Mitochondrial dysfunction and longevity in animals: untangling the knot

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ABSTRACT

Mitochondria generate ATP and are a source of potentially toxic reactive oxygen species (mtROS). It has been suggested that the gradual mitochondrial dysfunction that accompanies aging could be causal to the aging process itself. Here we review findings that suggest that age-dependent mitochondrial dysfunction is not sufficient to limit lifespan. Furthermore, mtROS are not always deleterious and can even stimulate pro-longevity pathways. Thus, mitochondrial dysfunction plays a complex role in regulating longevity but does not by itself cause aging.

The primary and most essential function of mitochondria is to produce energy for the cell. The oldest explanation for aging, the rate-of-living theory, postulates that aging and lifespan are regulated by the rate of energy metabolism, with lower rates leading to greater lifespans. However, the appeal of the rate-of-living theory has been weakened by its failure to predict sufficiently well the observed relationships between energy expenditure and lifespan. This is not to say that mitochondrial and energy metabolism don't play a crucial role in aging, but their relation to aging might not be simple. Mitochondria do much more besides energy production. Particularly relevant to aging, the mitochondrial electron transport chain (ETC) leaks electrons and generates reactive oxygen species (ROS) during normal respiration. Thus, there is a potential deleterious elevation of ROS production when the ETC function is perturbed. The mitochondrial free radical theory of aging posits that biological aging results from the production of ROS and the ensuing damage. However, direct manipulation of cellular ROS levels within the biologically significant range does not accelerate aging or decrease lifespan. Here we review the relationships between normal mitochondrial function, mitochondrial dysfunction, ROS generation and lifespan.

Deleterious mitochondrial dysfunction

Numerous studies have described damage to mitochondria in aged cells and organisms, including in human samples. This damage includes a gradual decline in respiratory chain capacity, decreased activities of individual ETC complexes, elevated oxidative damage, decreased mitochondrial content, morphological abnormalities in mitochondrial structure and increased fragility of aged mitochondria during experimental isolation (*1*)(Fig. 1). To explore the significance of these observations for the aging process, a key question is whether the observed damage and dysfunction is severe enough to cause the other degenerative phenotypes of aging.

There is no doubt that mitochondrial dysfunction can severely damage the organism. Human patients with mutations in mitochondrial DNA (mtDNA) or nuclear genes coding for proteins that function in the mitochondrial ETC are generally severely affected. They often show multisystem disorders that include myopathy, encephalopathy, stroke, and hearing loss (*2*). As most mitochondrial disorders present with neurological and muscular symptoms, it is generally postulated that cells with high energy demands, such as the central nervous system and oxidative muscles, are more susceptible to the reduced energy output of defective mitochondria and are thus more strongly affected by mitochondrial impairment. There is however considerable clinical variability among mitochondrial disease patients and some mutations only affect particular tissues, reflecting a diversity of distinct disease mechanisms, which are still poorly understood. To understand these conditions, a variety of mouse knockout (KO) models have been developed for nuclear-encoded mitochondrial proteins (*3*). These include mutants carrying KO mutations in genes that are required for the assembly and function of ETC complexes, those with defects in the production of mobile electron carriers (i.e., cytochrome c and ubiquinone), and those lacking necessary factors for the maintenance of mitochondrial dynamics or the integrity of mtDNA. In virtually every case, complete germline KO causes embryonic to perinatal lethality. Tissue-specific conditional KOs, mostly targeted to neurons or muscles, result in abnormal mitochondria with severe deficits in respiratory chain function, giving rise to a variety of disease phenotypes. Most show severe progressive loss of tissue function, such as progressive skeletal muscle weakening, movement impairment and neurobehavioral abnormalities. All result in death within the first year of life, with lifespans reduced to less than 40% of the normal (*3*).

Much research on why mitochondrial dysfunction gradually develops with time has focused on mtDNA. Mutations and deletions in mtDNA increase with age, and clonally expanded

