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FORUM REVIEW ARTICLE

Regulation of Lifespan by the Mitochondrial Electron Transport Chain: Reactive Oxygen Species-Dependent and Reactive Oxygen Species-Independent Mechanisms

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Abstract

Significance: Aging is a consequence of the accumulation of cellular damage that impairs the capacity of an aging organism to adapt to stress. The Mitochondrial Free Radical Theory of Aging (MFRTA) has been one of the most influential ideas over the past 50 years. The MFRTA is supported by the accumulation of oxidative damage during aging along with comparative studies demonstrating that long-lived species or individuals produce fewer mitochondrial reactive oxygen species and have lower levels of oxidative damage. ***Recent Advances:*** Recently, however, species that combine high oxidative damage with a longer lifespan (*i.e.*, naked mole rats) have been described. Moreover, most of the interventions based on antioxidant supplementation do not increase longevity, as would be predicted by the MFRTA. Studies to date provide a clear understanding that mitochondrial function regulates the rate of aging, but the underlying mechanisms remain unclear. ***Critical Issues:*** Here, we review the reactive oxygen species (ROS)-dependent and ROS-independent mechanisms by which mitochondria can affect longevity. We discuss the role of different ROS (superoxide, hydrogen peroxide, and hydroxyl radical), both as oxidants as well as signaling molecules. We also describe how mitochondria can regulate longevity by ROS-independent mechanisms. We discuss alterations in mitochondrial DNA, accumulation of cellular waste as a consequence of glyco- and lipoxidative damage, and the regulation of DNA maintenance enzymes as mechanisms that can determine longevity without involving ROS. ***Future Directions:*** We also show how the regulation of longevity is a complex process whereby ROS-dependent and ROS-independent mechanisms interact to determine the maximum lifespan of species and individuals. *Antioxid. Redox Signal.* 19, 1953–1969.

Introduction

Aging is a pathological process that affects our health and independence. Over time, we succumb to a myriad of age-related pathologies and eventually death. A rapidly aging global population puts pressure on our healthcare systems. Due to this burden, society's—and thus the scientific community's—interest in the mechanisms of aging has intensified greatly during the last 100 years. In addition to its social importance, the aging process is scientifically fascinating, and understanding the multifaceted processes responsible for it will contribute to our understanding in other aspects of biology and medicine.

Aging is a consequence of the progressive accumulation of deleterious changes that reduce an organism's ability to resist stress (65). According to the Mitochondrial Free Radical Theory of Aging (MFRTA), mitochondrial free radicals generated as byproducts of metabolism cause the accumulation of oxidative damage and aging (63, 64). However, it is currently accepted that the MFRTA can only partially explain the aging phenomenon (155). In this review, we present evidence for and against the MFRTA. We also review some variations (reactive oxygen species [ROS] dependent) and alternatives (ROS independent) to the MFRTA that consider mitochondria as central regulators of aging, and can be experimentally tested.

Mitochondria are not only the powerhouses of the cell but also the essential components of intermediary metabolism, where they are required for cellular differentiation, iron metabolism, and the central regulation of apoptosis (188). Mitochondrial diseases dramatically illustrate the results of mitochondrial dysfunction (122). To date, there is no doubt that mitochondria are altered during aging, but there is a lack of consensus regarding whether they initiate the process or are merely subjected to the consequences (155).

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Is the MFRTA Still Valid Today?

To determine whether mitochondria drive aging or whether their progressive dysfunction is simply a consequence of aging, the validity of MFRTA requires assessment. Two main observations support the MFRTA. First, there is an accumulation of oxidative damage with age (31, 51, 114, 156, 177), and secondly, long-lived individuals or species seem to produce fewer ROS and accumulate less damage than short-lived individuals (56, 152, 153, 169, 170). During the 1990s, Sohal's group published several correlations between the production of mitochondrial reactive oxygen species (mtROS) and lifespan in several mammalian (169, 170), bird (86), and insect species (168). Similar results have subsequently been reported by different groups in the same and other species (14, 89). However, important exceptions have also been described (89, 109, 110). The most notable of these exceptions is the naked mole rat, which lives seven times longer than the mouse, but produces the same amount of mtROS (89), and has very low levels of antioxidants (8) and high levels of oxidative damage (9). However, a major problem with all of these studies supporting or refuting the MFRTA is that they employed different methodologies, and most of them only measured levels of hydrogen peroxide (H_2O_2) in isolated mitochondria. Importantly, the main ROS produced by the animal electron transport chain (ETC) is superoxide and not H_2O_2 (46). Measuring H_2O_2 leads to errors in assessment, as the activity of superoxide dismutases may influence the levels detected. Interestingly, a recent study measuring superoxide in different primate species found a negative correlation with lifespan (36) similar to the original correlation described by Sohal *et al.* (170). *In vitro* studies support a role for Complex I, but not Complex III, in regulating longevity (153). Differences in ROS production between long-lived species or individuals are only apparent when substrates that allow the transfer of electrons through Complex I (*e.g.*, pyruvate and malate, or succinate without rotenone) are used (Fig. 1). These differences disappear when electron transfer through Complex I is inhibited (*e.g.*, using succinate plus rotenone) (153).

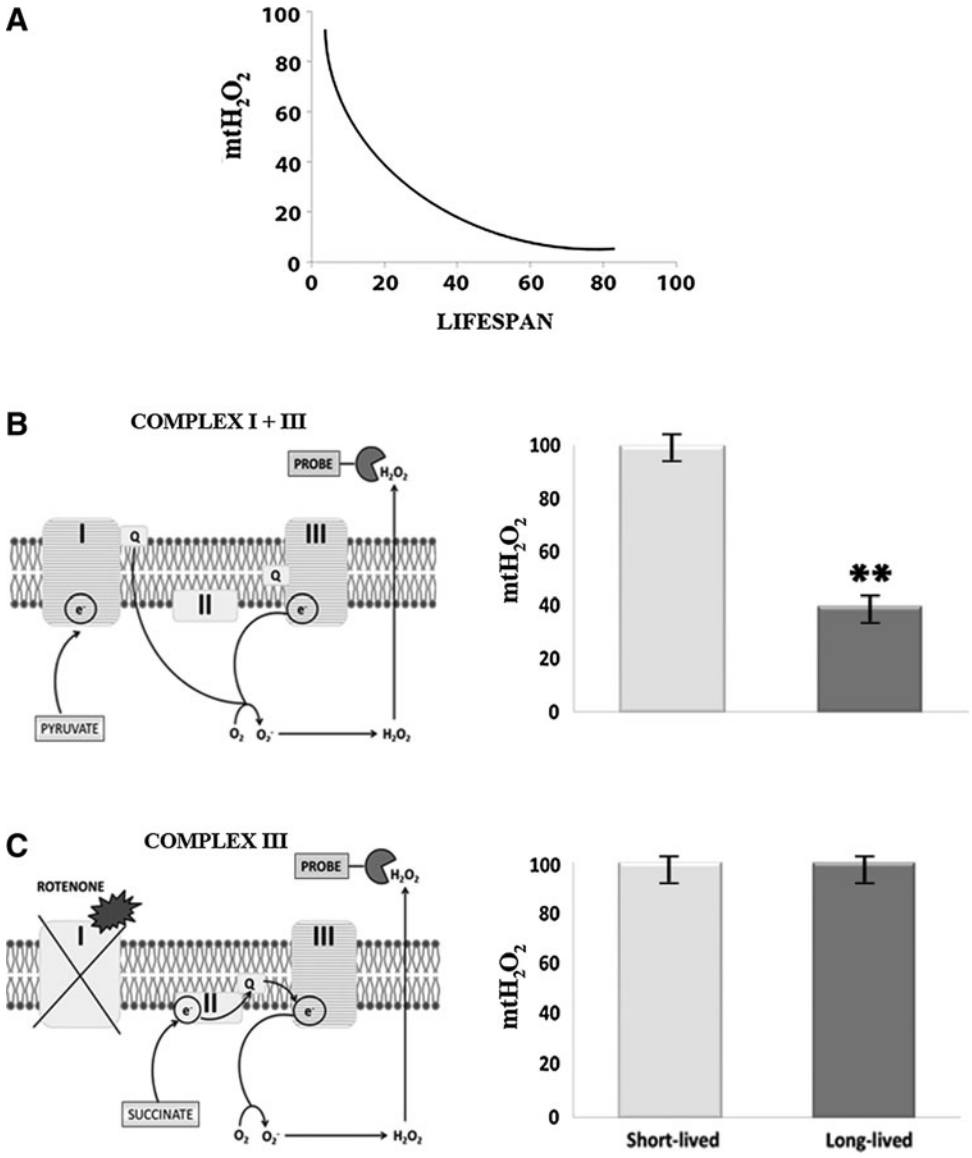

FIG. 1. Mitochondrial ROS production in isolated mitochondria. Most of the published reports studying the (negative) relationship between mtROS and longevity **(A)** have been conducted in isolated mitochondria. Differences in mtROS production are only found when the complex I is fed with electrons, using pyruvate + malate or succinate as substrates **(B)**. Under these conditions, complexes I and III produce ROS. When the pass of electrons through the complex I is inhibited using, for example, succinate + rotenone, the differences between long-lived species/individuals disappear **(C)**. Under these conditions, only complex III generates ROS. These results indicate that differences in aging-related ROS generation, at least *in vitro*, are exclusively due to complex I. **indicates statistically significant differences between the groups. H_2O_2, hydrogen peroxide; mtROS, mitochondrial reactive oxygen species; ROS, reactive oxygen species.

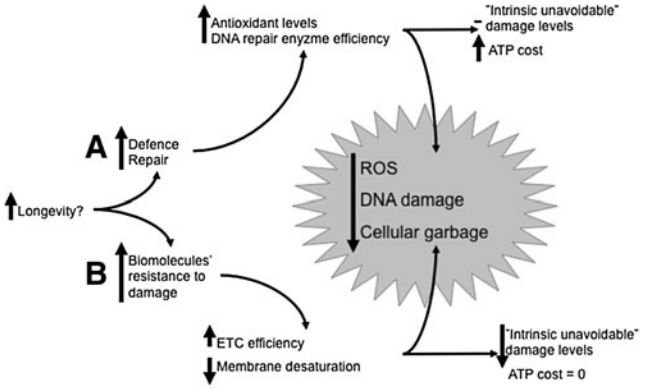

FIG. 2. Two possible strategies for increasing longevity. *Upward arrows* indicate an increase in a cellular component. *Downward arrows* indicate a decrease in that component. **(A)** Increasing cellular repair and defense against damaging agents by, for example, increasing DNA repair enzyme efficiency and antioxidant levels. **(B)** Decreasing the damage generation and increasing damage resistance of biomolecular targets by, for example, reducing the production of superoxide produced by Complex I or by decreasing membrane unsaturation, which renders membranes less vulnerable to oxidative damage. While both strategies will result in a decrease in ROS, DNA damage, and accumulation of cellular garbage, **(A)** will require a large increase in energy expenditure and will not decrease the rate at which intrinsic unavoidable damage occurs, whereas **(B)** will decrease the rate of intrinsic unavoidable damage accumulation by virtue of decreasing the rate at which all damage occurs, while having a negligible energetic cost. ETC, electron transport chain.

The accumulation of oxidative damage is not only dependent on ROS production. Individuals producing the same amount of free radicals may age slower if they express more antioxidants, have more efficient repair systems, or possess molecules that are more resistant to oxidation. In fact, there are two possible ways of extending lifespan: one is to reduce damage, and the other is to increase defense and repair (Fig. 2). In a competitive environment where resources are scarce, it seems logical that the most sensible strategy would be to optimize energy usage, and the most efficient use of energy would be to reduce damage. This hypothesis is supported by several independent studies that have shown that long-lived species do not seem to have more antioxidants or better repair systems (124, 151). In fact, these species are, in general, characterized by a reduction in the expression of antioxidant genes (23, 95, 123, 124, 151). Conversely, long-lived species also typically possess molecules that are more resistant to oxidation (132, 134, 135, 179, 180) (Fig. 3), as observed in naked mole rats (73, 126). For example, birds normally live longer than mammals of the same size and generally produce fewer mtROS (14, 86, 89, 153), but they also have cellular membranes that are more resistant to oxidation than those in mammals (73, 131, 132, 179). The trick to making a membrane more resistant to oxidation is simple: reduce the number of fatty acids with four or more double bonds to reduce the level of unsaturated fatty acids (73). This homeoviscous adaptation correlates with increased longevity (128), and it may be a strategy to reduce oxidative damage while maintaining the fluidity of the membrane (73). Similarly, long-lived mammals and birds are depleted of amino acids prone to oxidation, such as methionine or cysteine (112, 148).

