

The Mitochondrial Basis of Aging

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<http://dx.doi.org/10.1016/j.molcel.2016.01.028>

A decline in mitochondrial quality and activity has been associated with normal aging and correlated with the development of a wide range of age-related diseases. Here, we review the evidence that a decline in mitochondria function contributes to aging. In particular, we discuss how mitochondria contribute to specific aspects of the aging process, including cellular senescence, chronic inflammation, and the age-dependent decline in stem cell activity. Signaling pathways regulating the mitochondrial unfolded protein response and mitophagy are also reviewed, with particular emphasis placed on how these pathways might, in turn, regulate longevity. Taken together, these observations suggest that mitochondria influence or regulate a number of key aspects of aging and suggest that strategies directed at improving mitochondrial quality and function might have far-reaching beneficial effects.

Introduction

Scientists have long struggled to explain the evolutionary basis of aging. In particular, how can there be a genetic program of aging, if aging manifests itself long after the reproductive period has passed and, therefore, after all the forces of natural selection have long since abated? Potential explanations have come from Medawar's mutation accumulation hypothesis, Kirkwood's disposable soma theory, and the concept of antagonistic pleiotropy (Hughes and Reynolds, 2005). The latter hypothesis revolves around the notion that genes regulating aging in the old organism actually have a different, antagonistic function when the animal is young (Williams, 1957). In this scenario, those genes positively regulating growth and fertility in the young animal might serve to accelerate senescence and aging in the older animal. While, from an evolutionary viewpoint, this concept has been exclusively applied to our genetic inheritance, the notion of antagonistic pleiotropy actually provides a useful framework to understand the role of mitochondria in aging. Perhaps, no structure is so intimately and simultaneously connected to both the energy of youth and the decline of the old. The revelation of these complex and antagonistic functions of mitochondria has slowly transformed how we view this subcellular organelle. Mitochondria can no longer be viewed as simple bioenergetics factories but rather as platforms for intracellular signaling, regulators of innate immunity, and modulators of stem cell activity. In turn, each of these properties provides clues as to how mitochondria might regulate aging and age-related diseases. Here, we review how mitochondria participate in aging and how these insights may usher in a new era of mitochondrial-targeted therapies to potentially slow or reverse the aging process.

Mitochondrial Function during Aging

It has been long appreciated that aging in model organisms is accompanied by a decline in mitochondrial function and that this decline might, in turn, contribute to the observed age-dependent decline in organ function (Rockstein and Brandt, 1963). Similarly, a decline in mitochondrial function in humans

has also been observed; again, this decrement may pre-dispose humans to certain age-related diseases (Petersen et al., 2003). It is also known that mitochondrial mutations increase in frequency with age in both animal models and in humans (Cortopassi and Arnheim, 1990; Pikó et al., 1988), although the levels and kind of mutations appear to differ between tissues and even within tissues (Soong et al., 1992). While some have speculated that the increased levels of mitochondrial mutations contribute to aging and age-related diseases (Linnane et al., 1989), others have questioned whether these mutations ever reach a significant enough level to contribute to the aging process (Khrapko and Vijg, 2009). Indeed, since mtDNA exists in hundreds to thousands of copies per cell, the detection of mutant mtDNA does not, in itself, imply dysfunction, as it is generally believed that mutational load must exceed a threshold value (perhaps exceeding 60% of all mitochondria within a given tissue) for there to be a significant phenotype (Rossignol et al., 2003). Perhaps the strongest evidence for a potential causative role for mtDNA mutations in mammalian aging comes from analyzing the "mitochondrial mutator mice," which are knockin mice containing a mutated (D257A), proofreading-deficient form of the mtDNA polymerase POLG γ . This nuclear-encoded gene is the sole mtDNA polymerase, and the mutation at amino acid position 257 results in an enzyme that retains normal polymerase function but has impaired proofreading activity. Mice containing one or two copies of this proofreading-deficient POLG accumulate a significant level of mitochondrial mutations, and homozygous knockin mice exhibit an accelerated aging phenotype (Kujoth et al., 2005; Trifunovic et al., 2004). Nonetheless, while this model clearly links mitochondrial mutations to aging, it should be noted that the type and magnitude of mitochondrial mutations do not appear to faithfully replicate what is seen during normal aging (Williams et al., 2010). Thus, while the levels of mitochondrial mutations increase with age, it remains unclear whether this increase plays a fundamental role in the aging process.

Regardless of mtDNA, in humans, the link between mitochondrial function and aging has been, perhaps, best studied by analyzing skeletal muscle. While all studies are not in complete

concordance, the majority of reports have found that aging is generally accompanied by a decline in activity of mitochondrial enzymes (e.g., citrate synthase), a decrease in respiratory capacity per mitochondria (e.g., substrate-dependent oxygen consumption), an increase in reactive oxygen species (ROS) production, and a reduced phosphocreatine (PCr) recovery time (an *in vivo* measurement of mitochondrial respiratory capacity). Nonetheless, the literature is also filled with many counterexamples that may reflect differences in how the specific assays were performed or differences in the muscle fiber type studied (Hepple, 2014). Most studies have also noted that aging is accompanied by an accelerated rate of muscle loss, in terms of both mass and activity (e.g., strength). Although muscle strength over a lifetime declines at an average rate of roughly 1% per year, for patients in their 70s, that rate of decline can increase 2- to 4-fold (Goodpaster et al., 2006). At present, perhaps the best intervention to counteract this age-dependent decline in muscle function, termed sarcopenia, is physical exercise. Indeed, accumulating evidence from epidemiological studies and randomized clinical trials illustrates that regular physical activity and endurance exercise benefits a range of human age-related pathologies, including sarcopenia, as well as the age-dependent decline in cardiac and cognitive functions (Chakravarty et al., 2008; Kosmadakis et al., 2010; Rowe et al., 2014; Stessman et al., 2009; Willis et al., 2012). Interestingly, endurance exercise also conferred phenotypic protection and prevented the premature mortality observed in the mitochondrial mutator mice mentioned earlier (Safdar et al., 2011). The therapeutic effects of endurance exercise are accompanied by a number of physiological adaptations; however, one of the most beneficial effects appears to be stimulation of mitochondrial biogenesis in a wide variety of tissues, including the brain (Arany et al., 2005; Egan and Zierath, 2013; Rowe et al., 2014; Steiner et al., 2011; Wu et al., 2002). Mitochondrial biogenesis is largely coordinated by the transcriptional coactivator peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1 α) (Handschin and Spiegelman, 2008; Ruas et al., 2012). PGC-1 α , in turn, regulates the activity of several transcription factors involved in creating new mitochondria, including nuclear respiratory factors NRF1 and NRF2 and mitochondrial transcription factor A (TFAM) (Austin and St-Pierre, 2012). Increasing PGC-1 α levels in mouse skeletal muscle is sufficient to forestall the development of age-dependent sarcopenia, again emphasizing the potential importance of this pathway for aging biology (Wenz et al., 2009). The development of sarcopenia is not, however, solely an issue of impaired mitochondrial biogenesis (Argilés et al., 2015). Recently, it was also shown that phosphorylation—and, hence, activity of ATP citrate lyase (ACL), a key regulator of acetyl-coenzyme A (CoA) levels—was markedly reduced in sarcopenic muscle (Das et al., 2015). In this study, ACL phosphorylation was stimulated by insulin-like growth factor 1 (IGF-1), a growth factor known to increase muscle mass (Egerman and Glass, 2014) and also known to decline in the serum of aging men and woman (O'Connor et al., 1998). Increasing ACL levels in mice resulted in improved mitochondrial function, suggesting that this might be a complementary approach to combat the deleterious effects of skeletal muscle aging (Das et al., 2015).

