The biochemistry and cell biology of aging: metabolic regulation through mitochondrial signaling

Yun Chau Long, Theresa May Chin Tan, Inoue Takao, and Bor Luen Tang

Department of Biochemistry, Yong Loo Lin School of Medicine, National University Health System, Singapore; and NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, Singapore

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Abstract

Long YC, Tan TM, Takao I, Tang BL. The biochemistry and cell biology of aging: metabolic regulation through mitochondrial signaling. Am J Physiol Endocrinol Metab 306: E581–E591, 2014. First published January 22, 2014; doi:10.1152/ajpendo.00665.2013.—Cellular and organ metabolism affects organismal lifespan. Aging is characterized by increased risks for metabolic disorders, with age-associated degenerative diseases exhibiting varying degrees of mitochondrial dysfunction. The traditional view of the role of mitochondria generated reactive oxygen species (ROS) in cellular aging, assumed to be causative and simply detrimental for a long time now, is in need of reassessment. While there is little doubt that high levels of ROS are detrimental, mounting evidence points toward a lifespan extension effect exerted by mild to moderate ROS elevation. Dietary caloric restriction, inhibition of insulin-like growth factor-I signaling, and inhibition of the nutrient-sensing mechanistic target of rapamycin are robust longevity-promoting interventions. All of these appear to elicit mitochondrial retrograde signaling processes (defined as signaling from the mitochondria to the rest of the cell, for example, the mitochondrial unfolded protein response, or UPRmit). The effects of mitochondrial retrograde signaling may even spread to other cells/tissues in a noncell autonomous manner by yet unidentified signaling mediators. Multiple recent publications support the notion that an evolutionarily conserved, mitochondria-initiated signaling is central to the genetic and epigenetic regulation of cellular aging and organismal lifespan.

Mitochondria and aging; mitochondria; reactive oxygen species; mitochondria retrograde signaling; aging; mitokine

...THE SUM OF THE DELETERIOUS free radical reactions going on continuously throughout the cells and tissues constitutes the aging process or is a major contributor to it” was Denham Harman’s take on how aging occurs (65), which aptly summarizes the classical “Free Radical Theory of Aging” he proposed (64). The mitochondrial respiratory chain (or the electron transport chain, ETC) is the main cellular source of reactive oxygen species (ROS) such as superoxide, which could be converted to H2O2 by superoxide dismutases (SODs). The latter generates highly damaging hydroxyl radicals via the Fenton reaction (60) in the presence of transition metals like Fe. Among the cumulative deleterious effects of ROS are mutations to somatic mitochondrial DNA (mtDNA), which impair mitochondrial function over time, and form the basis of the modified theory of “Mitochondria Theory of Aging” (105). The central tenet of the theories has some experimental support. For example, overexpression of SOD and catalase extends Drosophila lifespan (130). Deletion of the highly expressed peroxisomal catalase gene ctl-2 in Caenorhabditis elegans accelerated an aging phenotype (140). Key experimental support for cumulative mtDNA damage as a cause of aging is provided by homozygous knockin mice that express a proofreading-deficient version of PolgA, the catalytic subunit of mtDNA polymerase. The mice had increased somatic mtDNA mutations, reduced lifespan, and premature onset of aging-related phenotypes (177). In line with the above results, the degree of oxidative damage to mtDNA in several mammalian species was also found to be inversely correlated with their maximum lifespan (5).

Although logical and immensely popular probably because of the perceived ease of intervention with antioxidants, more recent studies have, however, questioned these simplistic views of a direct relationship between ROS, oxidative damage, and aging (17, 29, 139, 169). Dissenting data come in several forms, and from studies in multiple species. First, overexpression or deletion of antioxidant genes did not always yield the expected results. Several prominent examples are cited below. Transgenic overexpression of Cu/ZnSOD in mice (76) and D. melanogaster (38) did not increase lifespan. On the other hand, worms that completely lack any SOD activity have normal lifespan (180). Homozygous knockout mice for the cytoplasmic Cu/ZnSOD do accumulate massive oxidative damage and do have reduced lifespan, which could be due to increased sarcopenia (124) and cancer incidence in late life (40). However, if extensive genetic manipulation experiments in multiple model organisms are considered in total, it is clear that a strict...
correlation between the level of antioxidant gene expression and lifespan does not exist, and that the level of oxidative damage is not inversely correlated with lifespan.

