Taking a “good” look at free radicals in the aging process

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Abstract

The mitochondrial free radical theory of aging (MFRTA) proposes that aging is caused by damage to macromolecules from mitochondrial reactive oxygen species (ROS). This is based on the observed association of the rate of aging and the aged phenotype with the generation of ROS and with oxidative damage. The theory has led to the strong conviction in the general public that the consumption of antioxidants is crucial to health and beneficial to lifespan. However, a variety of recent findings convincingly demonstrate that ROS generation and oxidative damage cannot be the cause of aging. Here we propose that ROS play a role in mediating a stress response to age-dependent damage, which could generate the observed correlation between aging and ROS without implying that ROS cause aging.

Redefining our understanding of the relationship between ROS and aging

Mitochondria are a major source of reactive oxygen species (ROS), a type of molecule that includes free radicals such as superoxide. ROS spontaneously oxidize and damage macromolecules such as proteins, lipids and nucleic acids. Cells and organisms are said to be sustaining oxidative stress when an imbalance between ROS generation and detoxification or repair leads to an increase in the level of ROS-dependent damage. The mitochondrial free radical theory of aging (MFRTA) has provided an attractive framework that integrates numerous observations about the generation, the toxicity and the detoxification of ROS, as well as about how these parameters change with the physiological state of cells and organisms and with chronological age. The theory proposes that aging is actually caused by the toxicity of ROS through a vicious cycle in which ROS damage to the constituents of mitochondria leads to the generation of more ROS. The theory is based on numerous observations, including (1) that there is a strong correlation between chronological age and the level of ROS generation and oxidative damage, (2) that mitochondrial function is gradually lost during aging, (3) that inhibition of mitochondrial function can enhance ROS production, and (4) that several age-dependent diseases are associated with severe increases in oxidative stress.

The strength of the MFRTA is that it provides a framework for many observations while also stating a plausible causal theory of aging. Furthermore, it provided a clear research program by proposing a theory to be tested as well as by suggesting that decreasing the generation of ROS will result in health benefits. In fact the MFRTA is often taught in textbooks and in

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university lectures as being the demonstrated true theory of aging, and it has entered public consciousness, mostly in the form of a certainty that antioxidants, in particular in foods, in nutritional supplements, or in beauty products can slow down the effects of the aging process. We will briefly review some of the results that have led to the formulation of the MFRTA as well as recent findings with invertebrates, mammals and human subjects that have failed to support and even disproved the MFRTA.

Showing that the MFRTA is erroneous leaves the field with many observations about ROS and aging that now are in want of an explanation (Box 1). For this we are proposing a new hypothesis that incorporates the observations that led to the MFRTA yet is based on some of the very research results that falsify it. We posit that aging is a loss of homeostasis due to the accumulation of molecular damage, which is accepted by most. On this premise we propose that ROS generation represents a stress signal in response to age-dependent damage, which is why ROS generation increases gradually with chronological age until it reaches a level at which the toxicity of ROS now participates in creating the damage that it was meant to help alleviate, for example by enhancing the susceptibility to age-dependent diseases. This gradual ROS response hypothesis is not a causal theory of aging, but, like the MFRTA, it provides a greatly needed research program that could help to identify the nature and the source of the molecular damage that leads to the gradual deterioration of cellular functions that is the hallmark of aging.

Box 1

The mitochondrial free radical theory of aging (MFRTA) is facing a dead end and needs to be reconciled with new results that refute it

Major observations that led to the formulation of the MFRTA

- There is a strong correlation between chronological age and the level of reactive oxygen species (ROS) generation and of oxidative damage.
- Mitochondrial function is gradually lost during aging.
- Inhibition of mitochondrial function can enhance ROS production.
- Several age-dependent diseases are associated with severe increases in oxidative stress.

Major results that led to the refutation of the MFRTA

- Decreasing ROS production has failed to increase lifespan.
- High ROS production has been linked to increased longevity.