Mitochondria as Regulators of Stem Cell Function

While aging is accompanied by a general decline in mitochondrial function in all tissues, the effects of mitochondrial dysfunction might be particularly important within certain specialized cell types. Since a decline in adult stem cell function is thought to contribute to various aspects of aging (López-Otín et al., 2013), the role of mitochondrial dysfunction in stem cell biology has become a subject of increasing interest. In the case of hematopoietic stem cells (HSCs), perhaps the best studied stem cell population, mitochondria are thought to play a relatively minor role in the resting bioenergetics profile of these cells (Suda et al., 2011). Quiescent HSCs are generally thought to, instead, rely on glycolytic metabolism as the major source of their ATP, presumably in keeping with the low oxygen environment of the HSC niche, and as a mechanism to minimize the long-term deleterious effects of mitochondrial ROS production (Suda et al., 2011). Indeed, a number of links suggest that a rise in ROS might be harmful for stem cell function (Ito et al., 2004; Liu et al., 2009; Tothova et al., 2007), although there are also increasing examples in which ROS appear to play a positive and necessary signaling role in stem cell biology (Bigarella et al., 2014).

One clear connection between mitochondria and stem cell function has come from the analysis of the previously described mtDNA mutator mice (Kujoth et al., 2005; Trifunovic et al., 2004). Several reports have analyzed the stem cell function of the POLG knockin mice and found a range of defects. These include the development of a severe and often fatal anemia in the mice, as well as abnormalities in B cells (Chen et al., 2009). A similar impairment was observed in neural stem cell populations derived from POLG knockin mice (Ahlgqvist et al., 2012). Several features of these analyses deserve mentioning. First, the stem cell defects could, at least, be partially ameliorated by the administration of the antioxidant N-acetylcysteine (Ahlgqvist et al., 2012). Indeed, follow-up studies have demonstrated that POLG knockin cells also have markedly impaired capacity to be reprogrammed into pluripotent stem cells, a defect again related to an increase in mitochondrial ROS production (Hämäläinen et al., 2015). The second point to emphasize is that the observed stem cell defects appear to arise because of cell-autonomous mitochondrial defects. This mutator mouse model affects a multitude of cell types, including the stem cell and their progeny, as well as the niche. Nonetheless, transplantation of POLG knockin HSCs into a normal host recapitulates the observed defect (Chen et al., 2009), and other mouse models that have large-scale mitochondrial deletions only within post-mitotic tissues do not exhibit any stem cell defects (Ahlgqvist et al., 2012). Thus, even though stem cells do not seem to rely on oxidative phosphorylation for their energetics, mitochondria are clearly required for the long-term function of these cells and their progenitors in a cell-autonomous capacity. Finally, as mentioned previously, it is important to note that these stem cell defects do not appear to accurately recapitulate aging (Norddahl et al., 2011). Indeed, from a histological viewpoint, the anemia observed in these animals looks less like the anemia of aging and more like the pre-leukemic abnormality known as myelodysplastic syndrome (Chen et al., 2009). It should also be noted, that the level of mitochondrial mutation seen in these models is also dramatically higher than that seen during the

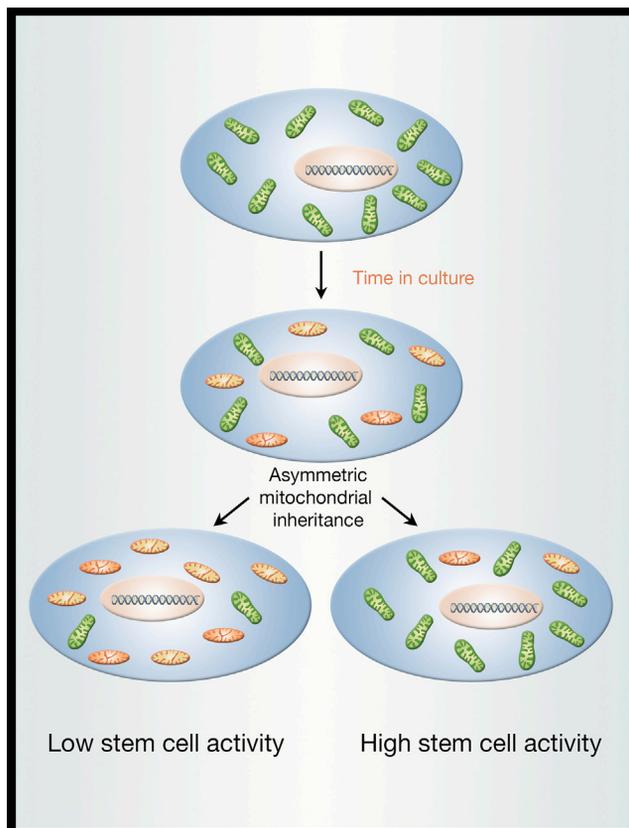


Figure 1. Stem Cells Exhibit Asymmetric Mitochondrial Inheritance

Analysis of stem-like cells within immortalized, transformed epithelial cultures revealed that young mitochondria (shown in green) and old mitochondria (depicted in orange) are not symmetrically distributed after the stem-like cell divides. Moreover, the daughter cell inheriting the younger mitochondria also exhibits higher stem-like activity. The molecular basis for this asymmetric mitochondrial distribution is not clear, nor is it known whether similar mechanisms exist *in vivo*.

normal aging process, which may account for why the observed stem cell defects do not faithfully recapitulate what is seen during normal aging.