Although interspecies differences in longevity may be explained by the differences in oxidative stress (153), it is possible that other mechanisms explain interindividual differences. Animals subjected to dietary restriction (DR) provide an excellent means to test this hypothesis. DR has been used for nearly a century to increase lifespan in laboratory animals (7), and most published studies show that rodents under DR had a decreased level of mtROS (12, 56, 61, 166); however, there is no consensus concerning lower organisms, such as flies or worms (84, 108). In fact, *in vivo* levels of mitochondrial H_2O_2 are not reduced by DR in flies (33). Nevertheless, as in long-lived species, animals subjected to DR are characterized by a long-term reduction in the levels of oxidative damage to proteins (91, 130), lipids (76, 140), and nucleic acids (43, 62). Paradoxically, the long-term reduction in oxidative damage could be caused by a short-term increase in mtROS production that elicits a prosurvival program involving the upregulation of antioxidant defenses (143, 158). Whether or not antioxidant defenses are upregulated during DR is controversial (55). Some studies in rodents indicate an upregulation of antioxidant defenses (160, 191), whereas others show downregulation or no change (116, 139). DR appears to have a different effect depending on the tissue

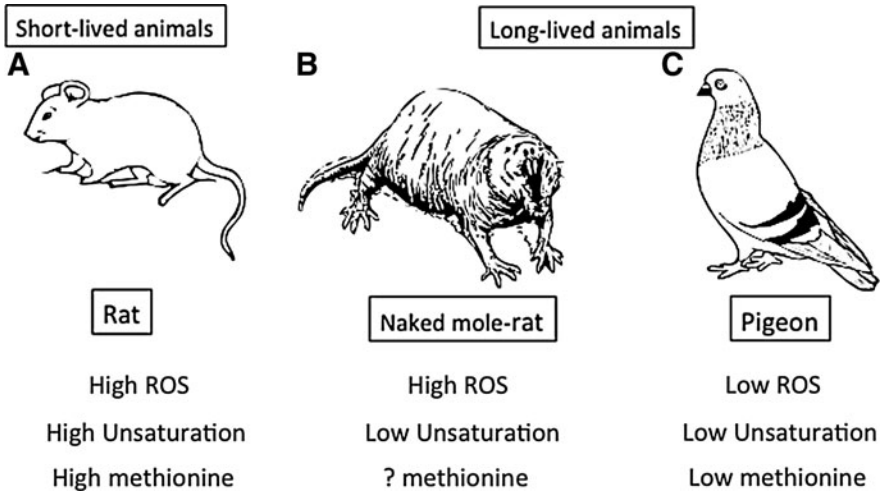

FIG. 3. Short-lived animals *versus* long-lived animals. Schematic representations of a rat **(A)**, naked mole rat **(B)**, and pigeon **(C)**. High levels of mtROS and molecules prone to oxidation characterize short-lived animals, such as rats. On the other hand, long-lived animals, such as pigeons, produce low levels of mtROS and have molecules that are more resistant to oxidation. Some species have high levels of mtROS and are long lived, such as naked mole rats. However, they have molecules that are more resistant to oxidation, and therefore can cope with higher levels of ROS.

analyzed and on the age at which DR is commenced (139). In conclusion, and at least in rodents, it is unclear if the long-term overexpression of antioxidants contributes to the extension of lifespan during DR, since overexpression of the same antioxidants without DR does not increase lifespan in the same animals (see below).

Although, DR appears to support a role of oxidative damage on lifespan determination, there is another physiological model that strongly argues against a role of antioxidants and pro-oxidants in the regulation of lifespan: exercise. Exhaustive exercise is well known to be deleterious for health either in rodents or in humans as professional sport (52). This kind of exercise has been related with overproduction of free radicals by both mitochondrial and extramitochondrial enzymes (187), which causes a burst of free radical generation, increasing oxidative stress (150). This supports the idea that overexposure to oxidative stress is deleterious. On the other hand, moderate exercise has positive effects on health. Moderate exercise increases mtROS production in the short-term, but also induces a general increase in antioxidant defenses (144) and a long-term reduction of oxidative stress (20) similar to the one described during DR and related with mitohormesis (see below) (143). Although exercise and DR have a similar effect on oxidative stress, they have a very different effect on lifespan on rodents. In rodents, DR increases lifespan up 50% (190); however, moderate exercise has no effect (69, 70). The lack of an effect on lifespan due to exercise contradicts the predictions made by the MFRTA, and as we will see there are other data against it.

The Antioxidant Paradox

The strongest evidence against the MFRTA arguably comes from studies that manipulate antioxidant levels. Many studies have shown that the administration of antioxidants fails to extend longevity (1, 98, 153), despite the fact that they are thought to be necessary for the initiation of the prosurvival response elicited by DR or alterations in insulin-like signaling pathways (194). The lack of an effect on lifespan could be the result of a failure to target the right place at the right time. Overexpression of endogenous antioxidants should overcome this deficiency, as they should boost defense systems that already exist. In fact, it is possible to extend the lifespan of worms or flies by overexpressing SOD1 (26), SOD2 (37), or the human methionine sulfoxide reductase A (MSRA) (147). Importantly, however, the increase in longevity with SOD1 and SOD2 is related to an increase, rather than a decrease, in oxidative stress and supports the idea of a mitochondrial signal that activates a prosurvival program (37) in response to mild cellular stress (194). The increase with MSRA is minor when the fly enzyme is used instead the human enzyme (32), and a different variant of the enzyme, MSRB, does not have any effect on lifespan (163). The inability of endogenous antioxidant overexpression to increase lifespan is clearer in mammals. There are currently no studies reporting a positive effect on lifespan (77, 114, 136), and the only study reporting an increase in lifespan by expressing catalase in mitochondria has not been replicated to date (30, 157).

The failure of supplementary antioxidants to extend lifespan could be due to endogenous antioxidants already being at an optimal level. Evolution may have selected for antioxidant levels that allow correct redox signaling while concomitantly preventing severe oxidative stress (Fig. 3). Thus, adding additional antioxidants may be detrimental due to the high-energetic cost or the alteration in cellular signaling. If this is true, then the elimination of existing defenses should have a dramatic effect on lifespan. However, this is not the case in observational studies. For example, it is possible to knockout all superoxide dismutase genes in *Caenorhabditis elegans* without shortening lifespan (182). In addition, although *Drosophila melanogaster* is more sensitive to the elimination of antioxidant defenses (114), it is still possible to reduce SOD2 activity to a minimal level in the nervous system without affecting longevity (101). However, the most relevant example comes from mammals, where total or partial knockout of the best-known antioxidants increases oxidative stress without reducing lifespan (78, 183, 196).

Reducing Damage Generation

One possible hypothesis is that there is always a certain amount of damage that cannot be prevented or repaired by antioxidants (endogenous or exogenous), and therefore it is possible that this intrinsic unavoidable damage regulates the rate of aging. Adding or removing antioxidants would not affect this type of damage. Available data suggest that evolution has favored the reduction of damage over the increase in repair or defense mechanisms in long-lived animals. However, to demonstrate whether or not a specific kind of damage regulates lifespan, the source of such damage should be specifically modified, and its effects on lifespan studied. For example, if we hypothesize that the generation of mtROS is responsible for aging, then we must specifically decrease mtROS generation to counteract the aging process. This is not straightforward, since very little is known regarding how mtROS are produced *in vivo*. Nevertheless, there are several approaches to address this difficulty (Fig. 4A, B). The simplest way is to remove the source of damage, such as by removing respiratory complexes that produce ROS. For obvious reasons, this is not possible in all animals. However, reducing the concentration of the complexes is easily achieved in invertebrate model organisms using RNAi techniques. Indeed, knockdown of Complex I, III, IV, or V has been shown to increase lifespan in both worms (41, 192) and flies (34). However, the mechanism underlying this phenomenon is not clear. Interestingly, knockdown of Complex II does not extend lifespan in worms (87). In fact, mutation of Complexes I or III subunits in worms also increases lifespan, despite concomitantly increasing superoxide levels (48, 192), whereas the mutation of Complex II subunits that also elevates oxidative stress has a negative effect on lifespan (74). It is possible that a specific ROS-mediated signal activates a prosurvival progam that overcomes the deleterious effects of increased oxidative damage (35) caused by mutations in Complexes I or III, but that this prosurvival program cannot compensate for alterations in the Kreb's cycle caused by mutations in Complex II.

An alternative approach consists of substituting a complex that is producing damage with a more efficient enzyme. The function of respiratory complexes can be replaced or substituted with so-called alternative enzymes (Fig. 4C, D). These enzymes are absent from the ETCs of most animals, but are normal regulators of respiration in plants or fungi. Alternative NADH dehydrogenases (NADH dehydrogenase internal 1 [NDI1], NADH dehydrogenase external 1 [NDE1], and

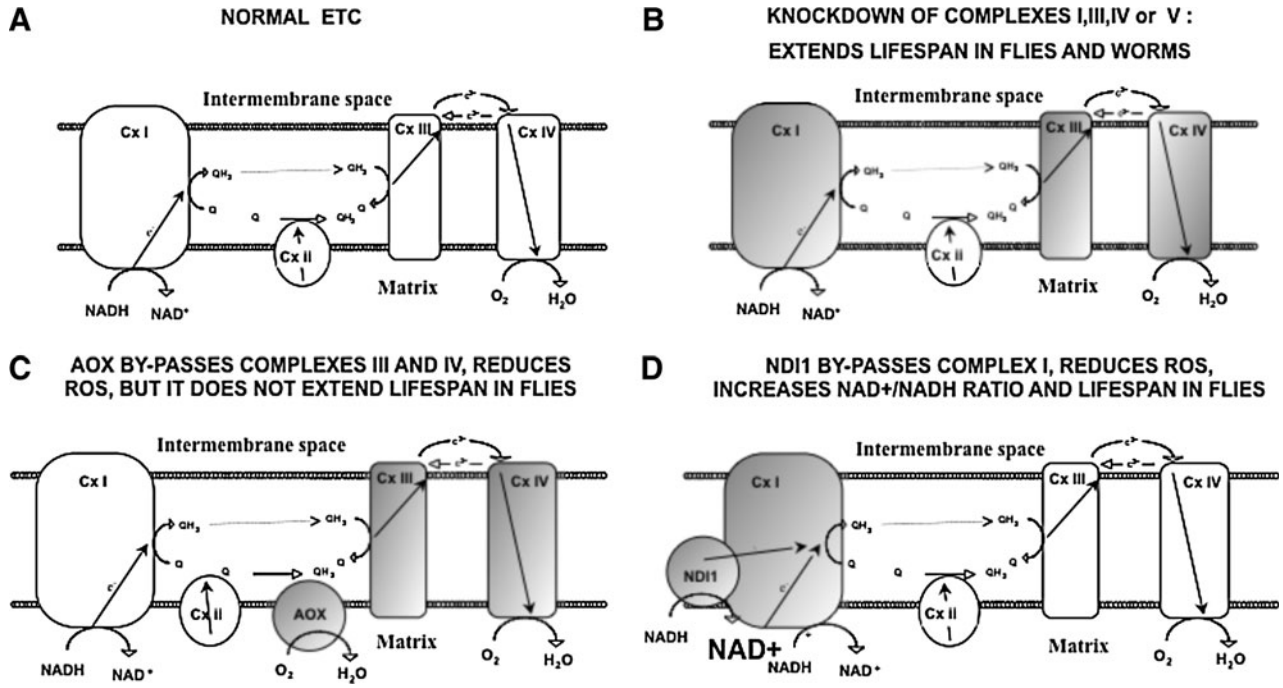

FIG. 4. Strategies to reduce ROS production. **(A)** Random mutagenesis of ETC subunits in wild-type animals is not a viable strategy. **(B)** However, it is possible to reduce the concentration of respiratory complexes using RNAi technology. **(C)** Another possibility is to use alternative enzymes, such as AOX, which bypass Complexes III and IV, or **(D)** NDI1, which bypasses Complex I. AOX, alternative oxidase; NDI1, NADH dehydrogenase internal 1.

NADH dehydrogenase external 2 [NDE2]) are located on both sides of the membrane and are able to feed electrons from NADH oxidation directly to the ubiquinone pool. The alternative oxidase (AOX) is able to bypass Complex III or IV, which reduces oxygen to water with electrons from ubiquinol. Knocking out Complex IV in *Podospora anserina* provokes the compensatory expression of AOX (44), which reduces mtROS production and stabilizes mitochondrial DNA (mtDNA), thereby preventing the accumulation of mutations. At the same time, knockout of this complex transforms *Podospora* into a virtually nonaging organism that grows continuously, compared to wild-type strains, which die after a couple of weeks. Interestingly, mutation of Complex I also extends the lifespan of *Podospora* (97); however, the increase is modest, and is NDI1 dependent as well as ROS independent. Different laboratories have successfully introduced alternative respiratory enzymes into worms (40), flies (49), mice (100), and human cells (38). These alternative enzymes are able to compensate mitochondrial dysfunction (149). Strikingly, the only positive effect on lifespan reported in animals has been bypassing respiratory Complex I using NDI1 (154). These results reinforce the role of Complex I in aging (171), and indicate that the engineer's approach is a valid option for prolonging lifespan.

ROS Modulation of Lifespan: Oxidative and Nonoxidative Mechanisms

As we have discussed, data regarding mtROS and longevity are contradictory. Initially, ROS were believed to be simply the deleterious byproducts of oxygen metabolism. It is now understood that ROS play a vital role in many cellular signaling processes, and that different types of ROS can elicit different physiological effects (118). Due to publication constraints, we will focus our discussion on three ROS: superoxide, H_2O_2, and hydroxyl radical.

The reactivity of superoxide is limited by its high rate of dismutation into H_2O_2, both spontaneously and when catalyzed by SODs. Furthermore, the negative charge of superoxide makes its diffusion across lipid bilayers unlikely; superoxide produced in a given compartment typically stays in that compartment, barring diffusion through pore complexes (96). On the other hand, the protonated form of superoxide, the hydroperoxyl radical (HOO•), is uncharged and can readily diffuse across membranes (5). Although only a small fraction of the superoxide population exists in the protonated form, the increased reactivity of the protonated form allows it to directly oxidize lipids (5). However, this increased reactivity also limits the diffusion distance, and as a result, the range over which a hydroperoxyl radical signal can act. Superoxide produced by Complex I is wholly directed into the mitochondrial matrix, whereas superoxide generated by Complex III is divided between the matrix and the intermembrane space (113). It has been proposed that relevant targets for aging, such as mtDNA, should be in the matrix (153).

