Cell. Mol. Life Sci. (2010) 67:1–8
DOI 10.1007/s00018-009-0138-8

Cellular and Molecular Life Sciences

VISIONS & REFLECTIONS (MINIREVIEW)

# When a theory of aging ages badly

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Received: 13 July 2009 / Revised: 14 August 2009 / Accepted: 18 August 2009 / Published online: 3 September 2009


**Abstract** According to the widely acknowledged mitochondrial free radical theory of aging (MFRTA), the macromolecular damage that results from the production of toxic reactive oxygen species (ROS) during cellular respiration is the cause of aging. However, although it is clear that oxidative damage increases during aging, the fundamental question regarding whether mitochondrial oxidative stress is in any way causal to the aging process remains unresolved. An increasing number of studies on long-lived vertebrate species, mutants and transgenic animals have seriously challenged the pervasive MFRTA. Here, we describe some of these new results, including those pertaining to the phenotype of the long-lived *Mclk1*$^{+/-}$ mice, which appear irreconcilable with the MFRTA. Thus, we believe that it is reasonable to now consider the MFRTA as refuted and that it is time to use the insight gained by many years of testing this theory to develop new views as to the physiological causes of aging.

## The mitochondrial free radical theory of aging (MFRTA): what does it claim?

Aging is a degenerative process that is very obvious in many living species. In their natural environment, animals rarely have the chance of fully experiencing the aging process because of the presence of many non-degenerative causes of death (starvation, disease, predation). Therefore, the molecular bases of aging are normally studied in protected environments. Under these conditions, where different model species can reach their maximal lifespan, aging can be defined as the steady increase with time in the probability of dying. This increased difficulty in remaining alive is believed to be the result of a gradual loss of the metabolic and genetic assets necessary to maintain the integrity and functionality of all cellular constituents. Understanding the mechanisms of aging has been at the center of some important biological research, and many theories of aging have been proposed [1, 2]. In order to receive consideration from the scientific community, the core of an aging theory must be a clear statement of what exactly initiates aging and causes the development of the aged phenotype. Theories also come always with additional affirmations and hypotheses to support their central statement. Discrimination between the core statement and the associated hypotheses of a theory is crucial because a theory could survive with some erroneous hypotheses, but should not survive with an inaccurate core statement. Strangely, even if aging has been suggested to be a multicausal process linked to a variety of molecular and cellular sources of damage [3–6], almost all popular theories of aging actually revolve around postulating a single physiological cause of aging. Of course, if aging is in fact multifactorial and does not have a single cause, then each of the theories that postulate a single cause might have some degree of truth.

Among all the single-cause theories that have been proposed, the MFRTA is without any doubt the most studied and the most recognized [7]. According to this theory, the cause of aging is the production of free radicals at the level of the electron transport chain that subsequently results in the formation of various reactive oxygen species (ROS) in the mitochondria of all tissues. The claim is that ROS will arise inevitably from normal mitochondrial

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energy metabolism to damage mitochondrial and cytosolic constituents and that the accumulation of this damage over time causes aging. To make ROS damage the cause of aging, additional implicit claims are that ROS are the only source of damage that is unavoidable or that can never be completely repaired. In addition to this core statement, the theory also encompass several associated hypotheses, for example, that the oxidative damage to mitochondrial constituents results in a deterioration of mitochondrial function over time, that the resulting mitochondrial dysfunction leads to more ROS generation and that global, including non-mitochondrial, oxidative damage, will also progressively accumulate with the aged phenotype (Fig. 1). As ROS have toxic properties and are indeed normally produced by mitochondria throughout life, it is easy to understand why the MFRTA has attracted so much interest in the last decades.

Since the MFRTA was proposed, its core statement, its satellite hypotheses and its observational foundations have all been tested in a variety of species [8, 9]. To date, the findings from these multiple analyses have led to two principal conclusions: (1) many associated hypotheses of the theory, and their observational foundations, are correct. (2) The core statement of the theory is wrong, as we will discuss in the following sections (Fig. 1). Note that a theory might be right until its core statement is proven to be wrong, following which the attempt to keep alive a weak version of the theory based on the truth of associated hypotheses and observations, as it was suggested for the MFRTA [9, 10], can only lead to misunderstandings and unclear research programs. In spite of understanding this, it is difficult for everyone to consider as refuted a theory that has been standing on the highest step of the podium for so many years [11]. Yet, the theory is now being openly challenged by many [12–17]. Here, we discuss a set of recent results that lead to conclusions that are particularly incompatible with the MFRTA and may actually suggest a new view of aging.