Another mechanism by which mitochondria might contribute to stem cell maintenance is through regulation of specific metabolites. Increasingly, there is evidence that metabolic intermediates play an important role in regulating the transcriptional and epigenetic states of cells. It is no presumed accident that chromatin modifications are largely dependent on the same carbon intermediates (e.g., methyl, acetyl, etc.) that are generated during normal mitochondrial metabolism. For example, one recent study demonstrated that, in mouse embryonic stem (ES) cells, the intracellular ratio of α -ketoglutarate (α KG) to succinate was important in maintaining pluripotency (Carey et al., 2015). Both of these metabolites are generated as a result of tricarboxylic acid (TCA) metabolism in the mitochondrial matrix. In turn, it was shown that levels of α KG modulated distinct chromatin modifications. This modulation was mediated, at least in part, by the activity of α KG-dependent demethylases, including Jumonji C (JmjC)-domain-containing enzymes and the ten-eleven translocation (Tet)-dependent DNA demethylases (Kae-

lin, 2011). Another important set of metabolites that connect stem cells to the mitochondria is the NAD^+ / $NADH$ ratio. Levels of NAD^+ appears to decline in tissues as they age (Mouchiroud et al., 2013; Yoshino et al., 2011). Analysis of neural stem cells (NSCs) has shown that reducing NAD^+ levels recapitulates at least some of the phenotypes of stem cell aging, while NAD^+ supplementation can restore function to old NSCs (Stein and Imai, 2014). These effects appear to be mediated, in part, by the sirtuin family of NAD -dependent enzymes. This connection has also been observed in HSC biology. SIRT3 is one of seven mammalian sirtuin family members and is found within the mitochondria, where it regulates the mitochondrial acetylome in an NAD -dependent fashion (Lombard et al., 2007). Interestingly, SIRT3 is highly enriched in HSCs, although its expression declines with age. Augmenting SIRT3 levels in old HSCs results in improved regenerative capacity in these aging stem cells (Brown et al., 2013). Similar results have been recently observed with overexpression of SIRT7 (Mohrin et al., 2015).

The unique properties of stem cells suggest that these cells might have mechanisms to ensure that these critical cells do not accumulate old and dysfunctional mitochondria. Preliminary evidence suggests that, in the brain, areas enriched for NSCs appear to have augmented rates of mitophagy (Sun et al., 2015). Another potential mechanism appears to be a unique capacity of adult stem cells to exclude older mitochondria. Indeed, a recent report studying the stem-like cells within immortalized human mammary epithelial cell cultures noted that there was an uneven distribution of mitochondria after cell division (Katajisto et al., 2015). This asymmetry was not a difference in mitochondrial number between the two daughter cells but, rather, a difference in the segregation of young and old mitochondria (Figure 1). Stem-like cells getting young mitochondria maintained their stem cell properties much more robustly than those cells receiving older mitochondria. This unequal distribution of mitochondria based on the age of the mitochondria was only seen within the stem-like cells in the culture, not the more differentiated mammary epithelial cells. In addition, this property was only seen with mitochondrial segregation and not with other organelles, such as lysosomes or ribosomes, or with cellular components such as chromatin. Currently, it is unclear whether this property is present *in vivo* and, if so, whether it is present in all, or just some, types of stem cells. However, it should be noted that, in yeast, where there is asymmetric division between the mother cell and the bud, there is also evidence of a corresponding asymmetric inheritance of both mitochondria (McFaline-Figueroa et al., 2011) and misfolded proteins (Clay et al., 2014).

Mitochondria and Cellular Senescence

As noted in the discussion of stem cell biology, mitochondria can regulate cellular aging through the modulation of the metabolic profile of the cell. Cellular senescence is accompanied by profound changes in the metabolome, and although different triggers of senescence all have a similar morphological appearance, the metabolic profiles of oncogene-induced senescence and replicative senescence appear distinct (Quijano et al., 2012). There is increasing evidence that these metabolic changes are casually related to the senescent state. For instance, p53 plays an important role in senescence, and evidence suggests that it

can also repress expression of mitochondrial malic enzyme (ME2), which converts the TCA metabolite malate to pyruvate via oxidative decarboxylation (Jiang et al., 2013). Moreover, knockdown of ME2 results in the induction of senescence, while forced expression allows cells to escape from senescence. This argues that the ability of p53 to mediate senescence may partially be through its ability to modulate TCA metabolism. Interestingly, previous observations have established that overexpression of malate dehydrogenase also results in lifespan extension in yeast (Easlon et al., 2008). The link between mitochondrial metabolism and senescence is also observed in oncogene-induced senescence (OIS). Analysis of cells undergoing OIS precipitated by expression of the BRAF oncogene demonstrated an increase in pyruvate oxidation that contributed to the generation of increased mitochondrial ROS and entry into the senescent state (Kaplon et al., 2013). This increase in pyruvate utilization was due to alteration in the phosphorylation state—and, hence, the activity—of the mitochondrial pyruvate dehydrogenase (PDH) complex. Again, gain- and loss-of-function studies suggest that these metabolic changes appear to be necessary for BRAF-induced senescence. Interestingly, the PDH complex is also regulated by the mitochondrial sirtuins, particularly SIRT3 and SIRT4 (Fan et al., 2014; Mathias et al., 2014). Similarly, from an organismal context, a recent large-scale screen of yeast single-gene deletion mutants uncovered a number of enzymes involved in the TCA cycle as potent regulators of lifespan (McCormick et al., 2015). Together, these argue that mitochondrial-induced metabolic changes might be necessary—and, in some cases, sufficient—to trigger cellular senescence and, potentially, to regulate overall longevity.

There is also a strong link between mitochondrial metabolism, ROS generation, and the senescent state. Almost 4 decades ago, it was noted that the lifespan of human cells in culture could be significantly extended by culturing the cells in a low-oxygen environment (Packer and Fuehr, 1977). A similar effect was also observed in mouse cells (Parrinello et al., 2003). Similarly, OIS triggered by Ras expression results in an increase in ROS levels, and OIS can be prevented by growing these cells in a low-oxygen state or supplementing the media with an antioxidant (Lee et al., 1999). Similar relationships have been observed between other regulators of senescence and ROS, including the p53 target and cell-cycle regulator p21, which also appears to regulate senescence in a redox-dependent fashion (Macip et al., 2002; Passos et al., 2010). All of these observations fit well with the long-standing notions of the free-radical theory of aging that postulated a causal role for ROS in the aging process (Harman, 1956). Nonetheless, there are a number of observations that suggest that the cellular effects of ROS, with regard to inducing senescence, do not unequivocally transfer to organismal aging. For instance, while in some animal models, scavenging mitochondrial oxidants appears to extend lifespan (Schriner et al., 2005), in other cases, a consistent relationship between ROS levels and lifespan was seemingly absent (Sanz et al., 2010; Yang et al., 2007). Moreover, in some cases, a rise in ROS appears to actually increase, rather than reduce, overall lifespan (Yang and Hekimi, 2010; Zarse et al., 2012).