Naturally, given these unexpected results, the perception of ROS being an unequivocally deleterious agent has also changed gradually (150, 181). ROS themselves act as signals (2, 46). While some of these signaling pathways promote cell death (85), others such as oxygen sensing and hypoxic response (18, 24) or induction of autophagy (158) promote cell survival. Therefore, in theory, low (nontoxic) levels of ROS can promote extension of lifespan by activating pathways that promote cellular resistance to various stresses.

Indeed, experiments in model organisms support this idea. While high levels of orally administered ROS generators caused premature death in worms, low levels increased lifespan through a pathway that involves DAF-16, a forkhead box O (FoxO) class of transcription factor, and the class III, NAD⁺-dependent deacetylase silent information regulator SIR2.1 (68). Generation of superoxide is elevated in C. elegans mitochondrial ETC mutants with increased lifespan. This elevation is apparently necessary and sufficient to increase longevity, since it is abolished by the antioxidants N-acetylcysteine and vitamin C and is also observed by treatment with low concentrations of the oxidative stressor paraquat (187). RNA interference (RNAi)-mediated silencing of ETC components could also promote longevity by activating hypoxia-inducible factor-1 (102). In yeast, it was also recently demonstrated that a small increase in the level of mitochondrial-produced superoxide protects against H₂O₂-induced stress. Overexpression of SOD increased sensitivity to H₂O₂, and this sensitivity was partially reversed by low concentrations of superoxide-generating menadione (175).

Recent work has further revealed that several pathways of lifespan extension, such as caloric restriction (CR) and insulin-like growth factor (IGF) or mechanistic target of rapamycin (mTOR) pathway inhibition, converge on a mitochondrial-like growth factor (IGF) or mechanistic target of rapamycin (mTOR) pathway inhibition, as signaling that involves low-level ROS and mitochondrially dependent signaling. This allows low energy metabolism to attenuate aging-associated disorders in a way that mimics caloric restriction (25, 26, 27, 69, 113, 163). These are associated with an upregulation of mitochondrial- and metabolism-related genes (70, 104). Although whether CR actually enhances mitochondrial biogenesis has been recently challenged (82, 97), there is apparently no impairment in terms of ATP production in CR-treated animals. It follows that an enhanced mitochondrial metabolic rate and efficiency are induced by CR.

A keenly anticipated possibility (and an intensively debated topic) in relation to CR is the possibility that the CR state could be pharmacologically achieved (89). The founding molecule of CR mimetic is the grape polyphenol resveratrol (26), which was first shown to prolong the replicative lifespan of budding yeast by activating Sir2 (75). The Sir2’s protein deacetylase activity on multiple substrates has been strongly implicated in cellular stress and survival responses, as well as in curbing aging and aging-associated disorders (37, 59, 173). Resveratrol administration has been shown by some authors to prolong lifespan of several invertebrate and vertebrate models (56, 148, 179, 185). Other reports, however, have argued against resveratrol having any significant lifespan extension effect (6, 83). Resveratrol’s direct activating effect on sirtuins has also been questioned (132), but recent results suggest that it could act as an allosteric activator of sirtuins in vivo (77). In mice, some groups have found that resveratrol reverses aging-related disorders by improving mitochondrial function (7, 94, 137) through activation of the mammalian Sir2 ortholog sirtuin 1 (Sirt1) (144). In these mice, average lifespan was not extended, but the vascular and metabolic disorders associated with aged mice were significantly reversed. Sirt1 activation appears to enhance the activity of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α, a key regulator of mitochondrial biogenesis (20, 94). However, others have also found that resveratrol had no effect on mitochondrial biogenesis in muscles, and deacetylation of PGC-1α by Sirt1 was shown to decrease, rather than increase, its transcriptional activity (71). Several other synthetic Sirt1 activators have also been shown to attenuate aging-associated disorders in a way that mimics CR (44, 121). Overall, although the actual lifespan extension effects of CR and CR mimetic for long-lived higher organisms has remained questionable, the entire body of evidence generally supports the notion that CR benefits health at the late stages of life.