ROS production, antioxidant defences and oxidative stress

Oxygen reduction during mitochondrial electron transport is a source of the superoxide radical ($O_2^{\bullet-}$), which mitochondrial superoxide dismutases (SODs) turn into hydrogen peroxide (H_2O_2) and O_2 , which can be reused to generate superoxide. In the presence of reduced transition metals H_2O_2 can be converted into the highly reactive hydroxyl radical

($\cdot\text{HO}$). The reactivity of ROS as well as their formation in different cellular compartments and from exogenous sources have been extensively documented and reviewed [1, 2].

Mitochondrial energy metabolism is recognized as an important source of ROS in the majority of eukaryotic cell types [3] (Figure 1). There are currently seven separate sites of mammalian mitochondrial ROS production that have been identified and characterized [4]. Importantly, because H_2O_2 is relatively stable and membrane-permeable, it can diffuse out of the mitochondria into the cytoplasm [5].

ROS can inflict damage to macromolecules, such as lipids, nucleic acids, and proteins. Polyunsaturated fatty acids are one of the most sensitive oxidation targets for ROS because once lipid peroxidation is initiated, a damaging chain reaction takes place [6]. DNA bases are also very susceptible to ROS attack, and oxidation of DNA bases is believed to cause mutations and deletions in both nuclear and mitochondrial genomes [7]. Almost all amino acid residues in a protein can be oxidized by ROS, with these modifications leading to losses of function [8].

Exposure to ROS appears to be unavoidable for cells living in an aerobic environment, and ROS toxicity is controlled by a complex network of non-enzymatic and enzymatic antioxidants, including the SODs, the glutathione peroxidases (GPxs), the peroxiredoxins (PRxs), catalase, and glutathione (GSH) itself, which can directly neutralize some species such as $\cdot\text{HO}$. In fact GSH is so central to detoxification that the ratio of GSH to GSSG (oxidized glutathione) is a good indicator of the redox status of the cell. Knowledge about these systems has been extensively reviewed [9, 10]. Oxidative stress can be defined as any imbalance between the production and the detoxification of ROS.

Correlation of ROS production, oxidative damage and the rate of aging

It is accepted by many that there exists a close association between ROS generation, ROS-related damage and aging [11]. For example, mitochondrial ROS generation is higher in isolated mitochondria from old animals [12], which also show a decrease in the GSH/GSSG ratio [13]. Aging is also associated with an increase in the levels of oxidatively damaged proteins, lipids and DNA [14]. In general, a negative correlation between mitochondrial ROS production and lifespan can be observed in a variety of types of organisms [15].

The logic of the mitochondrial free radical theory of aging (MFRTA)

Aging in mammals is universal, degenerative, and appears unavoidable even in very sheltered environments. Therefore, when looking for the cause of mammalian aging, it is reasonable to look for an intrinsic process that damages intracellular components. In fact this was the strong logic that was used by Harman when he first proposed [16] and later refined [17] his theory. Since then the core statement of the MFRTA has been reinforced by the discovery of superoxide dismutases, by the observation that mitochondria continuously produce ROS, and by the observation that oxidative damage increases with age [18]. Most recently, the lethal phenotype of mice that completely lack the mitochondrial superoxide dismutase SOD2, and the shortened lifespan of mice that completely lack the cytoplasmic SOD1 have also supported the MFRTA [19, 20].

The logic of the MFRTA also embodies a vicious cycle hypothesis [17, 21]. Since mitochondria are a source of ROS, the components of mitochondria, particularly mitochondrial DNA, should be a main target for oxidative damage. Furthermore a variety of experiments that were mainly conducted *in vitro* have shown that an impairment of mitochondrial electron transport could result in increased ROS production [2]. Thus mitochondrial ROS damage would lead to more ROS generation and further damage, initiating a runaway process. This notion is also consistent with the exponential increase in mortality that characterizes aging. Finally, high cellular ROS levels have been associated with several age-dependent human conditions, including cancer, neurodegeneration, diabetes, chronic inflammation and cardiovascular diseases.