The Mitochondrial Unfolded Protein Response and Longevity

Genetic screens in *C. elegans* have found that disruption of mitochondrial function often leads to an increase in overall lifespan (Dillin et al., 2002; Lee et al., 2003). In many ways, these observations seemed counterintuitive, especially given the wealth of data, as previously discussed, suggesting that a decline in mitochondrial function occurs with aging. Nonetheless, there are a growing number of experimental observations that suggest, in a wide range of organisms, that a modest decline or impairment in mitochondrial function leads to lifespan extension (Liu et al., 2005; Owusu-Ansah et al., 2013; Yee et al., 2014). Insight into this seeming paradox, perhaps another example of antagonistic pleiotropy, first came from examining worms that were long lived due to knockdown of a nuclear-encoded cytochrome C oxidase subunit (*cco-1*). Following *cco-1* knockdown, the impairment of electron transport in these animals appeared to trigger activation of the mitochondrial unfolded protein response (UPR^{mt}) (Durieux et al., 2011). The UPR^{mt} is a stress response pathway initially characterized in mammalian cells in which there was either a depletion of the mitochondrial genome or accumulation of misfolded proteins within the mitochondria (Martinus et al., 1996; Zhao et al., 2002). In either case, it was noted that this mitochondrial perturbation triggered a nuclear transcriptional response that included the increased expression of mitochondrial chaperone proteins. While initially described in mammalian cells (Zhao et al., 2002), the biochemistry and genetics of this pathway have been predominantly studied in *C. elegans*. It is now clear that the UPR^{mt} regulates a large set of genes that not only involve protein folding but also involve changes in ROS defenses, metabolism, regulation of iron sulfur cluster assembly, and, as discussed in the following text, modulation of the innate immune response (Nargund et al., 2015; Schulz and Haynes, 2015). In general terms, all of these changes allow for a restoration of mitochondrial function while, at the same time, re-wiring the cell to temporarily survive as best as possible without the benefit of full mitochondrial capacity. Nonetheless, the existence of this broad transcriptional response demonstrates that a means of communication and coordination exists between the mitochondria and the nucleus.

It is now known that, in worms, the UPR^{mt} is regulated, in part, by a unique transcription factor termed Activating Transcription Factor associated with Stress-1 (ATFS-1). ATFS-1 was identified initially in a screen for factors that mediate the UPR^{mt} in *C. elegans* (Haynes et al., 2010). It was subsequently demonstrated that ATFS-1 has both a nuclear localization targeting sequence and a mitochondrial targeting sequence (Nargund et al., 2012). While a mitochondrial localization predominates under basal conditions, mitochondrial stress results in reduced importation of ATFS-1, leading to nuclear accumulation and the transcriptional response delineated earlier. In addition to ATFS-1, in worms, the UPR^{mt} appears to require a number of other factors, including the homeobox transcription factor DVE-1, the ubiquitin-like protein UBL-5, the mitochondrial protease ClpP, and the inner mitochondrial membrane transporter HAF-1 (Jensen and Jasper, 2014).

The link between the UPR^{mt} and lifespan was made initially in the setting of attempting to explain why mutant worms, such as

those with knockdown of *cco-1*, live longer (Durieux et al., 2011). These results demonstrated that this mutant, as well as other long-lived mitochondrial mutants, all appeared to require activation of the UPR^{mt} for their lifespan extension. In contrast, other long-lived mutants, such as those involved in insulin/IGF signaling, appeared to extend lifespan independent of UPR^{mt} activation (Durieux et al., 2011). Remarkably, when *cco-1* was knocked down in one tissue (e.g., neurons), it appeared to activate induction of the UPR^{mt} in other, distal tissues (e.g., intestine). This suggested the existence of a circulating factor that signals, and perhaps coordinates, metabolism between tissues. The authors called this factor a mitokine (Durieux et al., 2011), although, to date, its molecular makeup remains undefined. Whether such factors exist in higher organisms is unclear, but there is clearly a growing interest in circulating factors that regulate aging, as evidenced by the renewed interest in parabiosis studies (Conboy et al., 2013). This notion of mitochondrial dysfunction in one tissue acting as a signal for other tissues has also been observed in *Drosophila*. In a recent example, muscle-specific impairment of a component of Complex I resulted in an increase in the overall lifespan of the fly (Owusu-Ansah et al., 2013). This mitochondrial stress resulted in the activation for at least two separate pathways that appeared to contribute to the observed longevity effects. In the muscle itself, disruption of Complex I resulted in the induction of the UPR^{mt} through what appeared to involve a redox-sensitive pathway. Indeed, overexpression of hydrogen-peroxide-scavenging enzymes, such as catalase or glutathione peroxidase, suppressed the induction of the UPR^{mt} and also abrogated the increased longevity seen with Complex I inhibition (Owusu-Ansah et al., 2013). These negative effects of redox scavengers are reminiscent of similar observations in humans where, for instance, the beneficial effects of exercise appear to be abrogated by antioxidant supplementation (Ristow et al., 2009). In addition to the induction of the UPR^{mt} in muscle, the authors also observed a systemic effect on insulin signaling mediated by changes in the level of a particular circulating IGF-binding partner (Owusu-Ansah et al., 2013). Again, these results argue that mitochondrial dysfunction in one tissue can signal through secreted factors in the circulation to alter the function of distal tissues. This inter-organ communication appears to be ultimately required for the observed increase in lifespan.

Another apparent way in which the UPR^{mt} appears to be activated is when there is a stoichiometric imbalance between mitochondrial and nuclear proteins (Houtkooper et al., 2013). This imbalance can be achieved experimentally in worms by knocking down a mitochondrial ribosomal gene (*Mrps5*), resulting in the selective translational impairment of mitochondrial transcripts. Houtkooper et al. (2013) observed that such a knockdown, or treatment with certain antibiotics that differentially effect mitochondrial and nuclear proteins, triggers induction of the UPR^{mt} and an increase in lifespan; these authors also observed a strong correlation between expression of *Mrps5* and murine lifespan. This suggests, as does other evidence (Wu et al., 2014), that elements of the UPR^{mt} appear to be very well conserved, even though the mammalian equivalent of ATFS-1 remains elusive. Additional evidence comes from analysis of the long-lived *Surf1* knockout mice (Dell'agnello et al.,

2007). *Surf1* is a cytochrome c oxidase assembly factor, and *Surf1*^{-/-} mice live 20% longer than controls, a phenotype that appears to be linked to the activation of a mitochondrial stress response pathway (Pulliam et al., 2014). Other potential relevant observations include a recent re-analysis of CLK-1, a monooxygenase that catalyzes the hydroxylation of 5-demethoxyubiquinone, an important step in the synthesis of ubiquinone. *Clk-1* null worms live longer (Felkai et al., 1999), as do mice who have lost one allele of the mammalian ortholog *COQ7* (Liu et al., 2005). Evidence suggests that the lifespan extension observed in *clk-1* null worms appears to involve activation of the UPR^{mt} (Nargund et al., 2012). While it was assumed that CLK-1 was exclusively mitochondrial, recent evidence suggests that CLK-1, as well as its mammalian ortholog *COQ7*, can also be found in the nucleus (Monaghan et al., 2015). In the nucleus, CLK-1 appears to help lower ROS levels and suppress the UPR^{mt}. Much like ATFS-1, CLK-1 and the mammalian *COQ7* can exist in both the nucleus and mitochondria and thereby appear uniquely suited to modulate the UPR^{mt}. The list of such factors is likely to grow. It will be of interest to see whether other mitochondrial mutants that are also long lived, including those with alterations in iron-sulfur proteins in Complex III (Hughes and Hekimi, 2011) or the outer mitochondrial membrane (Wu et al., 2012), also activate the UPR^{mt} or related pathways. Finally, two important caveats are worth noting. First, while a number of observations support a role for UPR^{mt} activation in modulating the aging process, it is important to note that this pathway is incompletely characterized at present, and evidence suggests that UPR^{mt} activation may not be sufficient by itself to extend lifespan (Bennett et al., 2014). Second, and perhaps relatedly, as recent observations in yeast suggest (Wang and Chen, 2015; Wrobel et al., 2015), the cellular response to mitochondrial perturbation is, undoubtedly, more complex than the currently conceived model of the UPR^{mt}.