H_2O_2 is relatively stable under physiological conditions, allowing it a much larger diffusion distance. However, despite its neutral charge, plasma membranes still act as a slight barrier to intercompartmental diffusion, leading to the formation of H_2O_2 gradients that depend on the rate of consumption between compartments (10). H_2O_2 is primarily consumed by glutathione peroxidases, which use H_2O_2 to oxidize two molecules of glutathione to glutathione disulfide, and catalase, which converts H_2O_2 into H_2O and O_2. These differences in stability and diffusion distance mean that H_2O_2

(generated from superoxide dismutation or by other means) is a more plausible messenger than other ROS (Fig. 5). H_2O_2 has been implicated as a vital mitochondrion-generated secondary messenger, whose range of communication is not limited to only the nucleus. Indeed, it has since been reported that H_2O_2 produced in the mitochondria can induce apoptosis in neighboring cells (137). Through signaling, mitochondrial ROS have been proposed as regulators of development (39) and longevity (94). Several cellular studies have shown that H_2O_2 (Fig. 5) may promote cellular proliferation (24), differentiation (92), senescence (50), or apoptosis (102). The effects of H_2O_2 depend on the cell type, cell cycle stage, and also concentration.

Neither superoxide nor H_2O_2 is reactive enough to directly attack lipids or mtDNA, but they do acquire it after reacting with free iron (22, 60). Iron–sulfur clusters are one of the few molecules that superoxide can attack directly. Intriguingly, the mitochondrion has plenty of these clusters, since it is the main location for the iron–sulfur cluster assembly (184). Important iron–sulfur cluster-containing mitochondrial proteins include respiratory Complexes I, II, and III, as well as aconitase, which are therefore direct targets of superoxide. The hydrophilic arm of Complex I possesses no less than nine iron–sulfur clusters, making it a prime target for superoxide. Electrons leaking from Complex I may react with oxygen to produce superoxide, which attacks iron–sulfur clusters of Complex I before antioxidants have the chance to intervene (Fig. 6). When iron–sulfur clusters are damaged, iron is released; Friedreich's ataxia is an excellent example of how excessively high free iron levels are deadly (11), because they favor the formation of hydroxyl radicals through Fenton's/Haber–Weiss reactions. In contrast to superoxide and, in particular, H_2O_2, the hydroxyl radical has an extremely short lifespan and diffusion distance (Fig. 5), owing to its ability to react with almost any cellular component. Hydroxyl radicals are extremely reactive, which is possibly why there are no specific enzymatic detoxification systems in place to deal with it, unlike other ROS. The hydroxyl radical can initiate lipid peroxidation by attacking fatty acids (6), cause protein aggregation (173), and promote deletions in mtDNA by causing double-strand breaks (Fig. 6) (29). Interestingly, aging is characterized by a decrease in respiration, increase in oxidative damage and free iron, and selective inhibition of enzymes such as aconitase (161, 193).

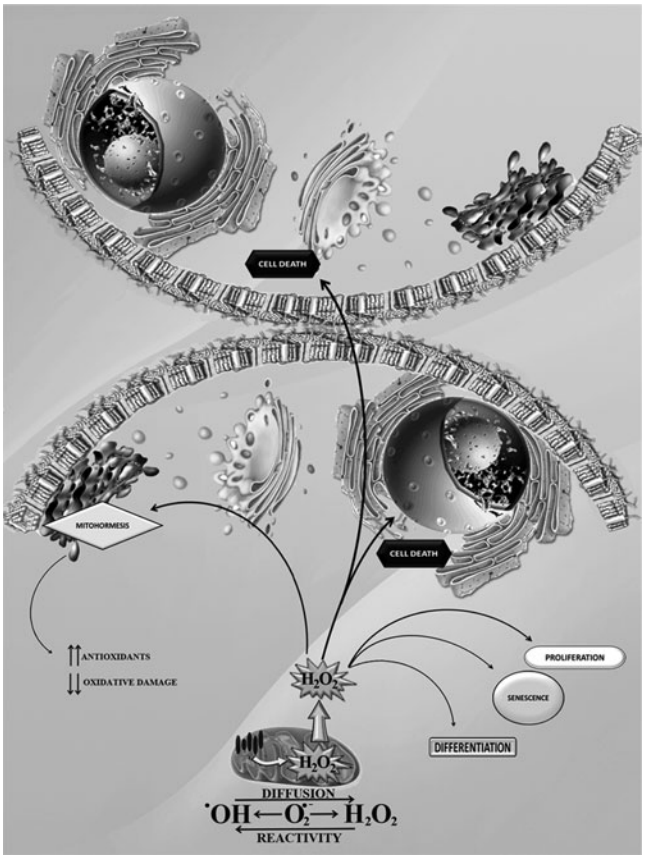

FIG. 5. Effect of ROS production on cellular signaling. ROS produced by mitochondria can act as cellular messengers either inside or outside the cell. The more reactive they are the shorter the distance they can diffuse, and the less suitable they are as messenger molecules. From this point of view, H_2O_2 is the best candidate, and hydroxyl radical is the worst. Interestingly, H_2O_2 may induce differentiation, proliferation, or cell death *in vitro*, and it has been related with the induction of mitohormesis *in vivo*, a cellular adaptation in response to stress that extends lifespan and is mediated by the generation of a mitochondrial signal.

As we mentioned, ROS are not only bad molecules but they also participate in cellular signaling, and are an essential component of cellular homeostasis. The difference in reactivity of each ROS species affects its lifespan and diffusion (Fig. 5). Less-reactive ROS are not only able to elicit a signal over much greater distances, but they are also able to form much larger concentration gradients over these distances. In this scenario, differential responses to local ROS concentrations could evoke a variety of changes to cellular physiology. Many cellular processes are now known to be redox regulated (167). Apoptosis, which relates mitochondrial function with aging (88), has a well-defined oxidative phenomenology. Oxidation of cysteine thiol groups is known to differentially regulate caspase activity (200), as well as the release of cytochrome c from mitochondria (121). Thiol and thioether groups in cysteine and methionine residues, respectively, are particularly vulnerable to oxidation and thus act as redox-switches, where structure and function are altered when the redox potential of the surrounding environment reaches a particular value whereupon oxidative modification of that group is stabilized (79). Different sulfur-containing groups have different redox potentials, with some being more difficult to oxidize than others. This leads to the situation where the right ROS signal must be produced to oxidize the relevant residue (*i.e.*, stronger oxidizing signal to oxidize residues with higher redox potentials) (167). Moreover, the signal must also be produced in the necessary proximity to the redox-modifiable entity. Production of the signal at too far a distance will result in it being consumed by the ubiquitous detoxifying systems before it reaches its intended target. The oxidative damage that accumulates with aging could therefore be viewed as the misfiring of ROS-signaling processes that subsequently affect targets other than those intended (79).

In contrast to what MFRTA predicts, there is evidence that ROS can have a positive effect on health and longevity through a process known as mitochondrial hormesis (mitohormesis) (143). This theory suggests that sublethal cellular stress provokes an ROS signal that induces stress response

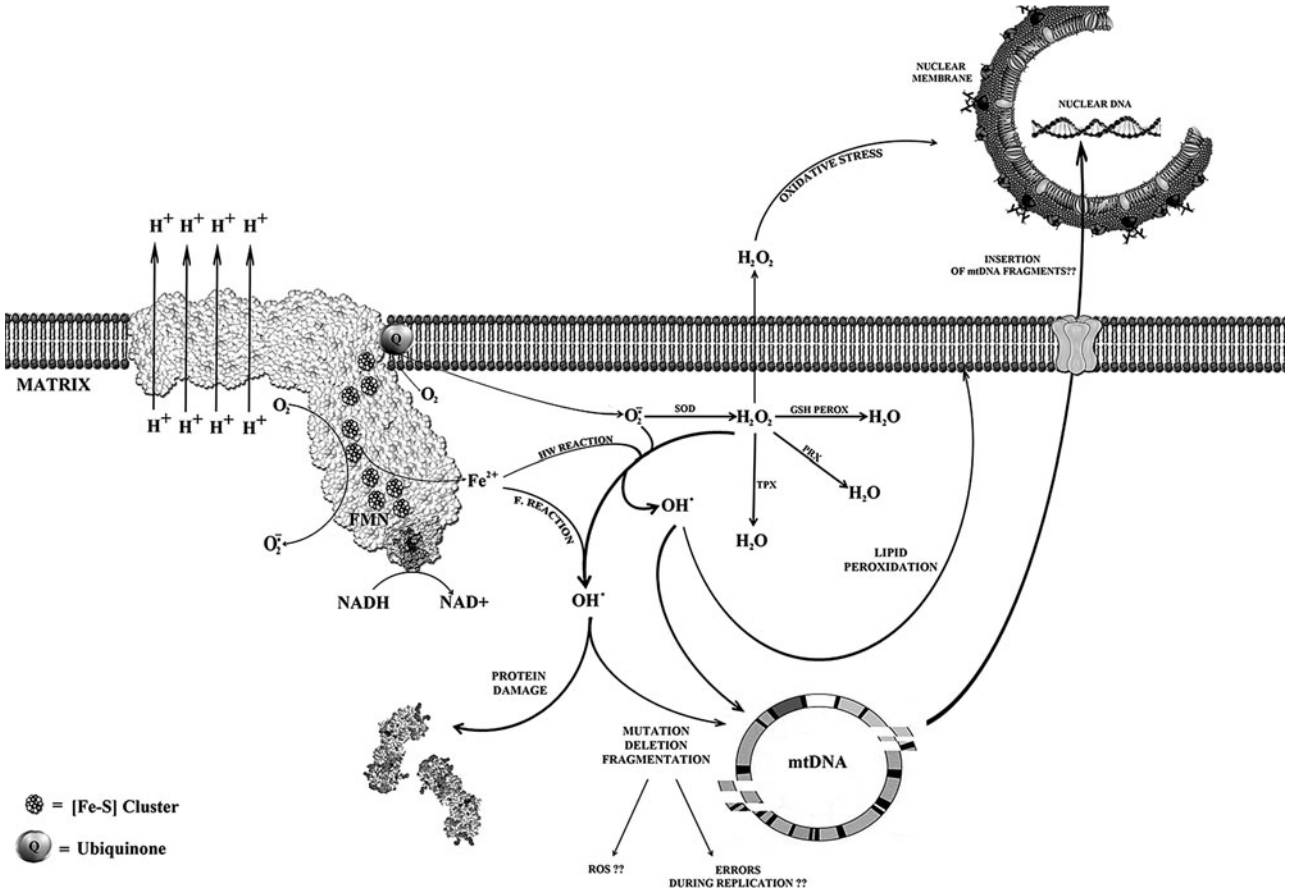

FIG. 6. Effects of ROS production on oxidative damage. Complex I is the main generator of superoxide in mitochondria. Superoxide is immediately dismuted to H_2O_2, either spontaneously or by the superoxide dismutases. H_2O_2 is then neutralized by peroxidases (using glutathione or thioredoxin as cofactors) or by peroxiredoxins. Despite its rapid dismutation, superoxide can still attack the iron–sulfur clusters of Complex I (or other enzymes) before antioxidants can neutralize it. Damaged iron–sulfur clusters release iron that can react both with H_2O_2 and superoxide to produce the extremely reactive hydroxyl radical. The hydroxyl radical can directly damage proteins, initiate lipid peroxidation, and even fragment mtDNA. H_2O_2 is not a free radical, so it can diffuse far away from the mitochondria causing damage in other places, that is, in the cellular nuclei after reacting with free iron or cupper. FMN, flavin mononucleotide; F. REACTION, Fenton's Reaction; GSH PEROX, glutathione peroxidase; HW REACTION, Haber–Weiss' reaction; PRX, peroxiredoxin; SOD, superoxide dismutase; TPX, thioredoxin peroxidase.

factors, which help maintain mitochondrial function and thus enhance longevity. In yeast, some forms of DR act through an increase in H_2O_2 levels mediated by upregulation of SOD2 activity (104), and a similar adaptation has been described in animals (158). A decrease in glucose availability causes an increase in the rate of oxidative phosphorylation (158), similar to the one that occurs during exercise (144), causing a mild physiological stress that is characterized by a temporary increase in the level of ROS (158). This increase initiates a long-term response that decreases oxidative damage and extends lifespan (143). Accordingly, mutations in Complex I in *C. elegans* (192), overexpression of SOD2 in *D. melanogaster* (37), and mutations in mitochondrial enzyme *COQ7* of *Mus musculus* (90) cause a common phenotype characterized by high levels of ROS without a reduction in longevity. It is possible that the initial increase in ROS generation serves to upregulate other processes that cause lifespan extension. The role of antioxidants in mitohormesis is not completely clear. If antioxidants themselves were responsible for the increased longevity, then administration or overexpression of the same antioxidants should increase lifespan, and as discussed above, this is not the case. Therefore, the upregulation of antioxidant defenses may simply be a means to ensure that the ROS signal is self-terminating. Given how ubiquitous redox signaling is currently known to be, the transient ROS signal proposed by the mitohormesis theory may act upon several different mechanisms to extend lifespan, including mitochondrial function, cell cycle regulation, target of rapamycin signaling, and protein homeostasis (proteostasis) (99). As we mentioned, exercise induces a mitohormetic adaptation characterized by an increase in antioxidant levels (20) without extension in lifespan (69, 70), whereas DR clearly extends it (190). It would thus be extremely useful to compare both treatments to find those pathways that should be specifically modulated to increase longevity.