Although the effect of CR on lifespan appears to be conserved in model organisms, the underlying biological basis of CR-induced lifespan extension has remained unclear. In particular, it is not obvious how decreased nutrient uptake translates into mechanisms of cellular protection and survival that lead to a longer lifespan. There are, however, tantalizing links between CR and two other methods of lifespan extension, as outlined below.

Impairment of the IGF-I signaling pathway is another well-known mode of lifespan extension, and this has been robustly demonstrated in C. elegans, Drosophila, and mice (72, 91, 92, 171, 174). The pituitary growth hormone (GH) regulates IGF-I release from the liver in mammals, and GH receptor mutations resulted in dwarf mice that have extended lifespan (107). IGF-I signaling through the IGF-I receptor and insulin receptor sub-
strate (IRS) activates the prosurvival phosphatidylinositol 3-kinase/Akt kinase axis, a key signaling pathway for cellular protection and survival (182). The fact that its attenuation is lifespan prolonging therefore constitutes a paradox that has not yet been convincingly resolved (172). This lifespan extension effect has been linked (116) to that induced by CR (15, 28, 166), with at least a partial overlap of the underlying mechanism. Interestingly, dwarf mice have increased mitochondrial respiration (184), and adipose tissue-specific insulin receptor knockout mice have increased mitochondrial gene expression and increased oxidative metabolism (88). A recent report showed that the C. elegans IGF-I receptor ortholog daf-2 mutants have enhanced mitochondrial metabolism and stress resistance. However, daf-2 mutant worms and mouse embryonic fibroblasts homozygous null for IRS-1 or heterozygous for insulin receptor have overall lower levels, rather than the expected higher levels of ROS compared with wild type. The authors showed that RNAi-mediated knockdown of daf-2 in adult C. elegans transiently increases ROS levels and that this transient ROS signal causes an adaptive response by inducing SOD and catalase, culminating in ultimately reduced ROS levels despite increased mitochondrial activity. Antioxidants attenuated the increased lifespan of the daf-2 mutant by up to 60% (190).

The serine threonine kinase mTOR is the central component of complexes that mediates nutrient sensing in the metazoan (191). Under nutrient-rich conditions, mTOR phosphorylates substrates such as the ribosomal S6 kinase and eukaryotic initiation factor 4E binding protein, thus promoting translation, growth, and proliferation. It is also a key modulator of aging disorders and lifespan (81). Rapamycin, or other methods of inhibiting mTOR activity, extended lifespan in yeast (84, 142), C. elegans (153), Drosophila (9, 87), and mice (66). Interfering with the downstream targets of mTOR such as inhibition of translation (62, 135) and deletion of S6 kinase/Sch9 (98, 165) could also extend lifespan. The beneficial effect of a diminished global translation on longevity is not simply a relief of protein synthesis-induced metabolic burden. Protected translation of distinctive genes may be sustained or induced in response to decreased overall protein translation (86). For example, the protein synthesis of GCN4 (which promotes the expression of enzymes involved in amino acid biosynthesis) is increased despite a global reduction in protein translation during starvation (133). The critical role of such distinct translational reprogramming is supported by the finding that deletion of GCN4 (gcn4) in yeast reduced the lifespan extension mediated by deletion of mTOR (TOR1) or 60S ribosomal subunits (170). Again, the mechanism underlying mTOR inhibition’s extension of lifespan is not yet clearly defined. It is conceivable that attenuation of mTOR signaling mimics a nutrient-deficient CR state, and mTOR inhibition may overlap or even underlie the lifespan extension effects of CR (11). mTOR inhibition also induces autophagy, a cellular response that clears damaged cellular components and is antiaging in its very nature (48).

Like the inhibition of IGF-I signaling discussed above, mTOR inhibition may affect mitochondrial metabolism. mTOR’s connection with mitochondria and mitochondrial metabolism is well established (33, 159) and stems from its physical localization to the organelle and its interaction with mitochondrial proteins (146). The lifespan extension effect of mTOR pathway inhibition has been associated with increased mitochondrial respiration and gene expression (14, 98). A recent report provided evidence that reduction of TOR signaling in growing yeast increases overall mitochondrial ETC activity and ROS production. While overexpression of mitochondrial MnSOD significantly attenuated chronological lifespan extension observed in tor1Δ strains, treatment with rapamycin or mitochondrial ROS-generating menadione could extend chronological lifespan in wild-type strains. The authors proposed that reduced TOR signaling constitutes an adaptive signal that prolongs lifespan (136).