Recent results that are incompatible with the MFRTA

Recent results that appear incompatible with the MFRTA have demonstrated : i) a lack of correlation between the level of ROS production and longevity in some species; ii) deleterious rather than beneficial effects on lifespan from the administration of antioxidants; iii) that the inactivation or overexpression of antioxidant activities in genetically engineered organisms fails to produce outcomes that supported the MFRTA, iv) the existence of long-lived mutants and species with high ROS production and high oxidative damage. The description of these results and their impact on the validity and the future of the theory is the main subject of recent reviews in the field [22–25].

Among these challenging observations some are particularly irreconcilable with the MFRTA because they target its core statement. For example, knockout mutations of the mitochondrial superoxide dismutase SOD-2 in the nematode *C. elegans* increase oxidative stress, yet these mutations not only fail to shorten lifespan, they can dramatically prolong it [26, 27]. Similarly, mice that lack one copy of *Sod2* (*Sod2*^{+/-}) display enhanced mitochondrial oxidative stress yet live as long as the wild-type [28]. Even *Sod2*^{+/-} *Gpx1*^{-/-} double mutant mice, which in addition to low SOD2 levels lack one of the main mitochondrial glutathione peroxidases, do not show a shortened lifespan [29]. Yet, if mitochondrial ROS cause aging then increased mitochondrial ROS should cause faster aging and a shorter lifespan.

Even the vicious cycle aspect of the MFRTA, at least in regard to mitochondrial DNA, has recently been specifically refuted. Indeed, mice in which the proof-reading function of the mitochondrial DNA polymerase PolG has been impaired rapidly accumulate mitochondrial mutations. This interferes with mitochondrial protein synthesis, which results in mitochondrial dysfunction and causing a variety of aging phenotypes and early death [30], but does not in fact increase ROS generation [31]. Additionally, the idea that oxidative damage to mtDNA is higher than to nuclear DNA due to close proximity of the principal source of ROS has also been refuted [32].

Need for an alternative point of view

From the above we believe it to be reasonable to contend that the MFRTA has been falsified [33]. However, there is no reason to doubt the validity of the observations on which the theory was based, including the data that suggest that ROS toxicity might sometimes be

involved in disease progression. We believe that the properties of ROS as signaling molecules, and more specifically of signaling molecules that function as stress signals in response to age-dependent damage can provide an alternative, testable, theoretical framework to explain a host of phenomena linked to ROS biology. Below we first review some of the crucial research results that suggest an alternative to the MFRTA.

The role of ROS in signal transduction

The toxicity of ROS is only one aspect of their action in living cells as ROS originating from mitochondria and from non-mitochondrial sites can also modulate the function of various signalling pathways (Figure 1). In fact ROS possess several typical characteristics of second messengers: they are controlled at the level of synthesis and removal, they have specific targets, and their signaling effects are reversible. These properties and the involvement of ROS in signal transduction have been extensively reviewed [5, 34]. In fact under physiological conditions, the transient generation of ROS, within boundaries, appears to be essential to maintain cellular homeostasis. ROS as messengers have been associated to signaling by insulin, cytokines and many growth factors [35, 36], whose activity regulates classic signaling cascades such as the extracellular ERK, JNK, and MAPK cascades, as well as the PI3-K/Akt, PLC- γ 1 and JAK/STAT pathways [37, 38]. These pathways in turn exert their phenotypic effects largely by modulating the activities of central transcription factors, among them NF- κ B, AP-1, Nrf2, FoxOs, HIF-1 α and p53 [39, 40]. Furthermore, the activities of enzymes such as catalase, GPxs and Prdx have been shown to be regulated by kinases and phosphatases that are susceptible to oxidative modifications, thus creating a regulatory network [9, 10].