Finally, while, as noted earlier, there is considerable interest in mitochondrial-to-nuclear signaling, there is also an important role for nuclear-to-mitochondrial signaling. For instance, telomere dysfunction results in impaired mitochondrial biogenesis through a pathway involving both p53 and *PGC-1 α* (Sahin et al., 2011). Conversely, cells lacking intact nuclear excision DNA repair (xeroderma pigmentosum group A; XPA) also exhibit mitochondrial dysfunction due to impaired mitophagy, mediated by a decline in NAD⁺ levels and sirtuin activity (Fang et al., 2014). Interestingly, XPA and the related Cockayne syndrome are DNA repair disorders that phenotypically manifest as accelerated aging conditions. Nonetheless, there is increasing evidence that these primary nuclear DNA repair disorders have profound metabolic consequences (Scheibye-Knudsen et al., 2014). Thus, between the nucleus and the mitochondria, signaling occurs in both directions, and further dissection of these pathways will likely yield important clues about organismal aging (Figure 2). Furthermore, signaling between the mitochondria and other organelles (e.g., lysosomes) is also emerging as a potential critical determinant of lifespan (Hughes and Gottschling, 2012).

Mitophagy in Aging

If misfolded proteins stemming from mtDNA mutations or proteotoxic stress accumulate to a level that exceeds the capacity

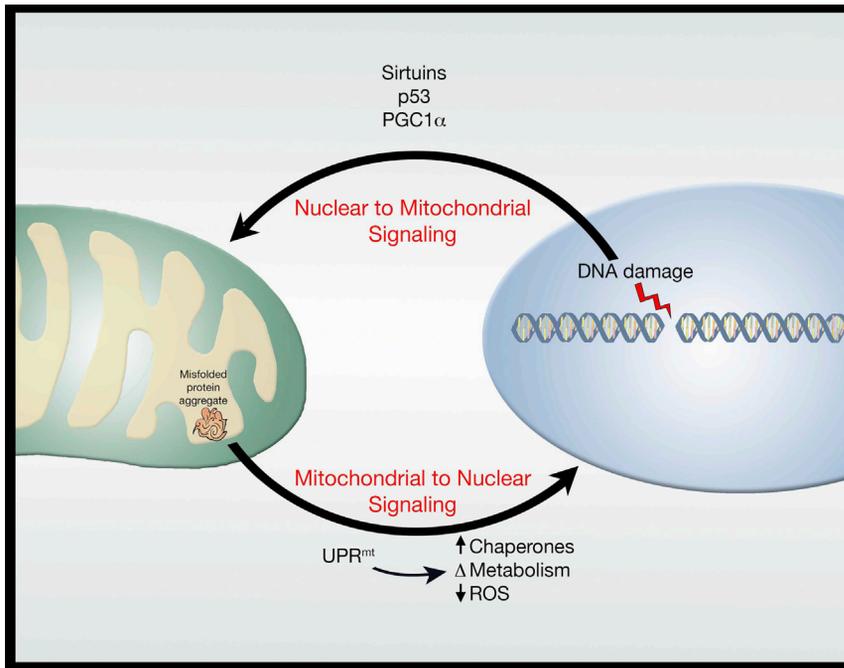


Figure 2. Bidirectional Signaling between the Nucleus and Mitochondria

Communication exists between the nucleus and the mitochondria, with evidence that nuclear stresses, such as DNA damage, trigger a mitochondrial response. Similarly, mitochondrial stresses, such as protein aggregates, stimulate a retrograde response to the nucleus. Both directions of this signaling paradigm appear intimately linked to longevity.

ubiquitination would, therefore, appear to be an attractive therapeutic avenue.

In contrast to man, loss of Parkin and PINK1 in mice does not lead to a neuronal phenotype. To assess endogenous Parkin function in mice under mitochondrial stress, POLG mutator mice were crossed into a Parkin null background. Although neither POLG mutator mice nor Parkin null mice displayed dopaminergic neuron loss, mutator mice in a Parkin null background lost ~40% of substantia nigral dopaminergic neurons by 1 year of age (Pickrell et al., 2015). The mutator/Parkin

of the UPR^{mt} , autophagy of mitochondria (mitophagy), or piecemeal autophagy of mitochondrial subdomains, appears to mitigate mitochondrial impairment. The biochemical steps of one mitophagy pathway have been mapped out in some detail (Pickrell and Youle, 2015). In higher eukaryotes, including man and insects, a mitochondrial kinase PINK1 senses damage and signals this to the cytosolic E3 ligase Parkin. Mitochondrial damage caused by misfolded mitochondrial matrix proteins or other stresses that lead to inner mitochondrial membrane depolarization inhibits protein import through the inner mitochondrial membrane. By avoiding mitochondrial import, PINK1 circumvents proteolytic degradation and accumulates on the impaired mitochondria, with the kinase domain facing the cytosol (Figure 3). There, it phosphorylates ubiquitin attached to mitochondrial outer membrane proteins. These phospho-ubiquitin chains bind to Parkin, recruiting it from the cytosol to the mitochondria and activating its latent E3 ubiquitin ligase activity. Parkin further ubiquitinates mitochondrial outer membrane proteins to recruit receptors such as optineurin and NDP52, which signal autophagosome assembly proximal to individual damaged mitochondria (Itakura et al., 2012; Lazarou et al., 2015). In humans, loss-of-function mutations in either PINK1 or Parkin lead to early onset Parkinson's disease, normally a disease associated with aging, suggesting that insufficient mitophagy may directly lead to the loss of dopaminergic neurons that causes the motor phenotype. Interestingly, the effects of Parkin can be reversed by a family of mitochondrial deubiquitinating enzymes (DUBs) including ubiquitin-specific protease 8 (USP8), USP15, and USP30 (Durcan and Fon, 2015). The best evidence to date comes from analyzing USP30, which appears to antagonize Parkin function, as evidenced by the fact that genetic inhibition of USP30 rescues Parkin-deficient flies (Bingol et al., 2014). Pharmacological manipulation of mitochondrial