CR, inhibition of IGF-I, and mTOR signaling are effective interventions to delay aging in a wide range of species, and they seem to induce a similar cellular metabolic milieu. CR induces an energy-limiting metabolic condition, and suppression of IGF-I or mTOR signaling also generates conditions that are somewhat similar to the dietary restriction state. There may be common components, or points of convergence, in the lifespan extension mechanism of these three pathways. The AMP-activated protein kinase (AMPK) is a master sensor of cellular energy that is activated in response to energy limitation (63) and has been implicated in mediating protection against aging. Overexpression of the wild-type or an activated form of AMPK is sufficient to slow aging in C. elegans (1, 53). The dietary restriction effect on lifespan extension in C. elegans is suppressed when one of the catalytic subunits of AMPK is deleted, and the authors find that AMPK mediates the antiaging effects via phosphorylation of FOXO/DAF-16 (53). In a separate study, genetic inactivation of mTORC1 in C. elegans induced a protective gene expression program that confers stress resistance and longevity via FOXO/DAF-16 and Nrf/SKN-1 (153). These results suggested that the distinctive catabolic AMPK and anabolic mTOR networks might inversely regulate FOXO/DAF-16 (which is also downstream of IGF-I signaling) in the modulation of lifespan extension. The antagonistic regulation of C. elegans CREB-regulated transcriptional coactivators (CRT-C1) on longevity transcriptional program by AMPK and calcineurin further supports the yin-and-yang relationship between the catabolic and anabolic network in lifespan extension. Knockdown of CRT-C1 extends lifespan in C. elegans, and AMPK directly phosphorylates and inactivates the transcriptional coactivator by inducing its sequestration in the cytoplasm (115). Conversely, calcineurin, which is presumably activated during nutrient abundance (19), dephosphorylates CRT-C1 at the conserved AMPK/calcineurin sites and induces its translocation to the nucleus (115). Expression of a constitutively nuclear CRT-C1 mutant rendered the worms to become refractory to lifespan extension that is mediated by genetic knockdown of calcineurin (tau-6) or activation of AMPK (aak-2) (115). The reciprocal relationship between AMPK and insulin signaling pathways has been demonstrated in mammalian tissues (35, 112); it remains to be demonstrated whether the balance between the opposite metabolic network regulates longevity in higher organisms.

Mitochondrial Hormesis, Mitonuclear Protein Imbalance, and Metabolic/Stress Control of Lifespan

There are multiple overlaps and points of connection between the signaling events induced by CR, IGF-I inhibition, and mTOR inhibition. Interestingly, as revealed by recent
findings, all of these appeared to elicit a heightened mitochondrial respiration that produces a mild to moderate elevation of ROS. Contrary to what might be expected from the free radical theory of aging, this elevation in ROS may underlie lifespan extension (136, 190). How could this be reconciled with current knowledge?

Before attempting to answer the above question, it is worth noting that manipulations having the opposite effect, namely a mild to moderate reduction in mitochondrial respiration, can also extend lifespan, although not necessarily through reduction of ROS (79). This has been demonstrated by ETC mutants of C. elegans (45, 95, 103, 161, 187, 188), Drosophila (31), and mice (110). In particular, the lifespan extension effect of clock 1 (clk-1)/coq-7 mutation, which encodes a mitochondrial hydroxylase required for the synthesis of coenzyme Q, is conserved from C. elegans to mice (110). clk-1 mutants are defective in mitochondrial respiration due to impaired electron transfer between complex I and complex III. This defect elevated the generation of ROS (127). The ROS thus generated apparently also did not shorten lifespan, but instead extended it. Intriguingly, therefore, ROS generation and lifespan extension can arise from dietary, pharmacological, or genetic manipulations that either increase or decrease mitochondrial respiration. Viewed generally, these are forms of metabolic and energetics stresses that might elicit cellular adaptive responses.