An mtROS-Independent Control of Longevity

We have reviewed some of the mechanisms by which ROS can contribute to longevity. Although there is a substantial

amount of evidence showing that ROS are deleterious for most species [*C. elegans* being a notable exception in the case of superoxide (182)], there is no clear proof that they are a main determinant of normal aging. Mitochondria could however regulate longevity by an ROS-independent mechanism. From this point of view, ROS would correlate with longevity, because it would correlate with the real entity responsible for aging. Various reports to date show that by modifying mitochondrial function, it is possible to modify the rate of aging. Intriguingly, some of these modifications have opposite effects on ROS production (see above).

mtDNA has long been thought of as key to the aging process, due to the fact that mutations in mtDNA accumulate faster than in nuclear DNA (nDNA) (81), and short-lived animals accumulate mutations faster than long-lived animals (189). However, this depends on the tissue and the mutation (53, 59). The oxidation levels of mtDNA, but not nDNA, correlate with both mtROS production and maximum lifespan in mammals (15), which has been used to propose a connection between superoxide generation and accumulation of mutations in mtDNA (153). In fact, it is commonly accepted that this is caused by the more oxidizing redox environment found inside the mitochondria (126). However, superoxide is not reactive enough to directly attack mtDNA (22). Furthermore, oxidative lesions are easily repaired and do not normally lead to an accumulation of mutations (85), alteration of mitochondrial function (172), or reduction in lifespan (183). Although, both superoxide and H_2O_2 can cause mutations or deletions through the generation of the hydroxyl radical, as described above, the pattern of mutations found in mtDNA during aging is better explained by errors made by polymerase-γ during replication (85, 198). This can explain how mutations in respiratory subunits that have opposite effects on ROS production extend lifespan in worms and flies (192). Those mutations that reduce the need for mitochondrial replication (causing a mild decrease in mitochondrial function) will increase lifespan by delaying the age-related accumulation of mutations, at least under laboratory conditions. Mutations in the proofreading subunit of the polymerase-γ cause an aging-like phenotype in mice (88, 178), supporting a major role of mutations in aging. Curiously, the higher rate of mutations in mtDNA does not cause more oxidative stress, but an increase in apoptosis in postmitotic tissues (88). Recently, it has been proposed that the mutator phenotype may be caused by a dysregulation in stem cell differentiation that would occur early during embryogenesis (3). Strikingly, the stem cell phenotype is partially rescued by administration of *N*-acetyl-L-cysteine, indicating a complex alteration in ROS or redox equilibrium (3). Nevertheless, the results from the mutator mouse should be interpreted with caution, because the level of mutations reported in the mutator mouse is never reached during normal aging in wild-type mice (82, 185). Nowadays, the influence of mtDNA mutations on aging is still controversial (54). In fact, the proportion of point mutations in human mtDNA is quite low (115) with the exception of hot spots in certain tissues (105, 174). These mutations accumulate by a mechanism of clonal expansion constituting more than 90% of total mtDNA in single cells within certain tissues as the heart, substantia nigra, stomach, or liver (47, 103, 120). Deletions appear much more abundant and important in human aging (85). Initially, the number of cells with high levels of mutated molecules was considered too few to substantially affect tissue function (75). However, recently, it has been shown that at least in some tissues, as the ones cited before, cells with a deficient ETC caused by mutations might be abundant enough to disrupt normal function (83). There is a good co-localization of cytochrome oxidase-negative cells and accumulation of deletions in mtDNA in different tissues, including skeletal muscle (66) and the brain (59), which support the idea that the mutation of mtDNA is responsible for the loss of oxidative capacity (Fig. 7). Although, it does not prove that deletions cause aging, since the deletor mouse, which accumulates mtDNA deletions during aging and consequently develops progressive respiratory dysfunction, does not have a shorter lifespan compared to wild-type controls (181). Once again results from mouse models should be interpreted with caution, since the accumulation of mutations in mice and humans is different (53, 59). These last results indicate that the role of point mutations and deletions in human aging must be investigated in more detail.

One alternative hypothesis that would reconcile these contradictory results concerning mtDNA and aging was

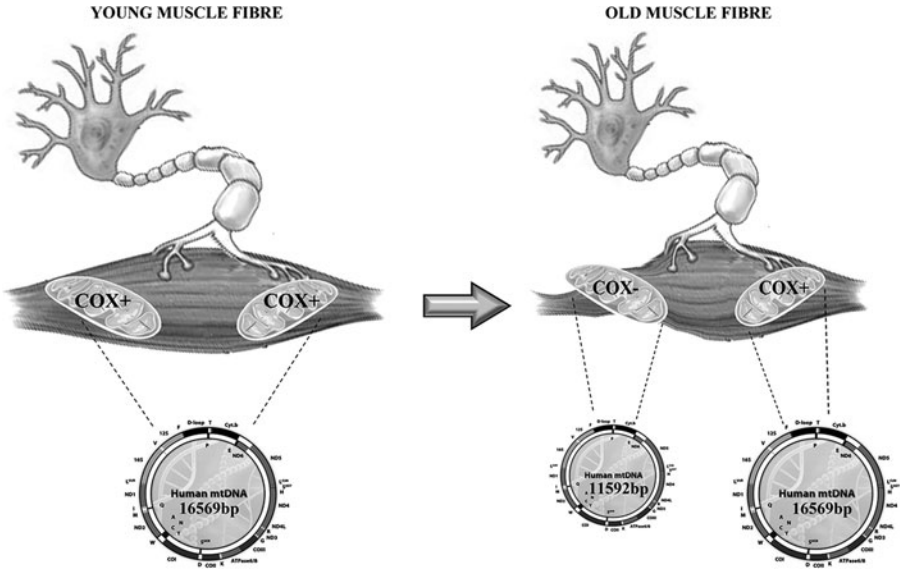

FIG. 7. Accumulation of mutations in mtDNA as a cause of aging. The role of mtDNA mutations in human aging is still controversial. Deletions appear to be much more abundant and important than point mutations in relation to human aging. Indeed, deletions accumulate with age in different tissues, for example, skeletal muscle, where there is a strong co-localization of COX-negative cells and accumulation of deletions. Accumulation of deletions would induce sarcopenia, increasing apoptosis in aged muscle. COX, cytochrome oxidase.

proposed by Richter in 1988 (142). According to his proposal, mtDNA fragments, which are produced by ROS damage (or by errors during replication), would escape mitochondria and accumulate in nDNA over time, thus inducing aging (Fig. 6). When outside of the mitochondria, mtDNA can cause inflammation (195) or even certain genetic syndromes through insertion into nDNA (106). Interestingly, an age-related increase in the presence of mtDNA fragments in nDNA has been described in both fungi (28) and rats (27). However, despite all of these observations, evidence that the insertion of mtDNA fragments into nDNA is relevant to aging has not been forthcoming.

Contradictory evidence concerning the mechanisms of aging may indicate that we are focusing on the wrong pathway. The Accumulative Waste Theory of Aging proposes that the accumulation of toxic products in the cell is the major force behind aging (175). Unicellular organisms, such as bacteria or yeast, divide asymmetrically to produce one old mother cell that accumulates damaged components and one daughter cell that is virtually new (2). This strategy allows these organisms to live forever. For obvious reasons, such a strategy is not valid in complex organisms with postmitotic tissues. Mitochondria are major determinants of the speed to which waste accumulates, and they can determine this without involving ROS produced by ETCs (68). Nonenzymatic glycation or glycoxidation of proteins (and other cellular components) is one of the main sources of cellular debris. Advanced glycoxidation end products (AGEs) are extremely reactive, causing the accumulation of products in the cytoplasm and in different organelles, including mitochondria, of components that the cell cannot eliminate (125). AGEs have long been known to be related to longevity. First, their levels are negatively correlated with lifespan in mammals (159). Secondly, they accumulate during aging (76, 129). Furthermore, their concentration is reduced in DR animals (129, 159). Glycoxidation is mainly determined by glycolytic flux. If it is interrupted, glycoxidation increases (68). There are several ways mitochondria can influence glycolytic flux, the most important being through the supply of NAD+ (67). Pyruvate produced during glycolysis is further oxidized to NADH, which is finally used to produce energy by the ETC. If NADH is not reoxidized to NAD+ by Complex I, there is a shortage of NAD+ available for the conversion of glyceraldehyde-3-phosphate into 1,3-biphosphoglycerate (Fig. 8). This immediately causes accumulation of dihydroxyacetone, which decomposes into methylglyoxal. Methylglyoxal is the main initiator of nonenzymatic glycation (18, 119). An efficient ETC, particularly an efficient Complex I, may guarantee that NAD+ is always available at the appropriate levels for glycolysis (171). In fact, patients suffering mitochondrial diseases are characterized by chronic lactic acidosis and accumulation of glycoxidative damage, which are also characteristics of old individuals (76, 146).

An appropriate balance between the reduced and oxidized forms of NADH is important not only for glycolysis. The ratio of NAD+/NADH, together with AMP/ATP levels (99), is a major sensor of the energetic and redox state of the cell (72). Based on the information provided by these sensors, cells can choose between running under a proaging or prosurvival program (Fig. 8). For example, DR is characterized by a change shifting the NAD+/NADH ratio to the left (45, 93), whereas aging is characterized by the opposite (21, 165). Different experimental reports support the notion that it is possible to regulate longevity by manipulating the levels of NADH and NAD+. In yeast, overexpression of the malate–aspartate NADH shuttle mimics DR, whereas its deletion suppresses lifespan extension (45). A similar phenomenon occurs in flies, where the knockdown of ETC complexes, which decreases the NAD+/NADH ratio, suppresses lifespan extension mediated by DR (199). On the other hand, the opposite strategy, an increase in the ratio *via* expression of NDI1 or overexpression of nicotinamidase in flies, notably extends lifespan (13, 154).

Another important contributor to the accumulation of cellular waste is lipoxidation (125). Lipoxidation is produced as a consequence of the oxidation of double bonds of fatty acids by free radicals. Polyunsaturated fatty acids are the most sensitive molecules to oxidation, and the more double bonds a fatty acid has, the more prone it is to oxidization (141). As with glycoxidation, the final result of lipoxidation is the formation of advanced lipoxidation end products (ALEs) (119). Like AGEs, ALEs are negatively correlated with longevity (133, 148) and accumulate during aging (130, 177), and this accumulation is delayed by DR (76, 130, 197). Most long-lived animals are characterized by low levels of mtROS, and membranes more resistant to oxidation (127). Intriguingly, these two phenomena are related at the molecular level due to the way desaturases operate. Desaturases require both electrons and energy to introduce double bonds into fatty acids (58). The reductive power is stored in NAD(P)H molecules (111). An effective ETC maintains low levels of NADH, limiting the activity of desaturases due to the lack of one basic supply (Fig. 8). Even animals where a lower level of ROS does not correlate with a longer lifespan are characterized by membranes more resistant to oxidation (127). This could be the result of more efficient mitochondria that operate efficiently even in an environment with high ROS levels or by the alternative strategy of reducing the activity of desaturases (134). An example of this is *C. elegans*, where lifespan is wholly independent of superoxide levels (182), but not of the unsaturation of the membranes (164). Long-lived worms have less unsaturated membranes, and reduction of desaturase activity alone is enough to extend lifespan (71, 164). Paradoxically, in flies where lifespan is directly correlated with superoxide levels, lipid membranes are characterized by very low levels of polyunsaturated fatty acids, lacking fatty acids with four or more double bonds such as docosahexaenoic or arachidonic acid (73). This could be an adaptation to the high levels of ROS produced during flying. Accordingly, birds are characterized by membranes with very low levels of unsaturation independent of ROS levels (73). Moreover, DR, which boosts respiration and increases the ratio of NAD+/NADH (45), also reduces membrane unsaturation and lipoxidative damage in mammals (130) and insects (197). In summary, lipoxidation can determine the rate of damage accumulation and can be controlled by mitochondria without modifying ROS production.