ROS signaling affects aging and lifespan

There is mounting evidence in several systems that ROS can stimulate beneficial responses to the cellular stresses produced by aging (Figure 2). In *C. elegans*, mutations in subunits of mitochondrial respiratory complexes can lead to increased lifespan [41, 42]. Two such mutations have now been found to result in an increase of superoxide generation by the mutant mitochondria [43]. Furthermore, and most importantly for the present discussion, this elevation of superoxide generation appears to be necessary and sufficient to increased longevity, as it is abolished by the antioxidants NAC and vitamin C [43], and is phenocopied by mild treatment with the prooxidant paraquat [43, 44]. These findings suggest that increased mitochondrial superoxide generation acts as a signal in young mutant animals to trigger changes of gene expression that prevent or attenuate the effects of subsequent aging. These findings were consistent with, and significantly extended, earlier studies in yeast [45] and in worms [46] that had suggested that an increase in ROS from mitochondria might be important in triggering the lifespan extension produced by glucose restriction.

Similar, if somewhat less direct evidence is also provided by studies in mice. *Mclk*^{+/-} mice lack one copy of an enzyme that is necessary for the synthesis of the antioxidant and redox co-factor ubiquinone [47]. This leads to numerous metabolic changes but in particular to an increase in mitochondrial oxidative stress [48], which is deleterious under some experimental conditions [49]. Surprisingly these mutant mice not only live longer than wild-

type siblings [50], they also show a much slower development of biomarkers of aging, including a slower loss of mitochondrial function [51]. Furthermore, recent evidence suggests that at least part of the beneficial effects of mitochondrial ROS in this system is mediated by their activity in elevating the levels of the protective transcription factor hypoxia-inducible factor 1 α (HIF-1 α) [52].

Another example involving mice links lifespan regulation to the biology of 4-hydroxynonenal (4-HNE). This is a product of lipid oxidation that has been shown to modulates ligand-independent signaling by membrane receptors and interacts with a variety of kinases [53]. Recently it was found that mice mutant for the glutathione transferase mGSTA4-4, an enzyme implicated in the detoxification of 4-hydroxynonenal (4-HNE) have an extended lifespan despite higher levels of this product of lipid oxidation [54].

The last example is the tantalising evidence that is provided by the biology of the very long-lived mole rats, which life up to 30 years, which is almost 10 times longer than laboratory mice. Indeed, these animals exhibit higher levels of ROS generation [55] and ROS-dependent macromolecular modifications than similar-sized but shorter-lived mice [56]. These observations are not only hard to reconcile with the MFRTA, they also suggest that ROS play a beneficial role in this species [24].

The gradual ROS response hypothesis

From the short reviews and analyses above we conclude: 1) that ROS do not cause aging, although high levels of ROS damage can contribute to the aged phenotype in particular in disease states, 2) that ROS are signaling molecules that can modulate stress response pathways, and 3) that increased ROS levels can result in positive effects, including on the cellular processes that limit lifespan. These facts suggest a hypothesis for how the biology of ROS could result in the observations that have formed the basis of the MFRTA (Figure 3). This hypothesis proposes that cellular constituents sustain a variety of age-dependent insults that trigger protective stress-response pathways that use ROS as second messengers. However, the protective mechanisms appear to be unable to fully prevent a gradual increase of age-related damage. The hypothesis thus further proposes that the gradual increase in damage should induce a gradually intensifying stimulation of stress-response pathways, and thus a gradually increasing or more sustained generation of ROS. Such a phenomenon could explain the striking correlation of the aged phenotype with ROS generation and ROS-dependent alterations of cellular constituents. The hypothesis also suggests that, as aging progresses, the level of ROS generation becomes maladaptive and ROS toxicity starts to contribute to the very damage production that the ROS-dependent stress pathways are meant to combat. This could even induce a run-away process in which ROS-dependent damage contributes to the stimulation of the ROS-dependent stress response pathways and thus leads to the further increase in ROS generation and ROS damage. A run-away process of this nature could explain the involvement of ROS in age-dependent diseases, which tend to develop only in the second half of lifespan. The possibility of a damaging run-away process also conforms to the general idea that the accelerating senescence and mortality that characterizes aging is due to damage being the source of more damage [57].