## Unexpected results that have shaken up the MFRTA

While many experiments have been performed in an attempt to validate the MFRTA, the results have not always supported the original hypothesis, but rather have sometimes provided strong evidence against the theory (Fig. 1). One kind of experiment that falls in this category is the dietary administration of compounds with antioxidant

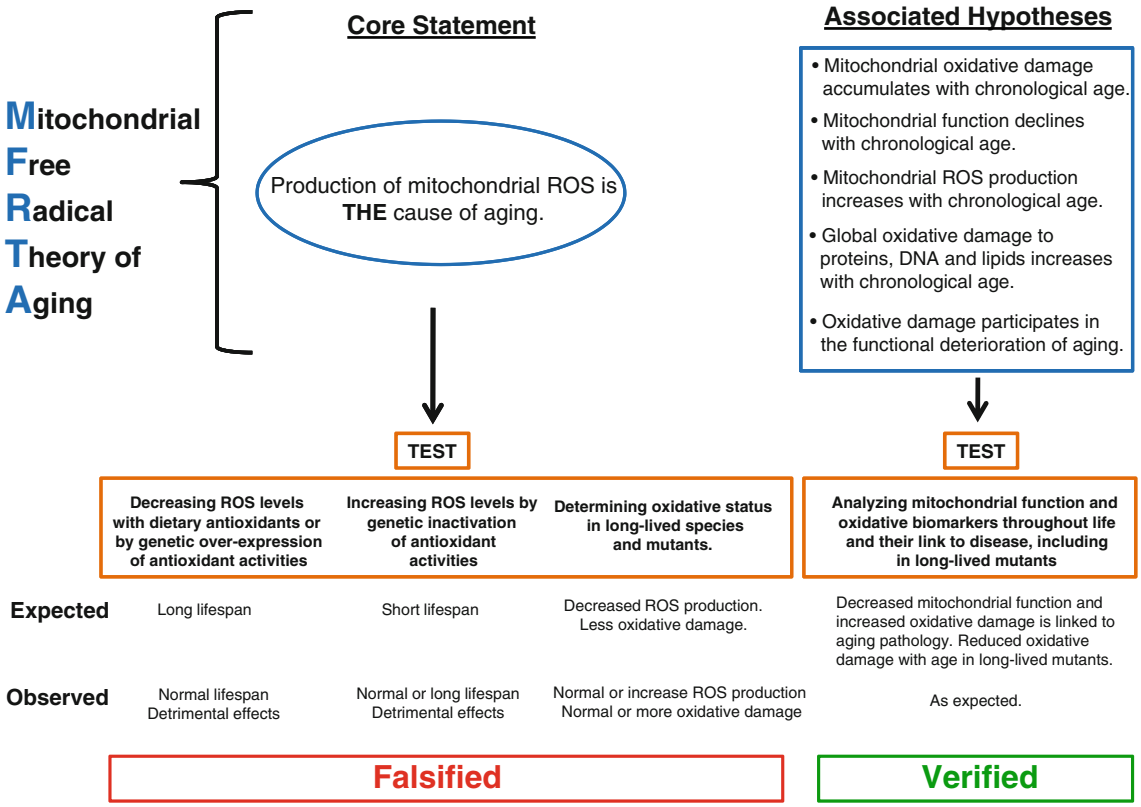


**Fig. 1** Schematic representation of the core statement and the associated hypotheses of the MFRTA, which have been tested by multiple studies in mammals. Taken together, the findings of these analyses have led to the conclusions that the core statement of the theory can now be considered as falsified in contrast to the associated hypotheses that have been verified by a variety of measures

properties in order to extend maximum lifespan. An increase in maximum lifespan directly in response to an exogenously supplemented antioxidant would have been a major finding in favor of the theory, but, to date, despite numerous experiments and clinical trials with promising compounds, there is no report of a successful study in mammals, and the results are strangely inconsistent in *Drosophila* [18, 19]. Worse, some undesirable effects, including disease and even an increased risk of death, have been shown to occur in clinical trials in humans, and this has resulted in the trials being urgently stopped [20–22]. Thus, even if a potentially promising compound with some antioxidant properties, such as resveratrol, has been shown to extend lifespans in invertebrate model organisms, similar treatment in mice has failed to slow the rate of aging in spite of some beneficial effects on health [23, 24]. Interestingly, directly targeting the mitochondria by feeding mice with coenzyme $Q_{10}$, a well-known mitochondrial antioxidant, has no effects on lifespan, even when mitochondrial coenzyme $Q_{10}$ content was successfully increased [25].

In addition to these pharmacological trials, a genetic approach in which antioxidant gene products are overexpressed with the same intention of decreasing ROS levels and ultimately reducing mitochondrial or global oxidative stress has also been taken. Again, a long lifespan of such transgenic animals would have been a crucial demonstration of the validity of the MFRTA. However, the over-expression of crucial antioxidant enzymes, such as SOD1, SOD2 and catalase, could again be shown to increase the lifespan in invertebrates organisms, such as *Drosophila* [26], but not in mice [9, 27]. Recently, it was also reported that even the overexpression of different combinations of major enzymatic antioxidants in double transgenic animals was still unable to slow down the aging process [28]. The main argument against the MFRTA that arose from these experiments is that the increased activity of antioxidant enzymes in fact succeeds in enhancing resistance to oxidative stress in these transgenic animals [29–31]. Most troubling is the finding that the overexpression of the mitochondrial superoxide dismutase (SOD2), which clearly represents the principal defense against mitochondrial superoxide, does not result in a lifespan extension in single transgenic mice or when overexpressed in combination with SOD1 [9].