The conceptual theory put forth to explain how metabolic stress may in fact promote lifespan extension is referred to as mitochondrial hormesis, or “mitohormesis” (151). In brief, metabolically induced low-level mitochondrial stress elicits cellular responses that increase the stress tolerance of the cell. These changes collectively suppress acute and prolonged ROS production and facilitate cellular survival. For multicellular organisms, lifespan extension presumably occurs when the benefits of cellular mitohormesis translates to survival at the organismal level. A number of “retrograde signaling” pathways are now known that sense and transmit mitochondrial signals to effect changes in nuclear gene expression. One example of such signaling occurs as a consequence of inhibition of mitochondria respiration and the disruption of mitochondrial membrane potential ($\Delta\Psi_m$) (57, 80, 111). Originally characterized in yeast, a drop in $\Delta\Psi_m$ initiates retrograde signaling through a set of retrograde (Rtg) regulation factors, which culminates in nuclear translocation of the heterodimer of two basic helix-loop-helix transcription factors, Rtg1 and Rtg3, which influences the expression of retrograde response target genes (80). A recent study using mammalian cybrid cells with mtDNA mutations identified 72 transcription factors that were potentially involved in mitochondrial retrograde signaling, including pathways not known to act in yeast (22). Activation of mitochondrial retrograde signaling extends the lifespan of yeast (93) and C. elegans (32), as well as human fibroblasts in culture (106). One form of ROS-mediated mitochondrial retrograde signaling is its activation of nuclear factor-$\kappa$B (NF-$\kappa$B) (51, 122), which heightens stress response and is survival promoting in many cell types (114). Mitochondrial ROS have also been shown to act through the mitogen-activated protein kinase p38 pertaining to hypoxia signaling in mammalian cells (41) and lifespan extension in C. elegans (161).

Stress and damage at the mitochondria also trigger the mitochondria-specific unfolded protein response (UPR$^{mt}$) (67, 138), a prominent mitochondria-nuclear signaling pathway that induces the expression of mitochondria protective molecular chaperones and other regulators of mitochondria homeostasis. The UPR$^{mt}$ is a potent transducer of ETC perturbation-based enhancement in lifespan (39). A recent report has shed light on how the UPR$^{mt}$ is initiated. Activating transcription factor associated with stress (ATF)-1 is a sensor of mitochondrial stress and has both a nuclear localization signal and a mitochondrial targeting sequence. During mitochondrial stress, its mitochondrial import efficiency was reduced, allowing ATF-1 to accumulate in the cytosol and translocate to the nucleus to enhance transcription of UPR$^{mt}$ genes (128).

Very recently, Houtkooper and colleagues have elaborated on the concept of “mitonuclear protein imbalance,” a stoichiometric imbalance between the expression of nuclear- and mitochondria-encoded proteins, as a conserved lifespan extension mechanism (74). Such an imbalance can be experimentally induced by knocking down mitochondrial ribosomal proteins. Mitonuclear protein imbalance, and consequentially UPR$^{mt}$, is also generated by pharmacological perturbation of mTOR, as well as CR mimetics (74). Induction of mitonuclear protein imbalance appears to also underlie the lifespan-promoting effect of preserved NAD$^+$ levels and sirtuin activity in aged animals (123). Using a Drosophila model, Owusu-Ansah and colleagues showed that mild muscle mitochondrial distress caused by transgenic RNAi against complex I components preserved muscle function and extended lifespan (131). These muscle-restricted phenotypes apparently involved redox-dependent induction of genes that regulate UPR$^{mt}$, as well as increased expression of the Drosophila gene Impl2. The latter encodes an insulin-like growth factor-binding protein, which repressed insulin signaling and enhanced mitophagy. This is an important illustration that mitohormesis occurring in a major organ of energy expenditure could prolong lifespan.

It should also be emphasized that UPR$^{mt}$ is by no means a standalone pathway as far as aging is concerned, and it is not surprising that its connection with the classical endoplasmic reticulum (ER) stress-induced UPR (ER UPR) has been recognized, particularly in chronic inflammatory diseases (149). The ER and mitochondria are physically and functionally linked through the ER-mitochondrial contact sites (155), which regulate the key cellular process of cell death (49) and autophagy (61). In yeast, it was recently reported that the ER may be a major source of ROS in the event of mitochondrial dysfunction (99). Chronic low-grade inflammation is characteristic of aging organs such as the brain (141) and underlies many aging-associated disorders in humans (21). The process of autophagy has been deeply connected with cellular and organismal aging (156). In C. elegans, it has been shown that signaling from IGF-I and mTOR pathways converges on the regulation of autophagy, and this appears to be a key component of lifespan regulation (176).