The hypothesis appears to be applicable to ROS signaling and ROS damage during aging whatever the origin of the ROS, from the mitochondria or from elsewhere in the cell. However, the hypothesis provides a particularly useful explanatory framework for a number of questions linked to mitochondrial ROS generation. For example, the proposed run-away process is akin to the vicious cycle hypothesis of ROS production without having to invoke that damage to the electron transport chain leads passively to increased ROS production. The hypothesis also provides a view of how an elevation of ROS in young mutant animals can lead to greater lifespan. We propose that in these cases the mutations turn on beneficial stress signals prematurely by elevating mitochondrial ROS generation already in young animals, and thus slow down the aging processes. In this way the mutations also delay the onset of the thresholds that lead to the run-away process and thus to a negative effect of ROS on the aging process. This could explain why both *C. elegans* and mouse mutants with elevated mitochondrial ROS generation [43, 48] have low levels of overall ROS damage [51, 58]. Similarly, the hypothesis also provides an explanation for why overexpression of antioxidant proteins or treatment with antioxidant drugs are unable to extend lifespan and rather cause cellular dysfunction [59] (Figure 1).

Hormesis has been proposed as the mechanism behind some of the observed effect of ROS on lifespan [60], but has been excluded in other cases [43]. Hormesis refers to a set of phenomena in which exposure to a low and/or repeated doses of a potentially harmful factor induces an adaptive beneficial effect on the cell or organism. The hypothesis of gradual ROS response that we are proposing here might share some molecular mechanisms with hormesis. However, our hypothesis proposes a process that is gradual, and that occurs as part of normal aging in wild type animals, and is thus conceptually quite distinct from hormesis.

Concluding Remarks

In the light of many recent findings it is now unrealistic to conclude that ROS cause aging. Therefore, a clear statement regarding the status of the MFRTA and alternative explanations for the well established correlation between ROS production and aging maybe useful. We hope that now that the MFRTA may be defunct, our hypothesis can provide a research program that uses its new interpretation of ROS biology to identify which deleterious mechanisms are most central to the initiation of the aging process and in this way lead us gradually to a new causal theory of aging.

References

1. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. *Nat Chem Biol.* 2008; 4:278–286. [PubMed: 18421291]
2. Turrens JF. Mitochondrial formation of reactive oxygen species. *J Physiol.* 2003; 552:335–344. [PubMed: 14561818]
3. Murphy MP. How mitochondria produce reactive oxygen species. *Biochem J.* 2009; 417:1–13. [PubMed: 19061483]
4. Brand MD. The sites and topology of mitochondrial superoxide production. *Exp Gerontol.* 2010; 45:466–472. [PubMed: 20064600]
5. Veal EA, et al. Hydrogen peroxide sensing and signaling. *Mol Cell.* 2007; 26:1–14. [PubMed: 17434122]