So far, the only experimental data resulting from the type of analyses just described that really support the mitochondrial oxidative theory is the overexpression in mice of human peroxisomal catalase targeted to mitochondria (MCAT). Indeed, these mutants have been shown to live 17–21% longer than their wild-type siblings [32]. An attenuation in the development of some age-related pathologies as well as in the rate of mitochondrial hydrogen peroxide production and in the level of aconitase inactivation following exposure to $H_2O_2$ was also reported [32]. Additionally, some beneficial effects on the health of the animals were noted, for example in the cardiovascular system [33]. However, there are some concerns about these experiments that make it unclear whether their results should be considered support for the core statement of the MFRTA. One problem is that the lifespan extension, originally evaluated after 2–4 backcrosses to C57B6 mice, became less evident after further backcrosses [32]. This means that the lifespan extension is at least in part the result of the interaction with specific alleles at other loci. This is a problem as reduced oxidative stress should always increase lifespan if the core statement of the MFRTA is correct. Also, non-targeted overexpression of catalase only or in combination with SOD2 has no effect on lifespan [28, 34]. It was also observed that the transgene was not equally expressed in all tissues, and the effects of MCAT expression are selective and do not impact all age-associated lesions [35]. The mitochondria from the transgenic mice seem resistant to oxidative stress, but aconitase activity in the absence of an exogenous treatment is not higher than in controls, thus suggesting that intramitochondrial oxidative status is unaffected by MCAT expression [32]. Moreover, knowing that catalase is a major hydrogen peroxide scavenger and that this particular ROS is now considered as an important mediator in mitochondrial signal transduction [36–38], it is difficult to exclude the possibility that the longevity phenotype did not directly result from reduced mitochondrial oxidative stress.

In addition to the overexpression of antioxidant activities, some studies have focused on heterozygous or homozygous mutant mice in which the activities of antioxidant gene products were reduced or removed entirely (Fig. 1). To date, the results that were obtained have also been disappointing, and sometimes even surprising. Indeed, the phenotypes that were obtained were principally lethal or resulted in severe pathology associated with short lifespan, and it is difficult to claim that these phenotypes result from accelerated aging and use these results as evidence for the theory [39]. Surprisingly, however, many of these mutants have been reported to have a normal lifespan [9]. One of the most interesting is the phenotype of the *Sod2*$^{+/-}$ mutant mice, which to our point of view is clearly incompatible with the MFRTA [40]. Indeed, instead of being short-lived as would have been predicted by the theory, the lifespan of these mutants for a crucial mitochondrial antioxidant is perfectly normal, in spite of increased oxidative stress [41, 42]. Recently, heterozygous mice for thioredoxin 2, a small cysteine-rich protein with antioxidant properties localized to the mitochondrial matrix, have been generated and are characterized by impaired mitochondrial function and high mitochondrial

oxidative stress [43]. The lifespan phenotype of these mitochondrial mutants, which a preliminary report describes as surprisingly unaffected [44], will be another new test for the theory.

## From incompatible to irreconcilable

As discussed in the previous section, many results that are not in accordance with the MFRTA have been generated over the years with antioxidant treatments or with transgenic animals. We believe these results to be incompatible with the truth of the theory because the lifespan of these animals is normal despite higher or lower levels of antioxidant expression and oxidative damage. These findings are of course serious challenges for the theory but one could still accept some arguments as to why they do not necessarily lead to a rejection of the core statement of the theory. For example, it was suggested that the incapacity of supplemented antioxidants to enhance longevity may be due to the difficulty of providing them at sufficient concentrations without inducing deleterious effects due to the regulatory functions of ROS [45]. Interestingly, the lack of lifespan phenotype resulting from antioxidant overexpression has also been related to the observation that, in some particular cases, the increased activity of antioxidant enzymes induced oxidative damage and other deleterious effects [30]. Paradoxically, pathological conditions linked to genetic inactivation of antioxidant that resulted in a shortened lifespan are considered by others to validate the theory [9]. Furthermore, it has been argued [9] that the normal lifespan of the *Sod2*$^{+/-}$ mice, despite documented increased oxidative damage in these animals, might be possible if not all oxidative biomarkers are elevated, with reference to unpublished results. However, we have recently confirmed that mitochondria of *Sod2*$^{+/-}$ mutant mice indeed exhibit high oxidative stress by a variety of measures [46]. In any case, if only one type of oxidative damage matters for lifespan the core statement of the MFRTA would have to be seriously amended.

Most recently, the MFRTA has faced a new form of challenge as high oxidative stress has been related to extended lifespan. Studies with the longest living rodents, the naked-mole rats (NMRs), have shown that rather than having low levels of oxidative stress, these animals exhibit higher levels of oxidative damage to lipids, DNA and proteins than mice at the same fraction of their maximum lifespan (physiologically matched) and equal to that of mice of the same chronological age [47]. Moreover, concentration of specific markers of lipid peroxidation are higher in young NMRs than in short-lived mice and, unexpectedly, did not accumulate with aging [48]. Similarly, significantly elevated mitochondrial ROS production was recently observed in the cardiovascular system of the long-lived Ames dwarf mice when compared to wild-type littermates [49].