null mice also displayed a substantial motor phenotype that was rescued by L-dopa treatment. Thus, endogenous Parkin preserves dopaminergic neurons from death stemming from mtDNA mutation accumulation. However, Parkin did not rescue the mutator mice from the accumulation of mtDNA mutations, suggesting that Parkin compensates for mitochondrial mutation accumulation, perhaps by clearing damaged proteins by autophagy. This model of Parkin removal of damaged proteins is consistent with the finding that ΔOTC , a misfolded matrix protein (deletion mutant of ornithine transcarbamylase) that induces the UPR^{mt} (Zhao et al., 2002), also induces PINK1 accumulation and Parkin translocation to mitochondria without depolarizing mitochondria (Jin and Youle, 2013). Parkin expression diminishes misfolded ΔOTC accumulation, suggesting that Parkin may function downstream of mtDNA mutation accumulation to clear proteotoxic stress during aging. Additional evidence that misfolded, mutated, or oxidized proteins can be selectively removed from mitochondria come from studies in *Drosophila* showing that Parkin functions via the autophagy machinery to eliminate select respiratory chain complex proteins (Vincow et al., 2013). How selective removal of mitochondrial proteins via autophagy occurs is not clear but may involve asymmetric mitochondrial fission (Twig et al., 2008) or mitochondrial derived vesicles (Sugiyama et al., 2014). Consistent with the suggestion that mitophagy protects animals from loss of mitochondrial function during aging, mitophagy rates decrease in the dentate gyrus with age and upon human huntingtin overexpression (Sun et al., 2015).

Although loss of Parkin expression has not been reported to exacerbate the aging phenotype of wild-type or POLG mutator mice, loss of Parkin expression in *Drosophila* decreases animal lifespan (Greene et al., 2003), and Parkin overexpression extends fly longevity without impairing fertility or food consumption (Rana et al., 2013). Parkin overexpression also reduces the levels

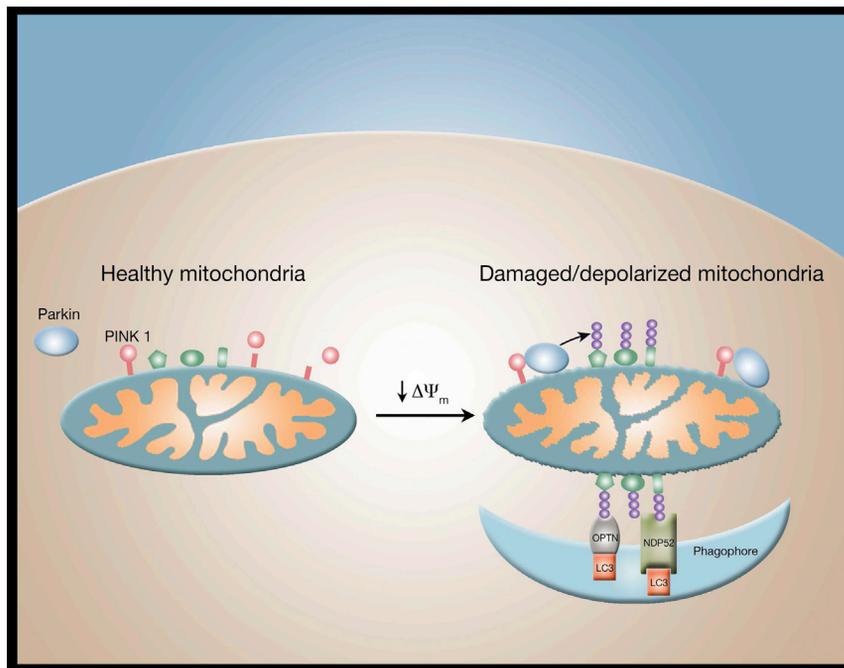


Figure 3. Parkin-Dependent Mitophagy

In healthy mitochondria, the PINK1 kinase is constitutively degraded. A fall in mitochondrial membrane potential ($\Delta\psi_m$) stabilizes PINK1, facilitating the recruitment of cytosolic Parkin to the mitochondrial outer membrane. Activation of Parkin results in the ubiquitination (purple balls) of multiple outer mitochondrial membrane proteins (shown in green). Once ubiquitinated, these proteins are recognized by specific mitophagy receptors such as optineurin (OPTN) and NDP52, which, along with LC3, direct the phagophore to surround the damaged mitochondria, allowing for its ultimate delivery to the lysosome for degradation via mitophagy.

of ubiquitin/protein aggregates that normally accumulate in *Drosophila* muscle with age. Thus, PINK1/Parkin-mediated mitophagy appears to mitigate deleterious consequences of mitochondria DNA mutation accumulation in mammals and foster longevity in flies.

PINK1/Parkin-independent mitophagy pathways have been also identified. One mitophagy process that occurs during mammal development induces the wholesale elimination of mitochondria from red blood cells. Expression levels of Nix, also called BNIP3L, increase dramatically during reticulocyte development, and mice lacking Nix retain mitochondria in mature RBCs (Schweers et al., 2007). Nix is localized on the outer mitochondrial membrane and exposes a domain toward the cytosol that binds to LC3 on autophagosomes and that participates, to some extent, in autophagic engulfment of mitochondria. Whether Nix functions only constitutively to eliminate mitochondria or is regulated post-translationally to mediate mitophagy remains unclear.

Expression of a predicted Nix homolog, PINK1, and Parkin in *C. elegans* appears to promote longevity (Palikaras et al., 2015). Although loss of Pink1, PDR-1 (a Parkin ortholog), and DCT-1, an outer mitochondrial membrane protein with domain organization similar to that of NIX, does not affect lifespan in wild-type worms, it decreases the lifespan of the long-lived *daf-2* mutant animals with a disrupted insulin-like signaling cascade and of the feeding-impaired—and, thus, calorie-restricted—*eat-2* mutant worms. This suggests that mitophagy is required for multiple distinct pathways that extend lifespan. Interestingly, loss of PINK1 or DCT-1 does decrease lifespan in *C. elegans* lacking a homolog of NRF2 called SKN-1. This indicates that mitochondrial biogenesis, mediated through SKN-1, compensates for a lack of mitophagy in wild-type nematodes. In mammals, the PINK1-Parkin axis requires SIRT1, the NAD-

dependent deacetylase previously linked to aging (Giblin et al., 2014), for full activity. Inhibition of SIRT1 decreases activation of PGC-1 α , leading to defective PINK1- and Parkin-mediated mitophagy (Fang et al., 2014).

Another link between mitochondrial quality control and lifespan has been observed in *Podospora anserine*, a well-established fungal model of aging. In this

model, increased expression of the mitochondrial matrix AAA+ protease LON can substantially extend lifespan without impairing growth, respiration, or fertility (Luce and Osiewacz, 2009). Both the LON protease and the ATP-dependent Clp protease (CLPP) are essential for protein homeostasis in the mitochondrial matrix, and deficits in their activity are tightly linked to a decline in mitochondrial function and to aging (Quirós et al., 2015). Interestingly, the LON protease is also known to regulate mitochondrial levels of PINK1 (Thomas et al., 2014), as well as being the dominant protease responsible for initially handling misfolded and aggregated proteins in the mitochondrial matrix (Bezawork-Geleta et al., 2015). The latter stimulus is the classic activator of the UPR^{mt}. Thus, while, for clarity, we have discussed mitophagy and the UPR^{mt} as distinct regulatory pathways, the aforementioned observation with LON proteases, as well as other evidence (Jin and Youle, 2013), suggests the existence of substantial cross-regulation between these various mitochondrial quality control pathways.