6. Niki E. Lipid peroxidation: physiological levels and dual biological effects. *Free Radic Biol Med.* 2009; 47:469–484. [PubMed: 19500666]
7. Fraga CG, et al. Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc Natl Acad Sci U S A.* 1990; 87:4533–4537. [PubMed: 2352934]
8. Ugarte N, et al. Oxidized mitochondrial protein degradation and repair in aging and oxidative stress. *Antioxid Redox Signal.* 2010; 13:539–549. [PubMed: 19958171]
9. Flohe L. Changing paradigms in thiology from antioxidant defense toward redox regulation. *Methods Enzymol.* 2010; 473:1–39. [PubMed: 20513470]
10. Yu BP. Cellular defenses against damage from reactive oxygen species. *Physiol Rev.* 1994; 74:139–162. [PubMed: 8295932]
11. Balaban RS, et al. Mitochondria, oxidants, and aging. *Cell.* 2005; 120:483–495. [PubMed: 15734681]
12. Sohal RS, Sohal BH. Hydrogen peroxide release by mitochondria increases during aging. *Mech Ageing Dev.* 1991; 57:187–202. [PubMed: 1904965]
13. Asensi M, et al. Ratio of reduced to oxidized glutathione as indicator of oxidative stress status and DNA damage. *Methods Enzymol.* 1999; 299:267–276. [PubMed: 9916205]
14. Halliwell B. The wanderings of a free radical. *Free Radic Biol Med.* 2009; 46:531–542. [PubMed: 19111608]
15. Lambert AJ, et al. Low rates of hydrogen peroxide production by isolated heart mitochondria associate with long maximum lifespan in vertebrate homeotherms. *Aging Cell.* 2007; 6:607–618. [PubMed: 17596208]
16. Harman D. Aging: a theory based on free radical and radiation chemistry. *J Gerontol.* 1956; 11:298–300. [PubMed: 13332224]
17. Harman D. The biologic clock: the mitochondria? *J Am Geriatr Soc.* 1972; 20:145–147. [PubMed: 5016631]
18. Muller FL, et al. Trends in oxidative aging theories. *Free Radic Biol Med.* 2007; 43:477–503. [PubMed: 17640558]
19. Elchuri S, et al. CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene.* 2005; 24:367–380. [PubMed: 15531919]
20. Li Y, et al. Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat Genet.* 1995; 11:376–381. [PubMed: 7493016]
21. Beckman KB, Ames BN. The free radical theory of aging matures. *Physiol Rev.* 1998; 78:547–581. [PubMed: 9562038]
22. Fukui H, Moraes CT. The mitochondrial impairment, oxidative stress and neurodegeneration connection: reality or just an attractive hypothesis? *Trends Neurosci.* 2008; 31:251–256. [PubMed: 18403030]
23. Blagosklonny MV. Aging: ROS or TOR. *Cell Cycle.* 2008; 7:3344–3354. [PubMed: 18971624]
24. Buffenstein R, et al. The oxidative stress theory of aging: embattled or invincible? Insights from non-traditional model organisms. *Age (Dordr).* 2008; 30:99–109. [PubMed: 19424860]
25. Van Raamsdonk JM, Hekimi S. Reactive Oxygen Species and Aging in *Caenorhabditis elegans*: Causal or Casual Relationship? *Antioxid Redox Signal.* 2010
26. Doonan R, et al. Against the oxidative damage theory of aging: superoxide dismutases protect against oxidative stress but have little or no effect on life span in *Caenorhabditis elegans*. *Genes Dev.* 2008; 22:3236–3241. [PubMed: 19056880]
27. Van Raamsdonk JM, Hekimi S. Deletion of the mitochondrial superoxide dismutase *sod-2* extends lifespan in *Caenorhabditis elegans*. *PLoS Genet.* 2009; 5:e1000361. [PubMed: 19197346]
28. Van Remmen H, et al. Life-long reduction in MnSOD activity results in increased DNA damage and higher incidence of cancer but does not accelerate aging. *Physiol Genomics.* 2003; 16:29–37. [PubMed: 14679299]
29. Zhang Y, et al. 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.* 2009; 64:1212–1220. [PubMed: 19776219]

