2007).
- [162] Sciacco M, Bonilla E, Schon EA, DiMauro S and Moraes CT. Distribution of wild-type and common deletion forms of mtDNA in normal and respiration-deficient muscle fibers from patients with mitochondrial myopathy. *Hum Mol Genet* 3: 13-19 (1994).
- [163] Anversa P, Hiler B, Ricci R, Guideri G and Olivetti G. Myocyte cell loss and myocyte hypertrophy in the aging rat heart. *J Am Coll Cardiol* 8: 1441-1448 (1986).
- [164] Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG and Capasso JM. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ Res* 67: 871-885 (1990).
- [165] Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, *et al.* Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res* 93: 604-613 (2003).
- [166] Strasser H, Tiefenthaler M, Steinlechner M, Eder I, Bartsch G and Konwalinka G. Age dependent apoptosis and loss of rhabdosphincter cells. *J Urol* 164: 1781-1785 (2000).
- [167] Porter MM, Vandervoort AA and Lexell J. Aging of human muscle: structure, function and adaptability. *Scand J Med Sci Sports* 5: 129-142 (1995).
- [168] Carlson BM. Denervation and the aging of skeletal muscle. *Basic Appl Myol* 14: 135-139 (2004).
- [169] Ishihara A and Araki H. Effects of age on the number and histochemical properties of muscle fibers and motoneurons in the rat extensor digitorum longus muscle. *Mech Ageing Dev* 45: 213-221 (1988).
- [170] Mattson MP, Pedersen WA, Duan W, Culmsee C and Camandola S. Cellular and molecular mechanisms underlying perturbed energy metabolism and neuronal degeneration in Alzheimer's and Parkinson's diseases. *Ann NY Acad Sci* 893: 154-175 (1999).
- [171] Yaghmaie F, Saeed O, Garan SA, Freitag W, Timiras PS and Sternberg H. Caloric restriction reduces cell loss and maintains estrogen receptor- α immunoreactivity in the pre-optic hypothalamus of female B6D2F1 mice. *Neuro Endocrinol Lett* 26: 197-203 (2005).
- [172] Qu B, Halliwell B, Ong CN, Lee BL and Li Q-T. Caloric restriction prevents oxidative damage induced by the carcinogen clofibrate in mouse liver. *FEBS Lett* 473: 85-88 (2000).
- [173] Li Y, Yan Q, Pendergrass WR and Wolf NS. Response of lens epithelial cells to hydrogen peroxide stress and the protective effect of caloric restriction. *Exp Cell Res* 239: 254-63 (1998).
- [174] Herrero A and Barja G. Sites and mechanisms responsible for the low rate of free radical production of heart mitochondria in the long-lived pigeon. *Mech Ageing Dev* 98: 95-111 (1997).
- [175] Herrero A and Barja G. H₂O₂ production of heart mitochondria and aging rate are slower in canaries and parakeets than in mice: sites of free radical generation and mechanisms involved. *Mech Ageing Dev* 103: 133-146 (1998).
- [176] Gredilla R, Barja G and Lopez-Torres M. Effect of short-term caloric restriction on H₂O₂ production and oxidative DNA damage in rat liver mitochondria and location of the free radical source. *J Bioenerg Biomembr* 33: 279-287 (2001).
- [177] Sanz A, Gomez J, Caro P and Barja G. Carbohydrate restriction does not change mitochondrial free radical generation and oxidative DNA damage. *J Bioenerg Biomembr* 38: 327-333 (2006).
- [178] Sanz A, Caro P, Ayala V, Portero-Otin M, Pamplona R and Barja G. Methionine restriction decreases mitochondrial oxygen radical generation and leak as well as oxidative damage to mitochondrial DNA and proteins. *FASEB J* 20: 1064-1073 (2006).
- [179] Judge S, Judge A, Grune T and Leeuwenburgh C. Short-term CR decreases cardiac mitochondrial oxidant production but increases carbonyl content. *Am J Physiol Regul Integr Comp Physiol* 286: R254-R259 (2004).
- [180] Gomez J, Caro P, Naudi A, Portero-Otin M, Pamplona R and Barja G. Effect of 8.5% and 25% caloric restriction on mitochondrial free radical production and oxidative stress in rat liver. *Biogerontology* 8: 555-566 (2007).
- [181] Csiszar A, Labinskyy N, Zhao X, Hu F, Serpillon S, Huang Z, *et al.* Vascular superoxide and hydrogen peroxide production and oxidative stress resistance in two closely related rodent species with disparate longevity. *Aging Cell* 6: 783-97 (2007).

- [182] Sohal RS, Ku H-H and Agarwal S. Biochemical correlates of longevity in two closely related rodent species. *Biochem Biophys Res Commun* 196: 7-11 (1993).
- [183] Sohal RS, Sohal BH and Orr WC. Mitochondrial superoxide and hydrogen peroxide generation, protein oxidative damage, and longevity in different species of flies. *Free Radic Biol Med* 19: 499-504 (1995).
- [184] Caro P, Gomez J, Lopez-Torres M, Sanchez I, Naudi´ A, Jove M, *et al.* Forty percent and eighty percent methionine restriction decrease mitochondrial ROS generation and oxidative stress in rat liver. *Biogerontology* 9: 183-196 (2008).
- [185] Lambert AJ and Merry BJ. Lack of effect of caloric restriction on bioenergetics and reactive oxygen species production in intact rat hepatocytes. *J Gerontol A Biol Sci Med Sci* 60: 175-180 (2005).
- [186] de Magalhaes JP, Costa J and Toussaint O. HAGR: the Human Ageing Genomic Resources. *Nucleic Acids Res* 33: D537-D543 (2005).

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