The accumulation of modifications in DNA, both genetic and epigenetic, has been proposed as a cause of aging (186). Two of the most studied enzymes related to aging, sirtuins and poly(ADP-ribose) polymerase (PARP), use NAD+ or some of its metabolites as cofactors (Fig. 8). This implies that a change in the ratio of NAD^+/NADH elicited by a change in ETC activity would alter the activity of these enzymes, thus

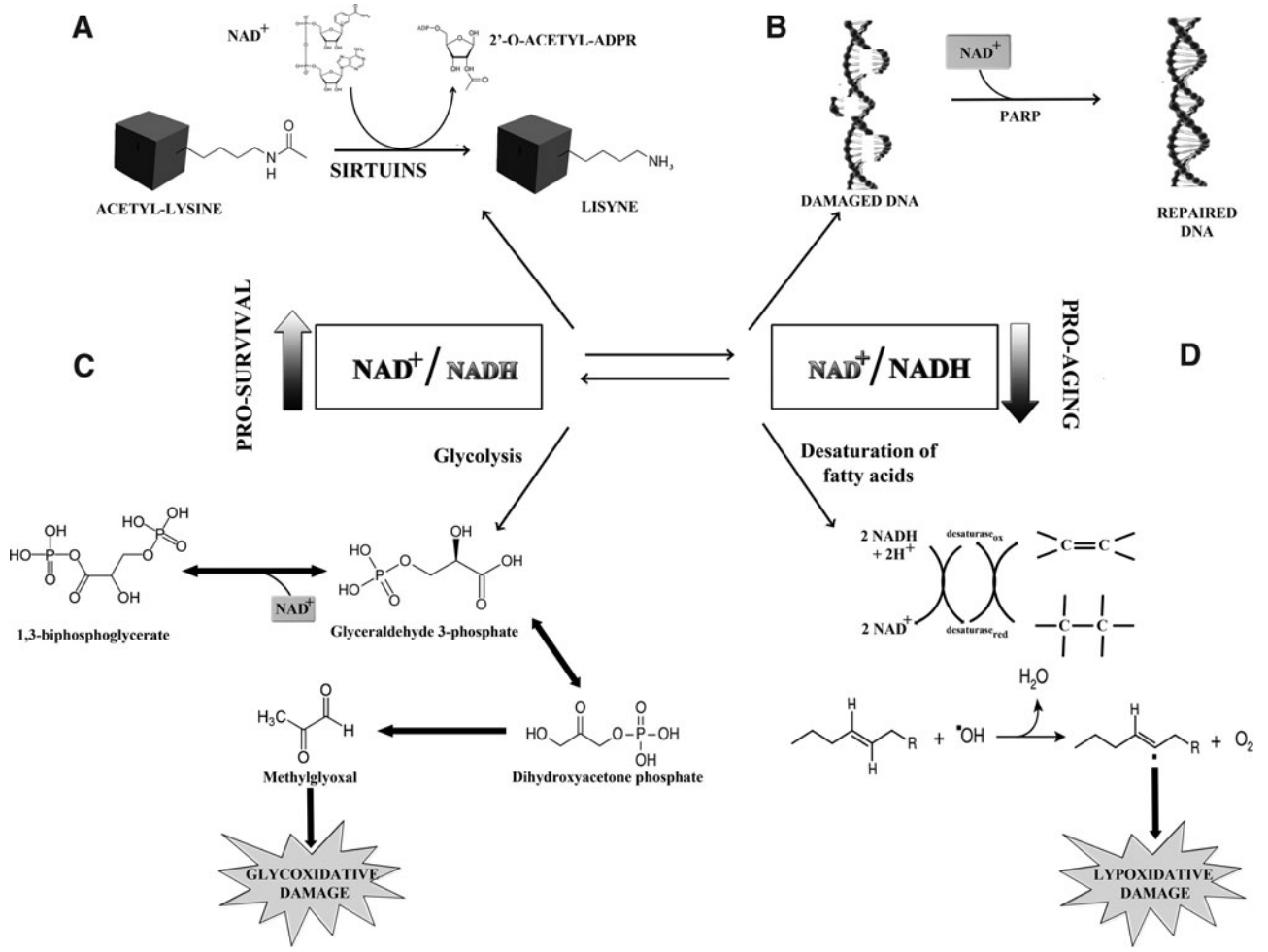

FIG. 8. Regulation of lifespan by a ROS-independent mechanism. Mitochondria can regulate longevity, which reduce the generation of damage caused by metabolic processes occurring in other cellular organelles. Through changes in the NAD+/NADH ratio, mitochondria can determine the activity of enzymes involved in DNA maintenance, such as **(A**, **B)** sirtuins or PARP, **(C)** glycolytic flux, or **(D)** regulate the activity of desaturases. A low NAD+/NADH ratio accelerates the accumulation of cellular debris by increasing glyco- and lipoxidation. On the other hand, a high NAD+/NADH ratio reduces the accumulation of damage by maintaining a high glycolytic flux and membranes that are more resistant to oxidation. PARP, poly (ADP-ribose) polymerase.

activating or repressing a prosurvival genetic program (57). Sir2/SIRT1 has been proposed as the fundamental link between the metabolic state of the cell and the necessary changes in the genetic program to adapt to it (42). Early reports described that Sir2 overexpression was able to extend longevity in lower animals (145, 176). Recently, this extension of lifespan has been put into question (25), and it has been clearly demonstrated that the proposed activator of SIRT1 activity—resveratrol—does not extend longevity (16), and that overexpression of SIRT1 itself does not extend lifespan (19) in mammals. Other sirtuins, such as the mitochondrial SIRT3, have been proposed as regulators of longevity (57). SIRT3 has been implicated in regulating the activity of several mitochondrial proteins related to longevity, including SOD2, the mitochondrial transition permeability pore, and even Complex I (4, 17, 138). SIRT3 should be able to coordinate changes in mitochondrial function necessary to prevent (or favor) cell death. Unfortunately, the effect of manipulation of SIRT3 function on longevity remains unknown. Manipulating the activity of other enzymes that use NAD+ or NADH as cofactors also alters longevity in fruit flies (154). Overexpression of PARP-1, which uses NAD+, extends *Drosophila* lifespan (162), whereas knockout of Apoptosis inducing factor (AIF), which oxidizes NADH, dramatically shortens it (80). In the future, it will be interesting to determine whether these enzymes are able to modify longevity in mammals.

We have discussed that mitochondria could regulate longevity by controlling damage production independently of ROS generation by the ETC. For space reasons, we have only commented on how the NAD+/NADH ratio can control accumulation of glycoxidative/lipoxidative damage and regulate the activity of enzymes that use them as cofactors. Interestingly, the activity of Complex I (and other enzymes producing ROS in the mitochondrial matrix) is also regulated by the NAD+/NADH ratio (117). For example, Complex I leaks more electrons when the ratio is low, because the subunits of the complex become over-reduced. This basically means that Complex I that is more effective at oxidizing NADH protects itself, reducing the leak of electrons (171). This creates a positive feedback where a more efficient

Complex I guarantees less ROS production and lower levels of unsaturated membrane, resulting in less lipoxidative damage. These are common characteristics among most of the long-lived species (127). Also, as demonstrated by the naked mole rat (107), it is feasible to have a higher ROS production by protecting molecules against oxidative damage using alternative approaches.

Summary

Based on studies to date, we can affirm that mitochondria are able to regulate the rate of aging, but we are still far away from a comprehensive understanding of exactly how this is achieved. While oxidative stress may or may not contribute to the progress of aging, it does act as a selection pressure during evolution to alter the mitochondrial physiology in different organisms to various extents. Intriguingly, similar changes (*e.g.*, reduction in the unsaturation of membranes) are observed in different species when prosurvival pathways are activated, such as during DR. Consequently, we can use oxidative stress-induced changes to track the final causes of aging, even if oxidative stress is not one of them. We have introduced alternative mechanisms by which mitochondria can contribute to longevity modulation using ROS-dependent or ROS-independent mechanisms. In the coming years, the implication of these and other still as yet-unknown mechanisms should be addressed to determine the precise role of mitochondria in aging.

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References

1. Abner EL, Schmitt FA, Mendiondo MS, Marcum JL, and Kryscio RJ. Vitamin E and all-cause mortality: a meta-analysis. *Curr Aging Sci* 4: 158–170, 2011.
2. Aguilaniu H, Gustafsson L, Rigoulet M, and Nystrom T. Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science* 299: 1751–1753, 2003.
3. Ahlqvist KJ, Hamalainen RH, Yatsuga S, Uutela M, Terzioglu M, Gotz A, Forsstrom S, Salven P, Angers-Loustau A, Kopra OH, Tyynismaa H, Larsson NG, Wartiovaara K, Prolla T, Trifunovic A, and Suomalainen A. Somatic progenitor cell vulnerability to mitochondrial DNA mutagenesis underlies progeroid phenotypes in Polg mutator mice. *Cell Metab* 15: 100–109, 2012.
4. Ahn BH, Kim HS, Song S, Lee IH, Liu J, Vassilopoulos A, Deng CX, and Finkel T. A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. *Proc Natl Acad Sci U S A* 105: 14447–14452, 2008.
5. Aikens J and Dix TA. Perhydroxyl radical (HOO.) initiated lipid peroxidation. The role of fatty acid hydroperoxides. *J Biol Chem* 266: 15091–15098, 1991.
6. Almeida AM, Bertoncini CR, Borecky J, Souza-Pinto NC, and Vercesi AE. Mitochondrial DNA damage associated with lipid peroxidation of the mitochondrial membrane induced by Fe2+-citrate. *Anais da Academia Brasileira de Ciencias* 78: 505–514, 2006.
7. Anderson RM and Weindruch R. The caloric restriction paradigm: implications for healthy human aging. *Am J Hum Biol* 24: 101–106, 2012.
8. 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.
9. Andziak B, O'Connor TP, Qi W, DeWaal EM, Pierce A, Chaudhuri AR, Van Remmen H, and Buffenstein R. High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell* 5: 463–471, 2006.
10. Antunes F and Cadenas E. Estimation of H_2O_2 gradients across biomembranes. *FEBS Lett* 475: 121–126, 2000.
11. Armstrong JS, Khdour O, and Hecht SM. Does oxidative stress contribute to the pathology of Friedreich's ataxia? A radical question. *FASEB J* 24: 2152–2163, 2010.
12. Ash CE and Merry BJ. The molecular basis by which dietary restricted feeding reduces mitochondrial reactive oxygen species generation. *Mech Ageing Dev* 132: 43–54, 2011.
13. Balan V, Miller GS, Kaplun L, Balan K, Chong ZZ, Li F, Kaplun A, VanBerkum MF, Arking R, Freeman DC, Maiese K, and Tzivion G. Life span extension and neuronal cell protection by *Drosophila* nicotinamidase. *J Biol Chem* 283: 27810–27819, 2008.
14. Barja G, Cadenas S, Rojas C, Perez-Campo R, and Lopez-Torres M. Low mitochondrial free radical production per unit O_2 consumption can explain the simultaneous presence of high longevity and high aerobic metabolic rate in birds. *Free Radic Res* 21: 317–327, 1994.
15. 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.
16. Baur JA, Pearson KJ, Price NL, Jamieson HA, Lerin C, Kalra A, Prabhu VV, Allard JS, Lopez-Lluch G, Lewis K, Pistell PJ, Poosala S, Becker KG, Boss O, Gwinn D, Wang M, Ramaswamy S, Fishbein KW, Spencer RG, Lakatta EG, Le Couteur D, Shaw RJ, Navas P, Puigserver P, Ingram DK, de Cabo R, and Sinclair DA. Resveratrol improves health and survival of mice on a high-calorie diet. *Nature* 444: 337–342, 2006.
17. Bell EL and Guarente L. The SirT3 divining rod points to oxidative stress. *Mol Cell* 42: 561–568, 2011.
18. Bento CF, Marques F, Fernandes R, and Pereira P. Methylglyoxal alters the function and stability of critical components of the protein quality control. *PLoS One* 5: e13007, 2010.
19. Bordone L, Cohen D, Robinson A, Motta MC, van Veen E, Czopik A, Steele AD, Crowe H, Marmor S, Luo J, Gu W, and Guarente L. SIRT1 transgenic mice show phenotypes resembling calorie restriction. *Aging Cell* 6: 759–767, 2007.
20. Boveris A and Navarro A. Systemic and mitochondrial adaptive responses to moderate exercise in rodents. *Free Radic Biol Med* 44: 224–229, 2008.
21. Braidy N, Guillemin GJ, Mansour H, Chan-Ling T, Poljak A, and Grant R. Age related changes in NAD+ metabolism oxidative stress and Sirt1 activity in wistar rats. *PLoS One* 6: e19194, 2011.
22. Brawn K and Fridovich I. Superoxide radical and superoxide dismutases: threat and defense. *Acta Physiol Scand Suppl* 492: 9–18, 1980.
23. 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.
24. Burdon RH. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic Biol Med* 18: 775–794, 1995.
25. Burnett C, Valentini S, Cabreiro F, Goss M, Somogyvari M, Piper MD, Hoddinott M, Sutphin GL, Leko V, McElwee JJ, Vazquez-Manrique RP, Orfila AM, Ackerman D, Au C, Vinti G, Riesen M, Howard K, Neri C, Bedalov A, Kaeberlein M, Soti C, Partridge L, and Gems D. Absence of effects of Sir2 overexpression on lifespan in *C. elegans* and *Drosophila*. *Nature* 477: 482–485, 2011.
26. Cabreiro F, Ackerman D, Doonan R, Araiz C, Back P, Papp D, Braeckman BP, and Gems D. Increased life span from overexpression of superoxide dismutase in *Caenorhabditis elegans* is not caused by decreased oxidative damage. *Free Radic Biol Med* 51: 1575–1582, 2011.
27. Caro P, Gomez J, Arduini A, Gonzalez-Sanchez M, Gonzalez-Garcia M, Borras C, Vina J, Puertas MJ, Sastre J, and Barja G. Mitochondrial DNA sequences are present inside nuclear DNA in rat tissues and increase with age. *Mitochondrion* 10: 479–486, 2010.
28. Cheng X and Ivessa AS. The migration of mitochondrial DNA fragments to the nucleus affects the chronological aging process of *Saccharomyces cerevisiae*. *Aging Cell* 9: 919–923, 2010.
29. Chevion M. A site-specific mechanism for free radical induced biological damage: the essential role of redox-active transition metals. *Free Radic Biol Med* 5: 27–37, 1988.
30. Choi CQ. Old mice hard to replicate. *Scientist* 21: 64, 2007.
31. Choksi KB, Nuss JE, Deford JH, and Papaconstantinou J. Age-related alterations in oxidatively damaged proteins of mouse skeletal muscle mitochondrial electron transport chain complexes. *Free Radic Biol Med* 45: 826–838, 2008.
32. Chung H, Kim AK, Jung SA, Kim SW, Yu K, and Lee JH. The *Drosophila* homolog of methionine sulfoxide reductase A extends lifespan and increases nuclear localization of FOXO. *FEBS Lett* 584: 3609–3614, 2010.
33. Cocheme HM, Quin C, McQuaker SJ, Cabreiro F, Logan A, Prime TA, Abakumova I, Patel JV, Fearnley IM, James AM, Porteous CM, Smith RA, Saeed S, Carre JE, Singer M, Gems D, Hartley RC, Partridge L, and Murphy MP. Measurement of H_2O_2 within living *Drosophila* during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. *Cell Metab* 13: 340–350, 2011.
34. Copeland JM, Cho J, Lo T, Jr., Hur JH, Bahadorani S, Arabyan T, Rabie J, Soh J, and Walker DW. Extension of *Drosophila* life span by RNAi of the mitochondrial respiratory chain. *Curr Biol* 19: 1591–1598, 2009.
35. Cristina D, Cary M, Lunceford A, Clarke C, and Kenyon C. A regulated response to impaired respiration slows behavioral rates and increases lifespan in *Caenorhabditis elegans*. *PLoS Genet* 5: e1000450, 2009.
36. Csiszar A, Podlutsky A, Podlutskaya N, Sonntag WE, Merlin SZ, Philipp EE, Doyle K, Davila A, Recchia FA, Ballabh P, Pinto JT, and Ungvari Z. Testing the oxidative stress hypothesis of aging in primate fibroblasts: is there a correlation between species longevity and cellular ROS production? *J Gerontol Ser A Biol Sci Med Sci* 67: 841–852, 2012.
37. Curtis C, Landis GN, Folk D, Wehr NB, Hoe N, Waskar M, Abdueva D, Skvortsov D, Ford D, Luu A, Badrinath A, Levine RL, Bradley TJ, Tavare S, and Tower J. Transcriptional profiling of MnSOD-mediated lifespan extension in *Drosophila* reveals a species-general network of aging and metabolic genes. *Genome Biol* 8: R262, 2007.
38. Dassa EP, Dufour E, Goncalves S, Paupe V, Hakkaart GA, Jacobs HT, and Rustin P. Expression of the alternative oxidase complements cytochrome c oxidase deficiency in human cells. *EMBO Mol Med* 1: 30–36, 2009.
39. de Magalhaes JP and Church GM. Cells discover fire: employing reactive oxygen species in development and consequences for aging. *Exp Gerontol* 41: 1–10, 2006.
40. DeCorby A, Gaskova D, Sayles LC, and Lemire BD. Expression of Ndi1p, an alternative NADH:ubiquinone oxidoreductase, increases mitochondrial membrane potential in a *C. elegans* model of mitochondrial disease. *Biochim Biophys Acta* 1767: 1157–1163, 2007.
41. Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, and Kenyon C. Rates of behavior and aging specified by mitochondrial function during development. *Science* 298: 2398–2401, 2002.
42. Donmez G and Guarente L. Aging and disease: connections to sirtuins. *Aging Cell* 9: 285–290, 2010.
43. Drew B, Phaneuf S, Dirks A, Selman C, Gredilla R, Lezza A, Barja G, and Leeuwenburgh C. Effects of aging and caloric restriction on mitochondrial energy production in gastrocnemius muscle and heart. *Am J Physiol Regul Integr Comp Physiol* 284: R474–R480, 2003.
44. Dufour E, Boulay J, Rincheval V, and Sainsard-Chanet A. A causal link between respiration and senescence in *Podospora anserina*. *Proc Natl Acad Sci U S A* 97: 4138–4143, 2000.
45. Easlon E, Tsang F, Skinner C, Wang C, and Lin SJ. The malate-aspartate NADH shuttle components are novel metabolic longevity regulators required for calorie restriction-mediated life span extension in yeast. *Genes Dev* 22: 931–944, 2008.
46. Esterhazy D, King MS, Yakovlev G, and Hirst J. Production of reactive oxygen species by complex I (NADH:ubiquinone oxidoreductase) from *Escherichia coli* and comparison to the enzyme from mitochondria. *Biochemistry* 47: 3964–3971, 2008.
47. Fellous TG, Islam S, Tadrous PJ, Elia G, Kocher HM, Bhattacharya S, Mears L, Turnbull DM, Taylor RW, Greaves LC, Chinnery PF, Taylor G, McDonald SA, Wright NA, and Alison MR. Locating the stem cell niche and tracing hepatocyte lineages in human liver. *Hepatology* 49: 1655–1663, 2009.
48. Feng J, Bussiere F, and Hekimi S. Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev Cell* 1: 633–644, 2001.
49. Fernandez-Ayala DJ, Sanz A, Vartiainen S, Kemppainen KK, Babusiak M, Mustalahti E, Costa R, Tuomela T, Zeviani M, Chung J, O'Dell KM, Rustin P, and Jacobs HT. Expression of the *Ciona intestinalis* alternative oxidase (AOX) in *Drosophila* complements defects in mitochondrial oxidative phosphorylation. *Cell Metab* 9: 449–460, 2009.
50. Furukawa A, Tada-Oikawa S, Kawanishi S, and Oikawa S. H_2O_2 accelerates cellular senescence by accumulation of acetylated p53 via decrease in the function of SIRT1 by NAD+ depletion. *Cell Physiol Biochem* 20: 45–54, 2007.
51. Gan W, Nie B, Shi F, Xu XM, Qian JC, Takagi Y, Hayakawa H, Sekiguchi M, and Cai JP. Age-dependent increases in the oxidative damage of DNA, RNA, and their metabolites in normal and senescence-accelerated mice analyzed by LC-MS/MS: urinary 8-oxoguanosine as a novel biomarker of aging. *Free Radic Biol Med* 52: 1700–1707, 2012.
52. Gomez-Cabrera MC, Domenech E, and Vina J. Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic Biol Med* 44: 126–131, 2008.