30. Larsson NG. Somatic mitochondrial DNA mutations in mammalian aging. *Annu Rev Biochem.* 2010; 79:683–706. [PubMed: 20350166]
31. Trifunovic A, et al. Somatic mtDNA mutations cause aging phenotypes without affecting reactive oxygen species production. *Proc Natl Acad Sci U S A.* 2005; 102:17993–17998. [PubMed: 16332961]
32. Lim KS, et al. Oxidative damage in mitochondrial DNA is not extensive. *Ann N Y Acad Sci.* 2005; 1042:210–220. [PubMed: 15965065]
33. Lapointe J, Hekimi S. When a theory of aging ages badly. *Cell Mol Life Sci.* 2010; 67:1–8. [PubMed: 19730800]
34. Groeger G, et al. Hydrogen peroxide as a cell-survival signaling molecule. *Antioxid Redox Signal.* 2009; 11:2655–2671. [PubMed: 19558209]
35. Sundaresan M, et al. Requirement for generation of H₂O₂ for platelet-derived growth factor signal transduction. *Science.* 1995; 270:296–299. [PubMed: 7569979]
36. Woo CH, et al. Tumor necrosis factor- α generates reactive oxygen species via a cytosolic phospholipase A₂-linked cascade. *J Biol Chem.* 2000; 275:32357–32362. [PubMed: 10934206]
37. Theopold U. Developmental biology: A bad boy comes good. *Nature.* 2009; 461:486–487. [PubMed: 19779443]
38. Droge W. Free radicals in the physiological control of cell function. *Physiol Rev.* 2002; 82:47–95. [PubMed: 11773609]
39. Hamanaka RB, Chandel NS. Mitochondrial reactive oxygen species regulate cellular signaling and dictate biological outcomes. *Trends Biochem Sci.* 2010; 35:505–513. [PubMed: 20430626]
40. Trachootham D, et al. Redox regulation of cell survival. *Antioxid Redox Signal.* 2008; 10:1343–1374. [PubMed: 18522489]
41. Feng J, et al. Mitochondrial electron transport is a key determinant of life span in *Caenorhabditis elegans*. *Dev Cell.* 2001; 1:633–644. [PubMed: 11709184]
42. Yang W, Hekimi S. Two modes of mitochondrial dysfunction lead independently to lifespan extension in *Caenorhabditis elegans*. *Aging Cell.* 2010; 9:433–447. [PubMed: 20346072]
43. Yang, WaHS. A Mitochondrial Superoxide Signal Triggers Increased Longevity in *Caenorhabditis elegans*. *PLoS Biol.* 2010 In press.
44. Lee SJ, et al. Inhibition of respiration extends *C. elegans* life span via reactive oxygen species that increase HIF-1 activity. *Curr Biol.* 2010; 20:2131–2136. [PubMed: 21093262]
45. Mesquita A, et al. Caloric restriction or catalase inactivation extends yeast chronological lifespan by inducing H₂O₂ and superoxide dismutase activity. *Proc Natl Acad Sci U S A.* 2010; 107:15123–15128. [PubMed: 20696905]
46. Schulz TJ, et al. Glucose restriction extends *Caenorhabditis elegans* life span by inducing mitochondrial respiration and increasing oxidative stress. *Cell Metab.* 2007; 6:280–293. [PubMed: 17908557]
47. Levavasseur F, et al. Ubiquinone is necessary for mouse embryonic development but is not essential for mitochondrial respiration. *J Biol Chem.* 2001; 276:46160–46164. [PubMed: 11585841]
48. Lapointe J, Hekimi S. Early mitochondrial dysfunction in long-lived *Mcl1*^{+/-} mice. *J Biol Chem.* 2008; 283:26217–26227. [PubMed: 18635541]
49. Budinger GR, et al. Epithelial Cell Death is an Important Contributor to Oxidant-Mediated Acute Lung Injury. *Am J Respir Crit Care Med.* 2010
50. Liu X, et al. Evolutionary conservation of the *clk-1*-dependent mechanism of longevity: loss of *mcl1* increases cellular fitness and lifespan in mice. *Genes Dev.* 2005; 19:2424–2434. [PubMed: 16195414]
51. Lapointe J, et al. Reversal of the mitochondrial phenotype and slow development of oxidative biomarkers of aging in long-lived *Mcl1*^{+/-} mice. *J Biol Chem.* 2009; 284:20364–20374. [PubMed: 19478076]
52. Wang D, et al. Elevated mitochondrial reactive oxygen species generation affects the immune response via hypoxia-inducible factor-1 α in long-lived *Mcl1*^{+/-} mouse mutants. *J Immunol.* 2010; 184:582–590. [PubMed: 20007531]