As mentioned previously, genetic over-expression of major antioxidants does not lead to lifespan extension, and this is considered as a serious challenge for the theory. Yet, we believe that the increased lifespan recently demonstrated for mice with partial inactivation of GPx4 represents an even greater challenge. Indeed, mice heterozygous for *GPx4*, the gene encoding the only mitochondrial enzymatic antioxidant that directly reduces membrane-bound lipid hydroperoxides, have an increased median lifespan despite higher levels of biomarkers of oxidative damage [50]. Interestingly, it was recently shown that inactivation of the crucial mitochondrial antioxidant SOD-2 increases oxidative damage to proteins and general sensitivity to oxidative stress in *C. elegans*, yet simultaneously prolongs lifespan of these animals [51]. Thus, we are left with findings that suggest, paradoxically as far as the MFRTA is concerned, that high mitochondrial oxidative stress can positively affect longevity.

By studying MCLK1, a mitochondrial enzyme necessary for ubiquinone (coenzyme Q) biosynthesis, our group recently provided further evidence for beneficial effects of mitochondrial oxidative stress. Mutational inactivation of *clk-1* in *Caenorhabditis elegans*, and partial inactivation of *Mclk1* in mice prolong average and maximum lifespan in these organisms [52, 53]. The lifespan extension was observed in three different genetic backgrounds. However, due to the relatively small number of mice that were originally tested and the shorter than normal median lifespan of controls in the C57BL/6J background, the strength of these results has been questioned [9]. However, we have recently presented a new aging study with a large sample size in a mixed background that showed again that *Mclk1*$^{+/-}$ mutants live significantly longer than controls [46]. To date, virtually all our findings resulting from the analysis of the phenotype of the long-lived *Mclk1*$^{+/-}$ mice appear irreconcilable with the MFRTA [46, 54]: (1) we found that mitochondria in young *Mclk1*$^{+/-}$ mutants are dysfunctional despite normal levels of ubiquinone: for example, they display slow electron transport, contain low levels of ATP and sustain high oxidative stress. Yet these mice are long-lived, and their altered function ultimately results in a significant attenuation of the rate of development of non-mitochondrial oxidative biomarkers of aging. (2) In spite of their high oxidative stress, the function of *Mclk1*$^{+/-}$ mitochondria declines less rapidly with age than that of the wild type, indicating that even age-dependent damage to mitochondria is not principally caused by mitochondrial oxidative stress. (3) The positive effects that we have observed on lifespan, biomarkers of aging and mitochondrial function are not the result of low

mitochondrial oxidative stress in the aged animal. Indeed, at 23 months of age the mitochondrial oxidative stress of *Mclk1*$^{+/-}$ is the same as that of the controls, not lower. (4) The partial loss of mitochondrial superoxide detoxification in *Sod2*$^{+/-}$ mutants enhances the protective effects of *Mclk1* heterozygosity on mitochondrial function, suggesting that the oxidative stress observed in *Mclk1*$^{+/-}$ mutants might be an integral part of the mechanism that allows for their slow rate of aging. Therefore, as the *Mclk1*$^{+/-}$ mutants live long and differ minimally from their *Mclk1*$^{+/+}$ siblings at the molecular level (reduced dosage of one naturally encoded gene), it is difficult to conceive of a mechanism by which the same minimal change could both decrease the rate of aging and increase mitochondrial oxidative stress if the latter is the cause of aging.

We have proposed a mechanism for the conjunction of low ATP levels, high mitochondrial oxidative stress, low non-mitochondrial oxidative damage and increased lifespan in *Mclk1*$^{+/-}$ mutants (Fig. 2). A slow rate of electron transport leads to low ATP production and reduced NAD(H) synthesis, which leads in turn to a deficit in those mechanisms of ROS detoxification that require NADPH regeneration (NADPH is regenerated via NADH), such as those that use glutathione. The deficit in detoxification leads to higher oxidative stress, which, in turn, together with the low levels of NAD(H), impairs electron transport further. We have also proposed that the low levels of ATP and NAD(H) might lead to a depression of cytoplasmic damage-generating processes, including ROS damage, to explain the mild decrease in non-mitochondrial oxidative damage that we observe in young *Mclk1*$^{+/-}$ animals. The longevity of these animals might therefore spring from a lower rate of accumulation of irreversible non-mitochondrial damage. Our findings support this hypothesis as we observed a slowing down of overall ROS damage accumulation with age without a reduction of mitochondrial oxidative stress below that of the wild type. Note that our observations do not lead to a "non-mitochondrial free radical theory of aging," but only supports the "damage accumulation theory of aging," because in this study we have only monitored oxidative stress, which might be only one of several sources of damage. In addition, we have shown that mitochondrial function is lost more slowly in the mutants than in the wild type. We interpret this to mean that the overall sparing due to reduced cytoplasmic damage, including reduced ROS damage, spares mitochondrial function. This is not surprising as a majority of the components of the mitochondria are synthesized in the cytoplasm and encoded in the nucleus in the case of proteins [55]. An overall lower level of cytoplasmic damage will benefit mitochondrial function as much as it will benefit other cellular processes. Our views, which overlap with the rate-of-living theory of aging, are also supported by numerous observations in other systems such as in the nematode *C. elegans* [56].