Mitochondria and Inflammation

One of the hallmarks of aging is the development of a low-grade, chronic, sterile inflammatory state often deemed “inflammaging.” The development of this state, characterized in part by increased circulating inflammatory biomarkers such as interleukin (IL)-6 and C-reactive protein, is a known risk factor for increased morbidity and mortality in the elderly (Franceschi et al., 2000). Increasingly, there is a connection between mitochondrial function and the activation of this enhanced age-dependent immune response. Mechanistically, this connection can, perhaps, be traced back to the bacterial origins of the present-day mitochondria. As opposed to nuclear DNA, mtDNA (like bacterial DNA) is not methylated. The immune system has adapted to this subtle difference and has evolved strategies to

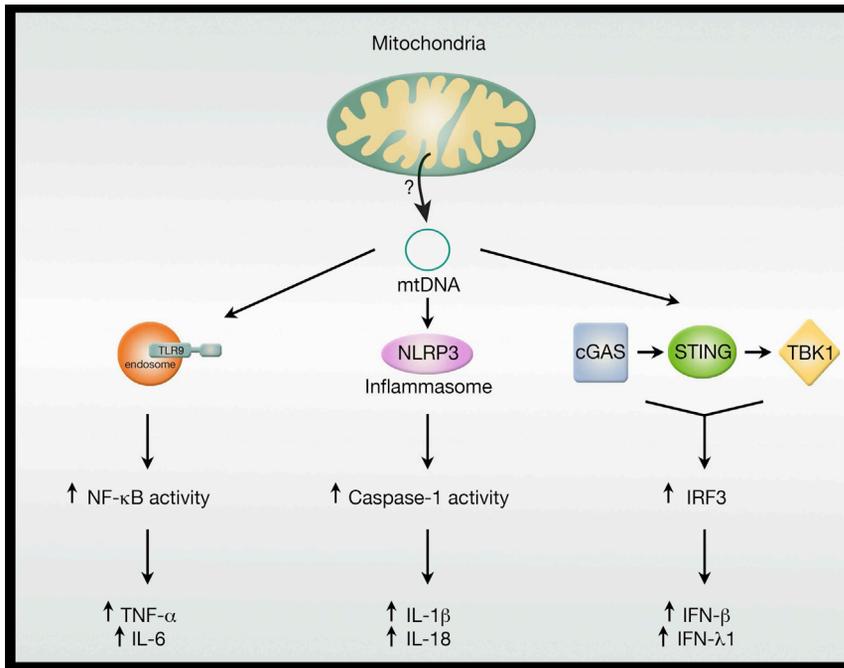


Figure 4. Mitochondria as Regulators of the Innate Immune Response

Release of mitochondrial DNA appears to trigger at least three distinct pathways linked to inflammation. The precise mechanism by which free mtDNA enters the cytosol to engage with various intracellular DNA sensors is currently unclear. Nonetheless, age-dependent breakdown of the mitochondrial membrane might allow escape of mtDNA and thereby help fuel the chronic, sterile inflammation associated with aging.

TBK1, activates the transcription factor IRF3 to induce production of type 1 interferons (IFNs) and IFN-stimulated gene products. A previous link between IFN signaling and mitochondria has been made when it was noted that an important component of retinoic-acid-inducible protein 1 (RIG-1)-like receptor (RLR) signaling was associated with mitochondria. In particular, the RLR adaptor protein MAVS (mitochondrial antiviral-signaling protein) was found to form a scaffold for signaling on the outer mitochondrial

membrane surface (Seth et al., 2005). The role of mtDNA in activating the STING pathway first came to light in more recent studies involving Bax/Bak-mediated apoptosis (Rongvaux et al., 2014; White et al., 2014). In these studies, caspase activation during apoptotic cell death was shown to suppress IFN production by preventing the ability of mtDNA to activate the cGAS-STING pathway. This caspase-mediated suppression ensures that apoptosis is immunologically silent. These results have been recently extended in a study characterizing the effect of haploinsufficiency of TFAM. Among other things, TFAM regulates mitochondrial nucleoid structure, abundance, and segregation. Cells expressing only one allele of TFAM (*Tfam*^{+/-}) were shown to have approximately 50% less mtDNA but no resting bioenergetics deficit (West et al., 2015). Interestingly, *Tfam*^{+/-} mouse embryonic fibroblasts exhibited constitutive activation of the cGAS-STING-IRF3 pathway (West et al., 2015). Moreover, in wild-type cells, herpes virus infection appears to trigger mitochondrial stress, including reducing TFAM levels, and this mitochondrial stress appears to be required to mount the full antiviral response (West et al., 2015). Again, these results argue for a central role of mitochondria—and, particularly, released mtDNA—in regulating the innate immune response. The precise nature, however, as to how mtDNA is released under these various conditions has not been well characterized.

While the aforementioned discussion has focused on seemingly permanent damage to mitochondria, resulting, presumably, in the rupture of the inner mitochondrial membrane and the subsequent release of mtDNA, other more reversible forms of mitochondrial dysfunction can also trigger an immune response. Those investigators interested in probing mitochondrial function have classically used chemicals such as antimycin and cyanide to block electron transport. The natural sources of these inhibitors are bacteria, which use these small molecules

recognize non-methylated DNA, primarily through members of the Toll-like receptors, including TLR9. Presumably, this response allows rapid activation of the immune system in the setting of bacterial infection. Besides releasing non-methylated DNA, damaged mitochondria, like bacteria, can release formyl peptides that can signal through the formyl peptide receptor-1 to trigger an immune response. Both mtDNA and mitochondrial formylated peptide can be viewed as mitochondrial-derived damage-associated molecular patterns (DAMPs) that are known to stimulate the innate immune system. The importance of this mitochondrial-elicited TLR9 response can be seen in a number of important medical inflammatory states, including trauma (Zhang et al., 2010) and heart failure (Oka et al., 2012).

mtDNA can also activate the NLRP3 inflammasome (Nakahira et al., 2011; Shimada et al., 2012). The inflammasome is a large multi-protein complex that controls caspase-1 activation, a step that is required for the subsequent processing and secretion of IL-1 β and IL-18. Interestingly, macrophages lacking mtDNA have severely impaired secretion of IL-1 β (Shimada et al., 2012). Moreover, genetic ablation of *Nlrp3* resulted in a diminished age-dependent activation of the innate immune system and protected animals from a number of age-related pathologies including bone loss, thymic involution, and loss of glycemic control (Youm et al., 2013). It is tempting to speculate that, in older tissues, the slow, chronic release of mtDNA or mitochondrial proteins might contribute to the age-dependent activation of the inflammasome and thereby contribute to the “inflammaging” milieu.