Interestingly, recent findings provided clear evidence that signaling from mitochondria could modulate aging in a noncell autonomous manner. In investigating the lifespan extension effect of RNAi-mediated knockdown of nuclear-encoded cytochrome c oxidase-1 subunit Vb/COX4 (cco-I) in C. elegans, Durieux et al. (39) found that knocking down cco-I in specific tissues like intestine and neurons significantly increased lifespan via upregulation of UPR$^{mt}$, but this is not so for cco-I knockdown in body-wall muscles. Intriguingly, mitochondrial perturbation in neurons resulted in upregulation of UPR$^{mt}$ in...
the intestine. It thus appears that mitochondrial respiratory stress in one tissue could activate the mitochondrial stress response pathway in not only surrounding cells within the same tissue but also those of a distal tissue. Indeed, mitochondrial ROS signaling through SKN-1/NRF2 from redox-sensitive neurons from the C. elegans head was shown to be sufficient for lifespan extension (161). The nature of this noncell autonomous signal, which Durieux et al. (39) termed a “mitokine,” is yet unclear. One possible candidate for a mitokine would be the ROS generated as a result of either mitochondrial respiratory impairment by genetic means or by CR and mTOR inhibition.

If ROS is one form of mitochondrial retrograde signaling that contributes to extension of lifespan, what is the nature of the species that is responsible for ROS-based signaling? The superoxide anion $\text{O}_2^-$ is extremely reactive and has a very short half-life. It is readily dismutated into $\text{H}_2\text{O}_2$ by both mitochondrial and cytoplasmic SODs and reacts with Fe-S clusters within mitochondrial proteins. The hydroxyl radical is even more short-lived and reactive. On the other hand, $\text{H}_2\text{O}_2$ is biochemically less toxic and has a longer half-life, and being uncharged allows it to passively diffuse across membranes, or through facilitated diffusion mediated by aquaporin channels (8, 120). Other than SOD-based reactions, $\text{H}_2\text{O}_2$ may also be generated by electron transfer processes involving molecules like the longevity gene product and signaling adaptor p66Shc (119). In response to oxidative stress, a fraction of the cytoplasmic p66Shc translocates to mitochondria, where it binds cytochrome c and acts as an oxidoreductase, transferring electrons from cytochrome c to molecular oxygen (50). Interestingly, $\text{H}_2\text{O}_2$ is also formed in the ER as a by-product of oxidative protein folding (178), or through the activity of ER NADPH oxidase-4 (186). Should ROS act as a signal, the exact oxidative chemical species of ROS involved may well be $\text{H}_2\text{O}_2$. $\text{H}_2\text{O}_2$ generated from 1-methylnicotinamide, a metabolite of nicotinamide generated by the activity of sirtuins on the mitochondrial genome-encoded, are therefore potential epigenetic factors up until the third generation (54).

Whereas the replicative lifespan of the budding yeast Schizosaccharomyces cerevisiae is defined as the total number of daughter cells generated by a mother cell, the chronological lifespan is the lifespan of a population of nondividing yeast (42). Pan and colleagues have shown that yeast chronological lifespan extension by TOR inhibition occurs via adaptive mitochondrial ROS signaling (136). The same group has recently demonstrated a direct link between mitochondrial ROS and epigenetic changes that would promote chronological lifespan extension in yeast. The authors showed that hormetic mitochondrial ROS inactivates the histone demethylase Rph1p specifically at subtelomeric heterochromatin and repression of subtelomeric transcription (162). This regulation occurs through the yeast DNA damage response proteins Tel1p and Rad53p, which are homologs of the mammalian DNA damage response kinases ataxia-telangiectasia mutated (ATM) and checkpoint kinase (Chk) 2. ROS could activate ATM/Tel1, which has unique roles in telomeric maintenance, by direct oxidation (58). Activated Tel1 phosphorylates Chk2/Rad53, which in turn phosphorylates Rph1p, thus inactivating the latter. Of note, the transcription effect of ROS is distinct from that induced by DNA-damaging agents. Tel1 activation by ROS occurs in the absence of nuclear DNA damage, and analysis of phosphoprotein profiles revealed that ROS induced phosphorylation of only a specific subset of phosphorylation target sites compared with treatment by a DNA-damaging agent. The authors also showed that ROS enhanced the association of the chromatin-silencing complex Sir3p with subtelomeric regions and that Sir3p was required for subtelomeric silencing following mitochondrial ROS signaling. These findings demonstrate that Rph1p-regulated subtelomeric silencing by Sir3p plays a key role in lifespan-extending hermetic ROS signaling.