## Relating the *Mclk1* model of longevity to long-lived animals and recognized concepts

Our laboratory has used the *C. elegans* system to show that the mechanism by which *clk-1* prolongs lifespan is distinct from that of the insulin signaling pathway but overlaps with that of caloric restriction [57, 58], which we will not further discuss here. Rather we would like to draw parallels to other

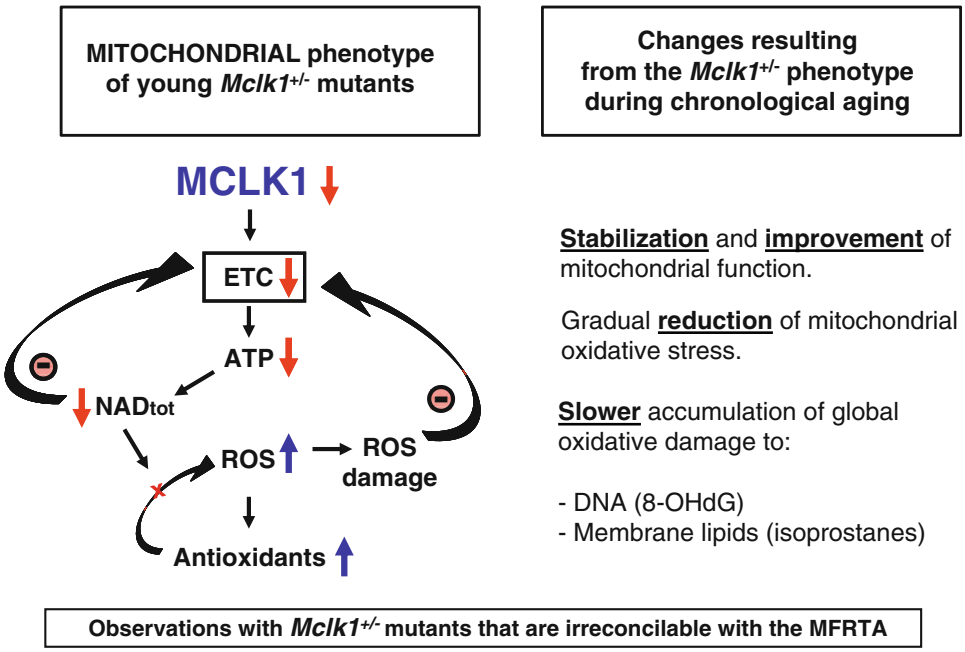


**Fig. 2** Regulation of mitochondrial function by MCLK1 determines the rate of aging in a manner that is irreconcilable with the MFRTA. Reduction of MCLK1 levels in *Mclk1*$^{+/-}$ mutants strongly affects mitochondrial function. The reduced mitochondrial function is linked to a reduction in the rate of electron transport, a decrease in energy production as well as to an increase in intra-mitochondrial oxidative stress. *Mclk1*$^{+/-}$ mutant animals also display a decrease in cytosolic and global ROS damage as well as in the rate of aging

studies in vertebrates, including some we have already mentioned. As pointed out above, long-lived naked mole rats (NMRs) exhibit high levels of oxidative damage even as young animals, similar to what we observe in *Mclk1*$^{+/-}$ mutants. Additionally, NMRs have markedly attenuated age-related accrual of oxidative damage, and the initial levels of damage are relatively stable throughout life [48]. Even at a young age, NMRs have a high tolerance to oxidative stress, that is, it does not readily affect functionality, and they are better able to maintain the structural and functional integrity of their proteins over time than can short-lived species [59]. Another model is that of long-lived *Gpx4*$^{+/-}$ mutants with high oxidative damage [50]. As overexpression of GPx4 protects mitochondrial ATP generation from oxidative stress [60], the increased lifespan of the *GPx4*$^{+/-}$ mutants, which have 50% reduction in the level of GPx4 [50], might find its source in reduced ATP generation and increased oxidative damage as observed in the *Mclk1*$^{+/-}$ mice. Finally, it was demonstrated that in mice lacking the mitochondrial NAD-dependent deacetylase SIRT3, mitochondria are dysfunctional, and basal levels of ATP in the heart, kidney and liver are reduced by about 50% [61]. This mitochondrial phenotype is similar to what we have reported for *Mclk1*$^{+/-}$, and it will be very interesting to learn about the mitochondrial oxidative status as well as the lifespan of this *Sirt3*$^{-/-}$ mutants.

The situation of *Mclk1*$^{+/-}$ mutants, which suffer from enhanced stress at an early stage but whose condition then proceeds to demonstrate paradoxical beneficial effects on biomarkers of aging, could be seen as hormesis. Hormesis has been defined, particularly in toxicology, as a process in which exposure to a low dose of a chemical agent or environmental factor that is damaging at higher doses induces an adaptive beneficial effect on the cell or organism [62]. This concept is now increasingly applied and studied in the context of aging research, and it was recently shown that single or multiple exposure to low doses of otherwise detrimental agents, such as irradiation, heat stress and ROS generators, might have a variety of anti-aging and longevity-extending effects [63, 64]. The term "mitohormesis" has further been proposed to described the effects of a high but sub-lethal level of mitochondrial ROS that might trigger other, ultimately beneficial, cellular events and results in transient cytoprotection or organismal longevity [65, 66]. It remains an open question whether it is judicious or confusing to use the concept of hormesis to describe the situation of genetic mutants whose condition is necessarily chronic.