53. Greaves LC, Barron MJ, Campbell-Shiel G, Kirkwood TB, and Turnbull DM. Differences in the accumulation of mitochondrial defects with age in mice and humans. *Mech Ageing Dev* 132: 588–591, 2011.
54. Greaves LC and Turnbull DM. Mitochondrial DNA mutations and ageing. *Biochim Biophys Acta* 1790: 1015–1020, 2009.
55. Gredilla R and Barja G. Minireview: the role of oxidative stress in relation to caloric restriction and longevity. *Endocrinology* 146: 3713–3717, 2005.
56. Gredilla R, Sanz A, Lopez-Torres M, and Barja G. Caloric restriction decreases mitochondrial free radical generation at complex I and lowers oxidative damage to mitochondrial DNA in the rat heart. *FASEB J* 15: 1589–1591, 2001.
57. Guarente L. Sirtuins and calorie restriction. *Nat Rev Mol Cell Biol* 13: 207, 2012.
58. Guillou H, Zadravec D, Martin PG, and Jacobsson A. The key roles of elongases and desaturases in mammalian fatty acid metabolism: insights from transgenic mice. *Prog Lipid Res* 49: 186–199, 2010.
59. Guo X, Kudryavtseva E, Bodyak N, Nicholas A, Dombrovsky I, Yang D, Kraytsberg Y, Simon DK, and Khrapko K. Mitochondrial DNA deletions in mice in men: substantia nigra is much less affected in the mouse. *Biochim Biophys Acta* 1797: 1159–1162, 2010.
60. Gutteridge JM. The role of superoxide and hydroxyl radicals in phospholipid peroxidation catalysed by iron salts. *FEBS Lett* 150: 454–458, 1982.
61. Hagopian K, Chen Y, Simmons Domer K, Soo Hoo R, Bentley T, McDonald RB, and Ramsey JJ. Caloric restriction influences hydrogen peroxide generation in mitochondrial sub-populations from mouse liver. *J Bioenerg Biomembr* 43: 227–236, 2011.
62. Hamilton ML, Van Remmen H, Drake JA, Yang H, Guo ZM, Kewitt K, Walter CA, and Richardson A. Does oxidative damage to DNA increase with age? *Proc Natl Acad Sci U S A* 98: 10469–10474, 2001.
63. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol* 11: 298–300, 1956.
64. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc* 20: 145–147, 1972.
65. Harman D. The free radical theory of aging. *Antioxid Redox Signal* 5: 557–561, 2003.
66. Herbst A, Pak JW, McKenzie D, Bua E, Bassiouni M, and Aiken JM. Accumulation of mitochondrial DNA deletion mutations in aged muscle fibers: evidence for a causal role in muscle fiber loss. *J Gerontol Ser A Biol Sci Med Sci* 62: 235–245, 2007.
67. Hipkiss AR. NAD+ availability and proteotoxicity. *Neuromol Med* 11: 97–100, 2009.
68. Hipkiss AR. Mitochondrial dysfunction, proteotoxicity, and aging: causes or effects, and the possible impact of NAD+-controlled protein glycation. *Adv Clin Chem* 50: 123–150, 2010.
69. Holloszy JO. Mortality rate and longevity of food-restricted exercising male rats: a reevaluation. *J Appl Physiol* 82: 399–403, 1997.
70. Holloszy JO. Longevity of exercising male rats: effect of an antioxidant supplemented diet. *Mech Ageing Dev* 100: 211–219, 1998.
71. Horikawa M, Nomura T, Hashimoto T, and Sakamoto K. Elongation and desaturation of fatty acids are critical in growth, lipid metabolism and ontogeny of *Caenorhabditis elegans*. *J Biochem* 144: 149–158, 2008.
72. Houtkooper RH, Canto C, Wanders RJ, and Auwerx J. The secret life of NAD+: an old metabolite controlling new metabolic signaling pathways. *Endocr Rev* 31: 194–223, 2010.
73. Hulbert AJ, Pamplona R, Buffenstein R, and Buttemer WA. Life and death: metabolic rate, membrane composition, and life span of animals. *Physiol Rev* 87: 1175–1213, 2007.
74. Ishii N, Fujii M, Hartman PS, Tsuda M, Yasuda K, Senoo-Matsuda N, Yanase S, Ayusawa D, and Suzuki K. A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. *Nature* 394: 694–697, 1998.
75. Jacobs HT. The mitochondrial theory of aging: dead or alive? *Aging Cell* 2: 11–17, 2003.
76. Jacobson J, Lambert AJ, Portero-Otin M, Pamplona R, Magwere T, Miwa S, Driege Y, Brand MD, and Partridge L. Biomarkers of aging in *Drosophila*. *Aging Cell* 9: 466–477, 2010.
77. Jang YC, Perez VI, Song W, Lustgarten MS, Salmon AB, Mele J, Qi W, Liu Y, Liang H, Chaudhuri A, Ikeno Y, Epstein CJ, Van Remmen H, and Richardson A. Overexpression of Mn superoxide dismutase does not increase life span in mice. *J Gerontol A Biol Sci Med Sci* 64: 1114–1125, 2009.
78. Jang YC and Remmen VH. The mitochondrial theory of aging: insight from transgenic and knockout mouse models. *Exp Gerontol* 44: 256–260, 2009.
79. Jones DP. Radical-free biology of oxidative stress. *Am J Physiol Cell Physiol* 295: C849–C868, 2008.
80. Joza N, Galindo K, Pospisilik JA, Benit P, Rangachari M, Kanitz EE, Nakashima Y, Neely GG, Rustin P, Abrams JM, Kroemer G, and Penninger JM. The molecular archaeology of a mitochondrial death effector: AIF in *Drosophila*. *Cell Death Differ* 15: 1009–1018, 2008.
81. Khrapko K, Coller HA, Andre PC, Li XC, Hanekamp JS, and Thilly WG. Mitochondrial mutational spectra in human cells and tissues. *Proc Natl Acad Sci U S A* 94: 13798–13803, 1997.
82. Khrapko K, Kraytsberg Y, de Grey AD, Vijg J, and Schon EA. Does premature aging of the mtDNA mutator mouse prove that mtDNA mutations are involved in natural aging? *Aging Cell* 5: 279–282, 2006.
83. Khrapko K and Vijg J. Mitochondrial DNA mutations and aging: devils in the details? *Trends Genet* 25: 91–98, 2009.
84. Kowaltowski AJ. Caloric restriction and redox state: does this diet increase or decrease oxidant production? *Redox Rep* 16: 237–241, 2011.
85. Krishnan KJ, Reeve AK, Samuels DC, Chinnery PF, Blackwood JK, Taylor RW, Wanrooij S, Spelbrink JN, Lightowlers RN, and Turnbull DM. What causes mitochondrial DNA deletions in human cells? *Nat Genet* 40: 275–279, 2008.
86. Ku HH and Sohal RS. Comparison of mitochondrial prooxidant generation and anti-oxidant defenses between rat and pigeon: possible basis of variation in longevity and metabolic potential. *Mech Ageing Dev* 72: 67–76, 1993.
87. Kuang J and Ebert PR. The failure to extend lifespan via disruption of complex II is linked to preservation of dynamic control of energy metabolism. *Mitochondrion* 12: 280–287, 2012.
88. Kujoth GC, Hiona A, Pugh TD, Someya S, Panzer K, Wohlgemuth SE, Hofer T, Seo AY, Sullivan R, Jobling WA, Morrow JD, Van Remmen H, Sedivy JM, Yamasoba T, Tanokura M, Weindruch R, Leeuwenburgh C, and Prolla TA. Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science* 309: 481–484, 2005.
89. Lambert AJ, Boysen HM, Buckingham JA, Yang T, Podlutsky A, Austad SN, Kunz TH, Buffenstein R, and Brand MD. Low rates of hydrogen peroxide production by

isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell* 6: 607–618, 2007.
90. Lapointe J, Stepanyan Z, Bigras E, and Hekimi S. Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mclk1+/− mice. *J Biol Chem* 284: 20364–20374, 2009.
91. Lass A, Sohal BH, Weindruch R, Forster MJ, and Sohal RS. Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. *Free Radic Biol Med* 25: 1089–1097, 1998.
92. Lee S, Tak E, Lee J, Rashid MA, Murphy MP, Ha J, and Kim SS. Mitochondrial H2O2 generated from electron transport chain complex I stimulates muscle differentiation. *Cell Res* 21: 817–834, 2011.
93. Lin SJ, Ford E, Haigis M, Liszt G, and Guarente L. Calorie restriction extends yeast life span by lowering the level of NADH. *Genes Dev* 18: 12–16, 2004.
94. Longo VD, Mitteldorf J, and Skulachev VP. Programmed and altruistic ageing. *Nat Rev Genet* 6: 866–872, 2005.
95. Lopez-Torres M, Perez-Campo R, Rojas C, Cadenas S, and Barja G. Maximum life span in vertebrates: relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, *in vivo* H_2O_2, and basal and maximum aerobic capacity. *Mech Ageing Dev* 70: 177–199, 1993.
96. Lustgarten MS, Bhattacharya A, Muller FL, Jang YC, Shimizu T, Shirasawa T, Richardson A, and Van Remmen H. Complex I generated, mitochondrial matrix-directed superoxide is released from the mitochondria through voltage dependent anion channels. *Biochem Biophys Res Commun* 422: 515–521, 2012.
97. Maas MF, Sellem CH, Krause F, Dencher NA, and Sainsard-Chanet A. Molecular gene therapy: overexpression of the alternative NADH dehydrogenase NDI1 restores overall physiology in a fungal model of respiratory complex I deficiency. *J Mol Biol* 399: 31–40, 2010.
98. Magwere T, West M, Riyahi K, Murphy MP, Smith RA, and Partridge L. The effects of exogenous antioxidants on lifespan and oxidative stress resistance in *Drosophila melanogaster*. *Mech Ageing Dev* 127: 356–370, 2006.
99. Mair W and Dillin A. Aging and survival: the genetics of life span extension by dietary restriction. *Annu Rev Biochem* 77: 727–754, 2008.
100. Marella M, Seo BB, Flotte TR, Matsuno-Yagi A, and Yagi T. No immune responses by the expression of the yeast Ndi1 protein in rats. *PLoS One* 6: e25910, 2011.
101. Martin I, Jones MA, Rhodenizer D, Zheng J, Warrick JM, Seroude L, and Grotewiel M. Sod2 knockdown in the musculature has whole-organism consequences in *Drosophila*. *Free Radic Biol Med* 47: 803–813, 2009.
102. Matsura T, Kai M, Fujii Y, Ito H, and Yamada K. Hydrogen peroxide-induced apoptosis in HL-60 cells requires caspase-3 activation. *Free Radic Res* 30: 73–83, 1999.
103. McDonald SA, Greaves LC, Gutierrez-Gonzalez L, Rodriguez-Justo M, Deheragoda M, Leedham SJ, Taylor RW, Lee CY, Preston SL, Lovell M, Hunt T, Elia G, Oukrif D, Harrison R, Novelli MR, Mitchell I, Stoker DL, Turnbull DM, Jankowski JA, and Wright NA. Mechanisms of field cancerization in the human stomach: the expansion and spread of mutated gastric stem cells. *Gastroenterology* 134: 500–510, 2008.
104. Mesquita A, Weinberger M, Silva A, Sampaio-Marques B, Almeida B, Leao C, Costa V, Rodrigues F, Burhans WC, and Ludovico P. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H_2O_2 and superoxide dismutase activity. *Proc Natl Acad Sci U S A* 107: 15123–15128, 2010.
105. Michikawa Y, Mazzucchelli F, Bresolin N, Scarlato G, and Attardi G. Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. *Science* 286: 774–779, 1999.
106. Millar DS, Tysoe C, Lazarou LP, Pilz DT, Mohammed S, Anderson K, Chuzhanova N, Cooper DN, and Butler R. An isolated case of lissencephaly caused by the insertion of a mitochondrial genome-derived DNA sequence into the 5′ untranslated region of the PAFAH1B1 (LIS1) gene. *Hum Genomics* 4: 384–393, 2010.
107. Mitchell TW, Buffenstein R, and Hulbert AJ. Membrane phospholipid composition may contribute to exceptional longevity of the naked mole-rat (*Heterocephalus glaber*): a comparative study using shotgun lipidomics. *Exp Gerontol* 42: 1053–1062, 2007.
108. Miwa S, Riyahi K, Partridge L, and Brand MD. Lack of correlation between mitochondrial reactive oxygen species production and life span in *Drosophila*. *Ann N Y Acad Sci* 1019: 388–391, 2004.
109. Montgomery MK, Hulbert AJ, and Buttemer WA. The long life of birds: the rat-pigeon comparison revisited. *PLoS One* 6: e24138, 2011.
110. Montgomery MK, Hulbert AJ, and Buttemer WA. Does the oxidative stress theory of aging explain longevity differences in birds? I. Mitochondrial ROS production. *Exp Gerontol* 47: 203–210, 2012.
111. Montgomery MR. Characterization of fatty acid desaturase activity in rat lung microsomes. *J Lipid Res* 17: 12–15, 1976.
112. Moosmann B and Behl C. Mitochondrially encoded cysteine predicts animal lifespan. *Aging Cell* 7: 32–46, 2008.
113. Muller FL, Liu Y, and Van Remmen H. Complex III releases superoxide to both sides of the inner mitochondrial membrane. *J Biol Chem* 279: 49064–49073, 2004.
114. Muller FL, Lustgarten MS, Jang Y, Richardson A, and Van Remmen H. Trends in oxidative aging theories. *Free Radic Biol Med* 43: 477–503, 2007.
115. Munscher C, Rieger T, Muller-Hocker J, and Kadenbach B. The point mutation of mitochondrial DNA characteristic for MERRF disease is found also in healthy people of different ages. *FEBS Lett* 317: 27–30, 1993.
116. Mura CV, Gong X, Taylor A, Villalobos-Molina R, and Scrofano MM. Effects of calorie restriction and aging on the expression of antioxidant enzymes and ubiquitin in the liver of Emory mice. *Mech Ageing Dev* 91: 115–129, 1996.
117. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J* 417: 1–13, 2009.
118. Murphy MP, Holmgren A, Larsson NG, Halliwell B, Chang CJ, Kalyanaraman B, Rhee SG, Thornalley PJ, Partridge L, Gems D, Nystrom T, Belousov V, Schumacker PT, and Winterbourn CC. Unraveling the biological roles of reactive oxygen species. *Cell Metab* 13: 361–366, 2011.
119. Naudi A, Jove M, Ayala V, Cassanye A, Serrano J, Gonzalo H, Boada J, Prat J, Portero-Otin M, and Pamplona R. Cellular dysfunction in diabetes as maladaptive response to mitochondrial oxidative stress. *Exp Diabetes Res* 2012: 696215, 2012.
120. Nekhaeva E, Bodyak ND, Kraytsberg Y, McGrath SB, Van Orsouw NJ, Pluzhnikov A, Wei JY, Vijg J, and Khrapko K. Clonally expanded mtDNA point mutations are abundant in individual cells of human tissues. *Proc Natl Acad Sci U S A* 99: 5521–5526, 2002.

121. Nie C, Tian C, Zhao L, Petit PX, Mehrpour M, and Chen Q. Cysteine 62 of Bax is critical for its conformational activation and its proapoptotic activity in response to H_2O_2-induced apoptosis. *J Biol Chem* 283: 15359–15369, 2008.
122. Nunnari J and Suomalainen A. Mitochondria: in sickness and in health. *Cell* 148: 1145–1159, 2012.
123. Page MM, Richardson J, Wiens BE, Tiedtke E, Peters CW, Faure PA, Burness G, and Stuart JA. Antioxidant enzyme activities are not broadly correlated with longevity in 14 vertebrate endotherm species. *Age (Dordr)* 32: 255–270, 2010.
124. Page MM and Stuart JA. Activities of DNA base excision repair enzymes in liver and brain correlate with body mass, but not lifespan. *Age (Dordr)* 34: 1195–1209, 2012.
125. Pamplona R. Advanced lipoxidation end-products. *Chem Biol Interact* 192: 14–20, 2011.
126. Pamplona R and Barja G. Highly resistant macromolecular components and low rate of generation of endogenous damage: two key traits of longevity. *Ageing Res Rev* 6: 189–210, 2007.
127. Pamplona R and Barja G. An evolutionary comparative scan for longevity-related oxidative stress resistance mechanisms in homeotherms. *Biogerontology* 12: 409–435, 2011.
128. Pamplona R, Barja G, and Portero-Otin M. Membrane fatty acid unsaturation, protection against oxidative stress, and maximum life span: a homeoviscous-longevity adaptation? *Ann N Y Acad Sci* 959: 475–490, 2002.
129. Pamplona R, Portero-Otin M, Bellmun MJ, Gredilla R, and Barja G. Aging increases nepsilon-(carboxymethyl)lysine and caloric restriction decreases nepsilon-(carboxyethyl)lysine and Nepsilon-(malondialdehyde)lysine in rat heart mitochondrial proteins. *Free Radic Res* 36: 47–54, 2002.
130. Pamplona R, Portero-Otin M, Requena J, Gredilla R, and Barja G. Oxidative, glycoxidative and lipoxidative damage to rat heart mitochondrial proteins is lower after 4 months of caloric restriction than in age-matched controls. *Mech Ageing Dev* 123: 1437–1446, 2002.
131. Pamplona R, Portero-Otin M, Requena JR, Thorpe SR, Herrero A, and Barja G. A low degree of fatty acid unsaturation leads to lower lipid peroxidation and lipoxidation-derived protein modification in heart mitochondria of the longevous pigeon than in the short-lived rat. *Mech Ageing Dev* 106: 283–296, 1999.
132. Pamplona R, Portero-Otin M, Riba D, Ledo F, Gredilla R, Herrero A, and Barja G. Heart fatty acid unsaturation and lipid peroxidation, and aging rate, are lower in the canary and the parakeet than in the mouse. *Aging (Milano)* 11: 44–49, 1999.
133. Pamplona R, Portero-Otin M, Riba D, Requena JR, Thorpe SR, Lopez-Torres M, and Barja G. Low fatty acid unsaturation: a mechanism for lowered lipoperoxidative modification of tissue proteins in mammalian species with long life spans. *J Gerontol Ser A Biol Sci Med Sci* 55: B286–B291, 2000.
134. Pamplona R, Portero-Otin M, Riba D, Ruiz C, Prat J, Bellmunt MJ, and Barja G. Mitochondrial membrane peroxidizability index is inversely related to maximum life span in mammals. *J Lipid Res* 39: 1989–1994, 1998.
135. Pamplona R, Prat J, Cadenas S, Rojas C, Perez-Campo R, Lopez Torres M, and Barja G. Low fatty acid unsaturation protects against lipid peroxidation in liver mitochondria from long-lived species: the pigeon and human case. *Mech Ageing Dev* 86: 53–66, 1996.
136. Perez VI, Cortez LA, Lew CM, Rodriguez M, Webb CR, Van Remmen H, Chaudhuri A, Qi W, Lee S, Bokov A, Fok W, Jones D, Richardson A, Yodoi J, Zhang Y, Tominaga K, Hubbard GB, and Ikeno Y. Thioredoxin 1 overexpression extends mainly the earlier part of life span in mice. *J Gerontol A Biol Sci Med Sci* 66: 1286–1299, 2011.
137. Pletjushkina OY, Fetisova EK, Lyamzaev KG, Ivanova OY, Domnina LV, Vyssokikh MY, Pustovidko AV, Vasiliev JM, Murphy MP, Chernyak BV, and Skulachev VP. Long-distance apoptotic killing of cells is mediated by hydrogen peroxide in a mitochondrial ROS-dependent fashion. *Cell Death Differ* 12: 1442–1444, 2005.
138. Qiu X, Brown K, Hirschey MD, Verdin E, and Chen D. Calorie restriction reduces oxidative stress by SIRT3-mediated SOD2 activation. *Cell Metab* 12: 662–667, 2010.
139. Radak Z, Takahashi R, Kumiyama A, Nakamoto H, Ohno H, Ookawara T, and Goto S. Effect of aging and late onset dietary restriction on antioxidant enzymes and proteasome activities, and protein carbonylation of rat skeletal muscle and tendon. *Exp Gerontol* 37: 1423–1430, 2002.
140. Rathod P, Hemnani T, and Parihar MS. Dietary restriction lowers endogenous levels of oxidative stress in different brain regions of adult mice. *Cell Mol Biol (Noisy-le-grand)* 57 Suppl: OL1575–1580, 2011.
141. Refsgaard HH, Tsai L, and Stadtman ER. Modifications of proteins by polyunsaturated fatty acid peroxidation products. *Proc Natl Acad Sci U S A* 97: 611–616, 2000.
142. Richter C. Do mitochondrial DNA fragments promote cancer and aging? *FEBS Lett* 241: 1–5, 1988.
143. Ristow M and Schmeisser S. Extending life span by increasing oxidative stress. *Free Radic Biol Med* 51: 327–336, 2011.
144. Ristow M, Zarse K, Oberbach A, Kloting N, Birringer M, Kiehntopf M, Stumvoll M, Kahn CR, and Bluher M. Antioxidants prevent health-promoting effects of physical exercise in humans. *Proc Natl Acad Sci U S A* 106: 8665–8670, 2009.
145. Rogina B and Helfand SL. Sir2 mediates longevity in the fly through a pathway related to calorie restriction. *Proc Natl Acad Sci U S A* 101: 15998–16003, 2004.
146. Ross JM, Oberg J, Brene S, Coppotelli G, Terzioglu M, Pernold K, Goiny M, Sitnikov R, Kehr J, Trifunovic A, Larsson NG, Hoffer BJ, and Olson L. High brain lactate is a hallmark of aging and caused by a shift in the lactate dehydrogenase A/B ratio. *Proc Natl Acad Sci U S A* 107: 20087–20092, 2010.
147. Ruan H, Tang XD, Chen ML, Joiner ML, Sun G, Brot N, Weissbach H, Heinemann SH, Iverson L, Wu CF, and Hoshi T. High-quality life extension by the enzyme peptide methionine sulfoxide reductase. *Proc Natl Acad Sci U S A* 99: 2748–2753, 2002.
148. Ruiz MC, Ayala V, Portero-Otin M, Requena JR, Barja G, and Pamplona R. Protein methionine content and MDA-lysine adducts are inversely related to maximum life span in the heart of mammals. *Mech Ageing Dev* 126: 1106–1114, 2005.
149. Rustin P and Jacobs HT. Respiratory chain alternative enzymes as tools to better understand and counteract respiratory chain deficiencies in human cells and animals. *Physiol Plant* 137: 362–370, 2009.
150. Sachdev S and Davies KJ. Production, detection, and adaptive responses to free radicals in exercise. *Free Radic Biol Med* 44: 215–223, 2008.
151. Salway KD, Page MM, Faure PA, Burness G, and Stuart JA. Enhanced protein repair and recycling are not correlated with longevity in 15 vertebrate endotherm species. *Age (Dordr)* 33: 33–47, 2011.