An emerging aspect of aging epigenetics is the involvement of micro-RNAs (miRNA) (36, 129). Following the initial study showing the significant influence of the miRNA lin-4 on the lifespan of C. elegans (13), other subsequent works have also provided evidence for roles of miRNAs in modulating conserved longevity pathways, including that of the insulin/IGF-I signaling and mTOR (55). In addition, there has also been extensive investigation on the roles of miRNAs in the regulation of Sir1 (101). The expression of certain mitochondrial genes is regulated by miRNAs, and some of these regulations are involved in metabolism and the aging process. For example, miR-210 regulates mitochondrial metabolism during hypoxia by repressing FE-S cluster assembly proteins (23). Mitochondrial SOD2 and thioredoxin reductase 2 are potential targets of miR-335 and miR-34a, which promote renal senescence (3). Another study showed that decreased miR-205 increased cell susceptibility to oxidative and ER stresses, which was associated with ROS induction and suppression of antioxidant enzymes (126). The mitochondrial localized miRNAs, or MitomiRs (4), which may be nuclear-encoded or mitochondrial genome-encoded, are therefore potential epigenetic regulators of ROS production and aging.
Epilogue

The preceding paragraphs provided an update of recent findings with regard to the role of mitohormesis and hormetic ROS production in modulation of aging and lifespan. It is now clear from multiple lifespan models that, while acute high levels of ROS production are damaging, low to moderate levels of mitochondrial respiratory/metabolic stress and ROS production generated by genetic and pharmacological manipulations could serve to provoke a signaling response from the mitochondria to the nucleus. This retrograde signaling promotes adaptive responses that would enhance cellular survival. This signaling process could elicit both gene expression changes via transcriptional and epigenetic mechanisms. Furthermore, mitohormetic signaling induced in one tissue could be sensed and followed by another tissue. A schematic summary of the above is depicted in Fig. 1. Taken together, it appears that the classic free radical theory of aging needs to be revised to incorporate the notion that an evolutionarily conserved mitochondria-based signaling process is central to regulation of cellular aging and organismal lifespan.

To better understand the mitochondrial signaling process such that this could be exploited for antiaging therapies, many unanswered questions require urgent resolution. How much (or how moderate) ROS is necessary and what is the threshold of ROS levels that would be beneficial rather than detrimental? Oral antioxidants have generally failed to provide any significant benefits on lifespan, and high-dosage antioxidant supplements have been associated with an increase in mortality in a meta-analysis of randomized trials (10). This paradox becomes less surprising when viewed in light of experiments in which lifespan extension effects of mitochondrial ROS are abolished by antioxidants (187). Interestingly, physical exercise has been viewed as a CR mimetic (78, 145) and strongly associated with health and longevity. Physical exercise induces oxidative stress and ROS production in muscles (143), and antioxidant supplements have been shown to reduce exercise-induced oxidative stress in aged muscles (157). However, the health-promoting effect of exercise could apparently also be nullified by antioxidants (52, 152). This again attests to the notion that suppressing systemic ROS generation may have the opposite effect to lifespan extension.

One of the most intriguing emerging notions is that mitochondrial respiratory stress in one tissue could act in a noncell autonomous manner to induce longevity-promoting mitohormesis in other tissues. What is the nature of the mitokine (39) that could signal mitohormesis in a noncell autonomous manner? If this is some form of ROS how could it be pharmacologically introduced? Which are the tissues that are best in initiating this systemic signaling process? Most of the work on mitochondria longevity signaling thus far is done in yeast and C. elegans aging models, but how would the results and their significance translate to vertebrates and humans? Pursuits...
of answers to these questions would likely dominate aging research in this and coming decades.

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