## Concluding and moving forward

It is difficult to doubt that mitochondria play a key role in the aging process [67, 68]. However, although it is well documented that irreversible oxidative damage accumulates during aging [69], it seems that the MFRTA's core statement that postulates that aging is triggered by the detrimental action of ROS produced during normal metabolism is simply wrong. It is not yet clear whether aging has a single cause or whether such a notion is misguided. In any case, the correlation between the presence of oxidative damage and the aged phenotype simply does not imply causation. Oxidative stress might be the consequence of aging, if aging indeed has some discrete cause, or causes, distinct from oxidative stress [40]. Alternatively, oxidative stress might result from the failure of one particular maintenance system of the organism and thus participate in causing aging, but no more, as is often proposed in multicausal or unifying theories of aging [3–6]. Therefore, there is no reason to believe that it could not be beneficial to health to counteract the deleterious effects induced by ROS, at least in pathological situations. However, any intervention will nonetheless have to be very critically evaluated as clearly revealed by the antioxidant supplementation trials and in light of the increasing number of studies showing the crucial roles of ROS in cellular signaling.

## References

1. Kirkwood TB (2005) Understanding the odd science of aging. Cell 120:437–447
2. Medvedev ZA (1990) An attempt at a rational classification of theories of ageing. Biol Rev Camb Philos Soc 65:375–398
3. Rattan SI (2006) Theories of biological aging: genes, proteins, and free radicals. Free Radic Res 40:1230–1238
4. Hayflick L (2007) Biological aging is no longer an unsolved problem. Ann NY Acad Sci 1100:1–13
5. Holliday R (2006) Aging is no longer an unsolved problem in biology. Ann NY Acad Sci 1067:1–9
6. Kirkwood TB (2008) A systematic look at an old problem. Nature 451:644–647
7. Harman D (1972) The biologic clock: the mitochondria? J Am Geriatr Soc 20:145–147
8. Gruber J, Schaffer S, Halliwell B (2008) The mitochondrial free radical theory of ageing—where do we stand? Front Biosci 13:6554–6579
9. Muller FL, Lustgarten MS, Jang Y, Richardson A, Van Remmen H (2007) Trends in oxidative aging theories. Free Radic Biol Med 43:477–503
10. Beckman KB, Ames BN (1998) The free radical theory of aging matures. Physiol Rev 78:547–581
11. Hekimi S, Guarente L (2003) Genetics and the specificity of the aging process. Science 299:1351–1354
12. de Magalhaes JP, Church GM (2006) Cells discover fire: employing reactive oxygen species in development and consequences for aging. Exp Gerontol 41:1–10
13. Blagosklonny MV (2008) Aging: ROS or TOR. Cell Cycle 7:3344–3354
14. Bonawitz ND, Shadel GS (2007) Rethinking the mitochondrial theory of aging: the role of mitochondrial gene expression in lifespan determination. Cell Cycle 6:1574–1578