152. Sanz A, Fernandez-Ayala DJ, Stefanatos RK, and Jacobs HT. Mitochondrial ROS production correlates with, but does not directly regulate lifespan in *Drosophila*. *Aging (Albany NY)* 2: 200–223, 2010.
153. Sanz A, Pamplona R, and Barja G. Is the mitochondrial free radical theory of aging intact? *Antioxid Redox Signal* 8: 582–599, 2006.
154. Sanz A, Soikkeli M, Portero-Otin M, Wilson A, Kemppainen E, McIlroy G, Ellila S, Kemppainen KK, Tuomela T, Lakanmaa M, Kiviranta E, Stefanatos R, Dufour E, Hutz B, Naudi A, Jove M, Zeb A, Vartiainen S, Matsuno-Yagi A, Yagi T, Rustin P, Pamplona R, and Jacobs HT. Expression of the yeast NADH dehydrogenase Ndi1 in *Drosophila* confers increased lifespan independently of dietary restriction. *Proc Natl Acad Sci U S A* 107: 9105–9110, 2010.
155. Sanz A and Stefanatos RK. The mitochondrial free radical theory of aging: a critical view. *Curr Aging Sci* 1: 10–21, 2008.
156. Sasaki T, Unno K, Tahara S, and Kaneko T. Age-related increase of reactive oxygen generation in the brains of mammals and birds: is reactive oxygen a signaling molecule to determine the aging process and life span? *Geriatr Gerontol Int* 10 Suppl 1: S10–S24, 2010.
157. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, and Rabinovitch PS. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science* 308: 1909–1911, 2005.
158. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, and Ristow M. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab* 6: 280–293, 2007.
159. Sell DR, Lane MA, Johnson WA, Masoro EJ, Mock OB, Reiser KM, Fogarty JF, Cutler RG, Ingram DK, Roth GS, and Monnier VM. Longevity and the genetic determination of collagen glycoxidation kinetics in mammalian senescence. *Proc Natl Acad Sci U S A* 93: 485–490, 1996.
160. Semsei I, Rao G, and Richardson A. Changes in the expression of superoxide dismutase and catalase as a function of age and dietary restriction. *Biochem Biophys Res Commun* 164: 620–625, 1989.
161. Seo AY, Xu J, Servais S, Hofer T, Marzetti E, Wohlgemuth SE, Knutson MD, Chung HY, and Leeuwenburgh C. Mitochondrial iron accumulation with age and functional consequences. *Aging Cell* 7: 706–716, 2008.
162. Shaposhnikov MV, Moskalev AA, and Plyusnina EN. Effect of PARP-1 overexpression and pharmacological inhibition of NF-kB on the lifespan of *Drosophila melanogaster*. *Adv Gerontol* 24: 405–419, 2011.
163. Shchedrina VA, Vorbruggen G, Lee BC, Kim HY, Kabil H, Harshman LG, and Gladyshev VN. Overexpression of methionine-R-sulfoxide reductases has no influence on fruit fly aging. *Mech Ageing Dev* 130: 429–443, 2009.
164. Shmookler Reis RJ, Xu L, Lee H, Chae M, Thaden JJ, Bharill P, Tazearslan C, Siegel E, Alla R, Zimniak P, and Ayyadevara S. Modulation of lipid biosynthesis contributes to stress resistance and longevity of *C. elegans* mutants. *Aging* 3: 125–147, 2011.
165. Sohal RS, Arnold L, and Orr WC. Effect of age on superoxide dismutase, catalase, glutathione reductase, inorganic peroxides, TBA-reactive material, GSH/GSSG, NADPH/NADP+ and NADH/NAD+ in *Drosophila melanogaster*. *Mech Ageing Dev* 56: 223–235, 1990.
166. Sohal RS, Ku HH, Agarwal S, Forster MJ, and Lal H. Oxidative damage, mitochondrial oxidant generation and antioxidant defenses during aging and in response to food restriction in the mouse. *Mech Ageing Dev* 74: 121–133, 1994.
167. Sohal RS and Orr WC. The redox stress hypothesis of aging. *Free Radic Biol Med* 52: 539–555, 2012.
168. 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.
169. Sohal RS, Svensson I, and Brunk UT. Hydrogen peroxide production by liver mitochondria in different species. *Mech Ageing Dev* 53: 209–215, 1990.
170. Sohal RS, Svensson I, Sohal BH, and Brunk UT. Superoxide anion radical production in different animal species. *Mech Ageing Dev* 49: 129–135, 1989.
171. Stefanatos R and Sanz A. Mitochondrial complex I: a central regulator of the aging process. *Cell Cycle* 10: 1528–1532, 2011.
172. 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.
173. Tabner BJ, Turnbull S, King JE, Benson FE, El-Agnaf OM, and Allsop D. A spectroscopic study of some of the peptidyl radicals formed following hydroxyl radical attack on beta-amyloid and alpha-synuclein. *Free Radic Res* 40: 731–739, 2006.
174. Taylor RW, Barron MJ, Borthwick GM, Gospel A, Chinnery PF, Samuels DC, Taylor GA, Plusa SM, Needham SJ, Greaves LC, Kirkwood TB, and Turnbull DM. Mitochondrial DNA mutations in human colonic crypt stem cells. *J Clin Investig* 112: 1351–1360, 2003.
175. Terman A, Kurz T, Navratil M, Arriaga EA, and Brunk UT. Mitochondrial turnover and aging of long-lived postmitotic cells: the mitochondrial-lysosomal axis theory of aging. *Antioxid Redox Signal* 12: 503–535, 2010.
176. Tissenbaum HA and Guarente L. Increased dosage of a sir-2 gene extends lifespan in *Caenorhabditis elegans*. *Nature* 410: 227–230, 2001.
177. Toroser D, Orr WC, and Sohal RS. Carbonylation of mitochondrial proteins in *Drosophila melanogaster* during aging. *Biochem Biophys Res Commun* 363: 418–424, 2007.
178. Trifunovic A, Wredenberg A, Falkenberg M, Spelbrink JN, Rovio AT, Bruder CE, Bohlooly YM, Gidlof S, Oldfors A, Wibom R, Tornell J, Jacobs HT, and Larsson NG. Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature* 429: 417–423, 2004.
179. Turner N, Else PL, and Hulbert AJ. An allometric comparison of microsomal membrane lipid composition and sodium pump molecular activity in the brain of mammals and birds. *J Exp Biol* 208: 371–381, 2005.
180. Turner N, Haga KL, Else PL, and Hulbert AJ. Scaling of Na+,K+-ATPase molecular activity and membrane fatty acid composition in mammalian and avian hearts. *Physiol Biochem Zool* 79: 522–533, 2006.
181. Tyynismaa H, Mjosund KP, Wanrooij S, Lappalainen I, Ylikallio E, Jalanko A, Spelbrink JN, Paetau A, and Suomalainen A. Mutant mitochondrial helicase Twinkle causes multiple mtDNA deletions and a late-onset mitochondrial disease in mice. *Proc Natl Acad Sci U S A* 102: 17687–17692, 2005.
182. Van Raamsdonk JM and Hekimi S. Superoxide dismutase is dispensable for normal animal lifespan. *Proc Natl Acad Sci U S A* 109: 5785–5790, 2012.

183. Van Remmen H, Ikeno Y, Hamilton M, Pahlavani M, Wolf N, Thorpe SR, Alderson NL, Baynes JW, Epstein CJ, Huang TT, Nelson J, Strong R, and Richardson A. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genom* 16: 29–37, 2003.
184. Veatch JR, McMurray MA, Nelson ZW, and Gottschling DE. Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell* 137: 1247–1258, 2009.
185. Vermulst M, Bielas JH, Kujoth GC, Ladiges WC, Rabinovitch PS, Prolla TA, and Loeb LA. Mitochondrial point mutations do not limit the natural lifespan of mice. *Nat Genet* 39: 540–543, 2007.
186. Vijg J. The role of DNA damage and repair in aging: new approaches to an old problem. *Mech Ageing Dev* 129: 498–502, 2008.
187. Vina J, Gimeno A, Sastre J, Desco C, Asensi M, Pallardo FV, Cuesta A, Ferrero JA, Terada LS, and Repine JE. Mechanism of free radical production in exhaustive exercise in humans and rats; role of xanthine oxidase and protection by allopurinol. *IUBMB Life* 49: 539–544, 2000.
188. Wallace DC. A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu Rev Genet* 39: 359–407, 2005.
189. Wang E, Wong A, and Cortopassi G. The rate of mitochondrial mutagenesis is faster in mice than humans. *Mutat Res* 377: 157–166, 1997.
190. Weindruch R, Walford RL, Fligiel S, and Guthrie D. The retardation of aging in mice by dietary restriction: longevity, cancer, immunity and lifetime energy intake. *J Nutr* 116: 641–654, 1986.
191. Xia E, Rao G, Van Remmen H, Heydari AR, and Richardson A. Activities of antioxidant enzymes in various tissues of male Fischer 344 rats are altered by food restriction. *J Nutr* 125: 195–201, 1995.
192. Yang W and Hekimi S. A mitochondrial superoxide signal triggers increased longevity in *Caenorhabditis elegans*. *PLoS Biol* 8: e1000556, 2010.
193. Yarian CS, Toroser D, and Sohal RS. Aconitase is the main functional target of aging in the citric acid cycle of kidney mitochondria from mice. *Mech Ageing Dev* 127: 79–84, 2006.
194. Zarse K, Schmeisser S, Groth M, Priebe S, Beuster G, Kuhlow D, Guthke R, Platzer M, Kahn CR, and Ristow M. Impaired insulin/IGF1 signaling extends life span by promoting mitochondrial L-proline catabolism to induce a transient ROS signal. *Cell Metab* 15: 451–465, 2012.
195. Zhang Q, Raoof M, Chen Y, Sumi Y, Sursal T, Junger W, Brohi K, Itagaki K, and Hauser CJ. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature* 464: 104–107, 2010.
196. Zhang Y, Ikeno Y, Qi W, Chaudhuri A, Li Y, Bokov A, Thorpe SR, Baynes JW, Epstein C, Richardson A, and Van Remmen H. Mice deficient in both Mn superoxide dismutase and glutathione peroxidase-1 have increased oxidative damage and a greater incidence of pathology but no reduction in longevity. *J Gerontol A Biol Sci Med Sci* 64: 1212–1220, 2009.
197. Zheng J, Mutcherson R, 2nd, and Helfand SL. Calorie restriction delays lipid oxidative damage in *Drosophila melanogaster*. *Aging Cell* 4: 209–216, 2005.
198. Zheng W, Khrapko K, Coller HA, Thilly WG, and Copeland WC. Origins of human mitochondrial point mutations as DNA polymerase gamma-mediated errors. *Mutat Res* 599: 11–20, 2006.
199. Zid BM, Rogers AN, Katewa SD, Vargas MA, Kolipinski MC, Lu TA, Benzer S, and Kapahi P. 4E-BP extends lifespan upon dietary restriction by enhancing mitochondrial activity in *Drosophila*. *Cell* 139: 149–160, 2009.
200. Zuo Y, Xiang B, Yang J, Sun X, Wang Y, Cang H, and Yi J. Oxidative modification of caspase-9 facilitates its activation via disulfide-mediated interaction with Apaf-1. *Cell Res* 19: 449–457, 2009.

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Abbreviations Used

AGEs = advanced glycoxidation end products
ALEs = advanced lipoxidation end products
AOX = alternative oxidase
COX = cytochrome oxidase
DR = dietary restriction
ETC = electron transport chain
FMN = flavin mononucleotide
H_2O_2 = hydrogen peroxide
MFRTA = Mitochondrial Free Radical Theory of Aging
MSRA A = methionine sulfoxide reductase A
mtDNA = mitochondrial DNA
mtROS = mitochondrial reactive oxygen species
NDE = NADH dehydrogenase external
NDI1 = NADH dehydrogenase internal 1
nDNA = nuclear DNA
PARP = poly(ADP-ribose) polymerase
PRX = peroxiredoxin
ROS = reactive oxygen species
SOD = superoxide dismutase
TPX = thioredoxin peroxidase