The sensing of free, intracellular mtDNA is not confined to TLR9 or the inflammasome, as, recently, a third pathway involving the adaptor protein STING has been described (Figure 4). In this pathway, the cytosolic sensor cGAS recognizes mtDNA and, through the adaptor protein STING and the kinase

membrane surface (Seth et al., 2005). The role of mtDNA in activating the STING pathway first came to light in more recent studies involving Bax/Bak-mediated apoptosis (Rongvaux et al., 2014; White et al., 2014). In these studies, caspase activation during apoptotic cell death was shown to suppress IFN production by preventing the ability of mtDNA to activate the cGAS-STING pathway. This caspase-mediated suppression ensures that apoptosis is immunologically silent. These results have been recently extended in a study characterizing the effect of haploinsufficiency of TFAM. Among other things, TFAM regulates mitochondrial nucleoid structure, abundance, and segregation. Cells expressing only one allele of TFAM (*Tfam*^{+/-}) were shown to have approximately 50% less mtDNA but no resting bioenergetics deficit (West et al., 2015). Interestingly, *Tfam*^{+/-} mouse embryonic fibroblasts exhibited constitutive activation of the cGAS-STING-IRF3 pathway (West et al., 2015). Moreover, in wild-type cells, herpes virus infection appears to trigger mitochondrial stress, including reducing TFAM levels, and this mitochondrial stress appears to be required to mount the full antiviral response (West et al., 2015). Again, these results argue for a central role of mitochondria—and, particularly, released mtDNA—in regulating the innate immune response. The precise nature, however, as to how mtDNA is released under these various conditions has not been well characterized.

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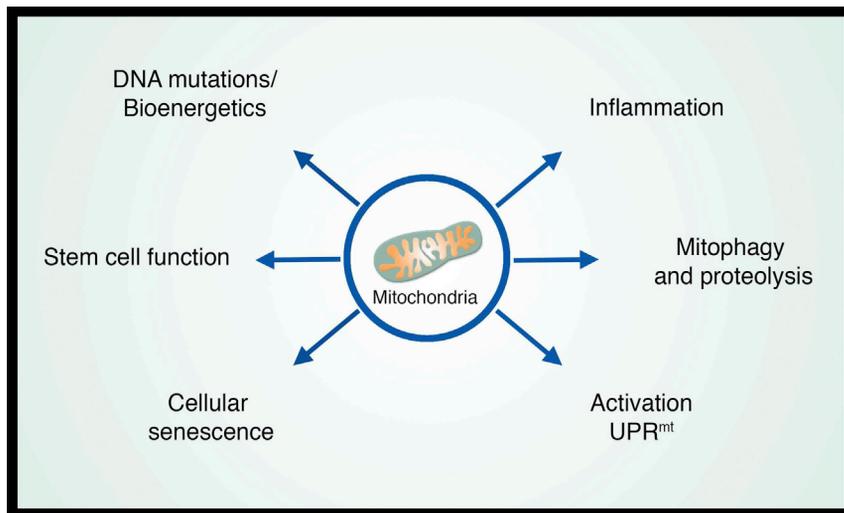


Figure 5. Mitochondria as Regulators of Organismal Aging

The contribution of mitochondria to the aging process occurs through multiple distinct pathways. Although depicted as separate pathways, clear intersections occur, as is evident between the connection between activation of the UPR^{mt} and the induction of the inflammatory response (see text for details).

to disable their host, allowing for more productive infections. Interestingly, when *C. elegans* are directly challenged with these mitochondrial inhibitors, this transient mitochondrial dysfunction appears to be interpreted as a pathogen attack and is sufficient to activate an innate immune response (Liu et al., 2014). In a related set of observations, infection of worms with the bacteria *Pseudomonas aeruginosa* was also shown to result in mitochondrial dysfunction and UPR^{mt} activation (Pellegrino et al., 2014). The latter response was shown to be critical for the worm to mount an effective immune response. Both observations suggest that, in *C. elegans*, mitochondrial dysfunction triggers activation of the innate immune response.

Conclusions

Taken together, these observations suggest that mitochondria can be intimately linked to a wide range of processes associated with aging, including senescence and inflammation, as well as the more generalized age-dependent decline in tissue and organ function (Figure 5). What specific perturbations of mitochondrial function are most relevant for the aging process requires additional clarification. As we have discussed, early studies in skeletal muscle concentrated on the accumulation of DNA mutations and the concomitant decline in electron transport function. Recent evidence has implicated triggering of the UPR^{mt} and quality control mechanisms, including mitophagy and proteolysis. Other processes not discussed here in depth include mitochondrial dynamics (Liesa and Shirihi, 2013; Pernas and Scorrano, 2015), as well as the biosynthetic properties of mitochondria. The best evidence for the relevance of the latter property comes from yeast, where mitochondrial regulation of iron-sulfur cluster biogenesis clearly modulates nuclear genomic integrity (Veitch et al., 2009). In many of the early studies, the association between mitochondria and the aging process was mostly correlative. Increasingly, however, causative connections are being established. This suggests that attempts to rejuvenate mitochondrial function or improve mitochondrial quality control might be an effective strategy to combat aging. Toward this goal, there are a number of ongoing efforts to develop small

molecules to therapeutically augment mitochondrial biogenesis (Suliman and Piantadosi, 2016). Similarly, raising NAD⁺ levels in older mice appears to restore mitochondrial function (Gomes et al., 2013). As such, there is considerable enthusiasm to develop methods to increase NAD⁺ levels, either through direct supplementation or by altering NAD⁺ metabolism (Cantó et al., 2015). Pharmacologic activation of mitophagy is another approach that might be widely beneficial in patients with age-related neurodegenerative disorders or to combat aspects of normal aging. With the relatively detailed molecular understanding of PINK1 and Parkin activation, efforts are underway in academia and industry to directly or indirectly modulate the activity of PINK1 (Hertz et al., 2013), Parkin, or USP30 (Bingol et al., 2014; Hasson et al., 2015) in order to promote mitophagic flux. As such, the next decade appears to hold considerable promise for developing a wide range of effective mitochondria-targeted therapies. With such agents, clinical trials can ultimately test the very tenable hypothesis that reversing the decline in mitochondrial function will slow, or even reverse, the rate at which we age.

AUTHOR CONTRIBUTIONS

N.S., R.J.Y., and T.F. all participated in the writing of this manuscript.

ACKNOWLEDGMENTS

We are grateful to members of the T.F. and R.J.Y. labs for helpful comments and to Ilsa Rovira for help with the preparation of the manuscript. This work was supported by NIH Intramural Funds and a Leduq Foundation Transatlantic Network Award.

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