15. Buffenstein R, Edrey YH, Yang T, Mele J (2008) The oxidative stress theory of aging: embattled or invincible? Insights from non-traditional model organisms. Age (Dordr) 30:99–109
16. Gems D, Doonan R (2009) Antioxidant defense and aging in *C. elegans*: is the oxidative damage theory of aging wrong? Cell Cycle 8
17. Fukui H, Moraes CT (2008) The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? Trends Neurosci 31:251–256
18. Howes RM (2006) The free radical fantasy: a panoply of paradoxes. Ann NY Acad Sci 1067:22–26
19. Magwere T, West M, Riyahi K, Murphy MP, Smith RA, Partridge L (2006) The effects of exogenous antioxidants on lifespan and oxidative stress resistance in Drosophila melanogaster. Mech Ageing Dev 127:356–370
20. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C (2007) Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 297:842–857
21. Collins R, Armitage J, Parish S, Sleigh P, Peto R (2003) MRC/BHF heart protection study of cholesterol-lowering with simvastatin in 5963 people with diabetes: a randomised placebo-controlled trial. Lancet 361:2005–2016
22. Omenn GS, Goodman GE, Thornquist MD, Balmes J, Cullen MR, Glass A, Keogh JP, Meyskens FL, Valanis B, Williams JH, Barnhart S, Hammar S (1996) Effects of a combination of beta carotene and vitamin A on lung cancer and cardiovascular disease. N Engl J Med 334:1150–1155
23. Pearson KJ, Baur JA, Lewis KN, Peshkin L, Price NL, Labinskyy N, Swindell WR, Kamara D, Minor RK, Perez E, Jamieson HA, Zhang Y, Dunn SR, Sharma K, Pleshko N, Woollett LA, Csiszar A, Ikeno Y, Le Couteur D, Elliott PJ, Becker KG, Navas P, Ingram DK, Wolf NS, Ungvari Z, Sinclair DA, de Cabo R (2008) Resveratrol delays age-related deterioration and mimics transcriptional aspects of dietary restriction without extending life span. Cell Metab 8:157–168
24. Wood JG, Rogina B, Lavu S, Howitz K, Helfand SL, Tatar M, Sinclair D (2004) Sirtuin activators mimic caloric restriction and delay ageing in metazoans. Nature 430:686–689
25. Sohal RS, Kamzalov S, Sumien N, Ferguson M, Rebrin I, Heinrich KR, Forster MJ (2006) Effect of coenzyme Q10 intake on endogenous coenzyme Q content, mitochondrial electron transport chain, antioxidative defenses, and life span of mice. Free Radic Biol Med 40:480–487
26. Sun J, Tower J (1999) FLP recombinase-mediated induction of Cu/Zn-superoxide dismutase transgene expression can extend the life span of adult Drosophila melanogaster flies. Mol Cell Biol 19:216–228
27. Huang TT, Carlson EJ, Gillespie AM, Shi Y, Epstein CJ (2000) Ubiquitous overexpression of CuZn superoxide dismutase does not extend life span in mice. J Gerontol A Biol Sci Med Sci 55:B5–B9
28. Perez VI, Van Remmen H, Bokov A, Epstein CJ, Vijg J, Richardson A (2009) The overexpression of major antioxidant enzymes does not extend the lifespan of mice. Aging Cell 8:73–75
29. Mele J, Van Remmen H, Vijg J, Richardson A (2006) Characterization of transgenic mice that overexpress both copper zinc superoxide dismutase and catalase. Antioxid Redox Signal 8:628–638
30. Raineri I, Carlson EJ, Gacayan R, Carra S, Oberley TD, Huang TT, Epstein CJ (2001) Strain-dependent high-level expression of a transgene for manganese superoxide dismutase is associated with growth retardation and decreased fertility. Free Radic Biol Med 31:1018–1030
31. Shan X, Chi L, Ke Y, Luo C, Qian S, Gozal D, Liu R (2007) Manganese superoxide dismutase protects mouse cortical neurons from chronic intermittent hypoxia-mediated oxidative damage. Neurobiol Dis 28:206–215
32. Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, Coskun PE, Ladiges W, Wolf N, Van Remmen H, Wallace DC, Rabinovitch PS (2005) Extension of murine life span by overexpression of catalase targeted to mitochondria. Science 308:1909–1911
33. Dai DF, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, Gollahon K, Martin GM, Loeb LA, Ladiges WC, Rabinovitch PS (2009) Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. Circulation 119:2789–2797
34. Chen X, Liang H, Van Remmen H, Vijg J, Richardson A (2004) Catalase transgenic mice: characterization and sensitivity to oxidative stress. Arch Biochem Biophys 422:197–210
35. Treuting PM, Linford NJ, Knoblaugh SE, Emond MJ, Morton JF, Martin GM, Rabinovitch PS, Ladiges WC (2008) Reduction of age-associated pathology in old mice by overexpression of catalase in mitochondria. J Gerontol A Biol Sci Med Sci 63:813–822
36. Connor KM, Subbaram S, Regan KJ, Nelson KK, Mazurkiewicz JE, Bartholomew PJ, Aplin AE, Tai YT, Aguirre-Ghiso J, Flores SC, Melendez JA (2005) Mitochondrial $H_2O_2$ regulates the angiogenic phenotype via PTEN oxidation. J Biol Chem 280:16916–16924
37. Chandel NS, Budinger GR (2007) The cellular basis for diverse responses to oxygen. Free Radic Biol Med 42:165–174
38. Guzy RD, Schumacker PT (2006) Oxygen sensing by mitochondria at complex III: the paradox of increased reactive oxygen species during hypoxia. Exp Physiol 91:807–819
39. Miller RA (2004) 'Accelerated aging': a primrose path to insight? Aging Cell 3:47–51
40. Hekimi S (2006) How genetic analysis tests theories of animal aging. Nat Genet 38:985–991
41. 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, Richardson A (2003) Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. Physiol Genomics 16:29–37
42. Williams MD, Van Remmen H, Conrad CC, Huang TT, Epstein CJ, Richardson A (1998) Increased oxidative damage is correlated to altered mitochondrial function in heterozygous manganese superoxide dismutase knockout mice. J Biol Chem 273:28510–28515
43. Perez VI, Lew CM, Cortez LA, Webb CR, Rodriguez M, Liu Y, Qi W, Li Y, Chaudhuri A, Van Remmen H, Richardson A, Ikeno Y (2008) Thioredoxin 2 haploin sufficiency in mice results in impaired mitochondrial function and increased oxidative stress. Free Radic Biol Med 44:882–892
44. Jang YC, Remmen VH (2009) The mitochondrial theory of aging: insight from transgenic and knockout mouse models. Exp Gerontol 44:256–260
45. Harman D (2009) Origin and evolution of the free radical theory of aging: a brief personal history, 1954–2009. Biogerontology
46. Lapointe J, Stepanyan Z, Bigras E, Hekimi S (2009) Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived Mclk1+/− mice. J Biol Chem 284(30):20364–20374
47. Andziak B, O'Connor TP, Qi W, DeWaal EM, Pierce A, Chaudhuri AR, Van Remmen H, Buffenstein R (2006) High oxidative damage levels in the longest-living rodent, the naked mole-rat. Aging Cell 5:463–471