53. Dwivedi S, et al. Role of 4-hydroxynonenal and its metabolites in signaling. *Redox Rep.* 2007; 12:4–10. [PubMed: 17263900]
54. Singh SP, et al. Disruption of the mGsta4 gene increases life span of C57BL mice. *J Gerontol A Biol Sci Med Sci.* 2010; 65:14–23. [PubMed: 19880816]
55. Labinskyy N, et al. Comparison of endothelial function, O₂·⁻ and H₂O₂ production, and vascular oxidative stress resistance between the longest-living rodent, the naked mole rat, and mice. *Am J Physiol Heart Circ Physiol.* 2006; 291:H2698–2704. [PubMed: 17090784]
56. Andziak B, et al. High oxidative damage levels in the longest-living rodent, the naked mole-rat. *Aging Cell.* 2006; 5:463–471. [PubMed: 17054663]
57. Cadenas E, Davies KJ. Mitochondrial free radical generation, oxidative stress, and aging. *Free Radic Biol Med.* 2000; 29:222–230. [PubMed: 11035250]
58. Yang W, et al. A Measurable increase in oxidative damage due to reduction in superoxide detoxification fails to shorten the life span of long-lived mitochondrial mutants of *Caenorhabditis elegans*. *Genetics.* 2007; 177:2063–2074. [PubMed: 18073424]
59. Gutteridge JM, Halliwell B. Antioxidants: Molecules, medicines, and myths. *Biochem Biophys Res Commun.* 2010; 393:561–564. [PubMed: 20171167]
60. Ristow M, Zarse K. How increased oxidative stress promotes longevity and metabolic health: The concept of mitochondrial hormesis (mitohormesis). *Exp Gerontol.* 2010; 45:410–418. [PubMed: 20350594]

Figure 1. Sources and targets of reactive oxygen species (ROS)

A variety of exogenous and endogenous factors can stimulate ROS production from the mitochondria and other compartments. Cellular ROS levels are controlled by a complex network of antioxidant activities. The reaction of ROS with macromolecules can produce two diametrically opposed outcomes: ROS can act as modulators in several signaling pathways implicated in stress-responses and other functions, but ROS can also inflict damage to lipids, nucleic acids, and proteins.

Figure 2. High ROS phenotypes that affect aging and lifespan positively

The *C. elegans* *nuo-6* mutants produce more mitochondrial superoxide, yet display an increased lifespan that, furthermore, can be suppressed by the antioxidant NAC[43]. *Mclk1*^{+/-} mice sustain high mitochondrial oxidative stress but live longer than their wild type siblings [48, 50]. High levels of oxidative damage have been observed in several tissues of very long-lived naked mole rats (NMRs) [56].

Figure 3. The gradual ROS response hypothesis

Cellular constituents sustain a variety of age-dependent damage that trigger ROS-dependent, protective, stress-response pathways. The ROS generation that is triggered by these mechanisms is well handled by the cellular detoxification systems and is therefore not deleterious (left side of the figure). These protective mechanisms appear unable to fully prevent the gradual accumulation of age-related damage. Thus the gradual increase in damage induces an ever greater stimulation of ROS production as the cell attempts to enhance its stress response. As aging progresses, ROS generation partially escapes control by the antioxidant systems and ROS toxicity starts to participate in causing the very damage that the ROS-dependent stress pathways are meant to neutralize (right side of the figure). This could trigger a toxic runaway process that might form the basis of the involvement of ROS in age-dependent diseases, which tend to develop only in the second half of lifespan.