48. Andziak B, Buffenstein R (2006) Disparate patterns of age-related changes in lipid peroxidation in long-lived naked mole-rats and shorter-lived mice. Aging Cell 5:525–532
49. Csiszar A, Labinskyy N, Perez V, Recchia FA, Podlutsky A, Mukhopadhyay P, Losonczy G, Pacher P, Austad SN, Bartke A, Ungvari Z (2008) Endothelial function and vascular oxidative stress in long-lived GH/IGF-deficient Ames dwarf mice. Am J Physiol Heart Circ Physiol 295:H1882–H1894
50. Ran Q, Liang H, Ikeno Y, Qi W, Prolla TA, Roberts LJ, Wolf N, Van Remmen H, Richardson A (2007) Reduction in glutathione peroxidase 4 increases life span through increased sensitivity to apoptosis. J Gerontol A Biol Sci Med Sci 62:932–942
51. Van Raamsdonk JM, Hekimi S (2009) Deletion of the mitochondrial superoxide dismutase sod-2 extends lifespan in *Caenorhabditis elegans*. PLoS Genet 5:e1000361
52. Liu X, Jiang N, Hughes B, Bigras E, Shoubridge E, Hekimi S (2005) Evolutionary conservation of the clk-1-dependent mechanism of longevity: loss of mclk1 increases cellular fitness and lifespan in mice. Genes Dev 19:2424–2434
53. Wong A, Boutis P, Hekimi S (1995) Mutations in the clk-1 gene of *Caenorhabditis elegans* affect developmental and behavioral timing. Genetics 139:1247–1259
54. Lapointe J, Hekimi S (2008) Early mitochondrial dysfunction in long-lived Mclk1$^{+/-}$ mice. J Biol Chem 283(38):26217–26227
55. Johnson DT, Harris RA, French S, Blair PV, You J, Bemis KG, Wang M, Balaban RS (2007) Tissue heterogeneity of the mammalian mitochondrial proteome. Am J Physiol Cell Physiol 292:C689–C697
56. Hekimi S, Burgess J, Bussiere F, Meng Y, Benard C (2001) Genetics of lifespan in C elegans: molecular diversity, physiological complexity, mechanistic simplicity. Trends Genet 17:712–718
57. Lakowski B, Hekimi S (1996) Determination of life-span in *Caenorhabditis elegans* by four clock genes. Science 272:1010–1013
58. Lakowski B, Hekimi S (1998) The genetics of caloric restriction in *Caenorhabditis elegans*. Proc Natl Acad Sci USA 95:13091–13096
59. Perez VI, Buffenstein R, Masamsetti V, Leonard S, Salmon AB, Mele J, Andziak B, Yang T, Edrey Y, Friguet B, Ward W, Richardson A, Chaudhuri A (2009) Protein stability and resistance to oxidative stress are determinants of longevity in the longest-living rodent, the naked mole-rat. Proc Natl Acad Sci USA 106:3059–3064
60. Liang H, Van Remmen H, Frohlich V, Lechleiter J, Richardson A, Ran Q (2007) Gpx4 protects mitochondrial ATP generation against oxidative damage. Biochem Biophys Res Commun 356:893–898
61. Ahn BH, Kim HS, Song S, Lee IH, Liu J, Vassilopoulos A, Deng CX, Finkel T (2008) A role for the mitochondrial deacetylase Sirt3 in regulating energy homeostasis. Proc Natl Acad Sci USA 105:14447–14452
62. Mattson MP (2008) Hormesis defined. Ageing Res Rev 7:1–7
63. Gems D, Partridge L (2008) Stress–response hormesis and aging: "that which does not kill us makes us stronger". Cell Metab 7:200–203
64. Rattan SI (2008) Hormesis in aging. Ageing Res Rev 7:63–78
65. Schulz TJ, Zarse K, Voigt A, Urban N, Birringer M, Ristow M (2007) Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. Cell Metab 6:280–293
66. Tapia PC (2006) Sublethal mitochondrial stress with an attendant stoichiometric augmentation of reactive oxygen species may precipitate many of the beneficial alterations in cellular physiology produced by caloric restriction, intermittent fasting, exercise and dietary phytonutrients: "Mitohormesis" for health and vitality. Med Hypotheses 66:832–843
67. Navarro A, Boveris A (2007) The mitochondrial energy transduction system and the aging process. Am J Physiol Cell Physiol 292:C670–C686
68. Lambert AJ, Brand MD (2007) Research on mitochondria and aging, 2006–2007. Aging Cell 6:417–420
69. Sohal RS, Mockett RJ, Orr WC (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. Free Radic Biol Med 33:575–586