mtDNA damage is more abundant in those areas of aged tissue that also show mitochondrial ETC dysfunction (*4*). These findings and many earlier studies led to the notion that continuous accumulation of mtDNA damage may play a causal role in the aging process. One particular model that has been used to study this is the 'mutator' mouse. In these mice the proofreading function of the mtDNA polymerase gamma (*Polg*) is defective, which leads to the accumulation of random mutations and deletions in mtDNA (*5, 6*). Decreased lifespan and an array of phenotypes reminiscent of normal aging have been observed in homozygous mutator mice ($Polg^{D257A/D257A}$). The mice exhibit decreased ETC complex content and activity, lower respiratory chain capacity and lower ATP levels, as well as activation of apoptotic pathways (*5, 6*). However, most studies of the mutator mice have reported negligible increases in oxidative stress, which often accompanies disruption of ETC function in the KO mouse models described above (*6*). This is of interest because oxidative stress, whether or not causative of aging, has been commonly regarded as a reliable biomarker of aging. In fact, it has often been suggested that ROS could be responsible for aging by acting as mutagens on mtDNA in somatic cells, inducing a vicious cycle where the mtDNA mutations lead to defective ETC function, producing even more mtDNA-damaging ROS, etc. It is noteworthy therefore, that a recent study using a mitochondria-targeted mass spectrometry probe, detected an increase in hydrogen peroxide in the mutator mice close to the end of their life span (*7*). However, no increase was found in young mutator mice despite their already elevated level of mtDNA mutations, nor was any increase found in old wild-type mice. Thus, though high mitochondrial ROS may well contribute to the shorter lifespan of mutator mice, which is supported by beneficial effects of some antioxidant interventions (*8*), its role in normal aging is probably not of great significance. In Drosophila and human brain tissue age-related increases of mtDNA

mutations are not caused by oxidative stress (*9, 10*), further weakening the vicious cycle hypothesis of mitochondrial aging.

Beyond this, the quantitative findings with the mutator mouse rather militate against the notion that mtDNA cause aging. In the homozygous mutator mice lifespan is shortened but the mtDNA mutation load is much higher than that detected in aged animals or elderly humans (*11*). Heterozygous mutator mice are born with a 30-fold higher mutation burden than aged wild-type mice and yet lack overt phenotypes and have a normal lifespan (*11*). This calls into question whether the slowly accumulating naturally occurring age-related mtDNA mutations have a leading role in causing aging, rather than representing only one of the types of damage accumulation that accompanies aging. A different mouse strain, the mtDNA deletor mouse, is also relevant in this context. These mice accumulate large-scale mtDNA deletions in postmitotic tissues, but do not show a shortened life span although they exhibit late-onset respiratory dysfunction in a subset of tissues (*12*), further undermining the notion that damage to mtDNA or mitochondrial dysfunction are sufficient to accelerate aging.

Uncoupling mitochondrial dysfunction and aging

Not all partial losses of mitochondrial function result in shortened lifespan, and some can even result in increased lifespan (*13*). Ubiquinone (UQ) is an obligate electron carrier in the ETC, and MCLK1 (a.k.a. COQ7) is the penultimate enzyme in the mitochondrial UQ biosynthetic pathway. Mice missing one copy of *Mclk1* appear superficially normal and live longer than their wild type littermates, despite markedly reduced mitochondrial respiration. Overall UQ concentration in whole mitochondria extracts is normal in these heterozygous mice, but it is lower in the inner membrane fraction. This causes a decrease in respiratory chain capacity, which in turn results in

low ATP generation. Interestingly, production of mtROS appears to be increased in the mutant while overall ROS levels are not. The extended longevity of these mutants is also associated with a slow development of biomarkers of aging, high macrophage expression of HIF-1α, and an enhanced immune response (*13*). However, it is not known whether these phenotypes are necessary or sufficient for the observed increased longevity.

SURF1 is an inner mitochondrial membrane protein required for complex IV assembly, the protein complex needed for oxidative phosphorylation. A knockout model of *Surf1*, in which a premature stop codon was inserted into exon 7, resulted in viable mice with increased lifespan (*14*). These mice exhibit the anticipated decrease in complex IV activity, which was down to 30%-50% of normal levels. Mitochondrial respiration was mildly affected in some tissues, but no change in mitochondrial ROS production was detected. Other phenotypic features include lower fat mass, elevated protein expression of the mitochondrial biogenesis regulator PGC-1α (BOX 1), increased insulin sensitivity, resistance to calcium-induced neuron death, and an increase in expression of some of the proteins involved in the mitochondrial unfolded protein response (UPR^{mt}) (*14-16*). Interestingly, there are other indications that the UPR^{mt} could participate in lifespan determination in mammals, as conserved longevity-promoting interventions, such as overexpression of Sirt1, rapamycin and resveratrol treatments induce the UPR^{mt} in mammalian cells (*17, 18*). However, if remains unclear how loss of SURF1 extends lifespan.

SOD2 is a mitochondrial matrix superoxide dismutase that serves as a first line of defense against oxidative stress by converting superoxide to hydrogen peroxide. *Sod2* heterozygous mice ($Sod2^{+/-}$) mice show increased oxidative stress as indicated by inactivation of ROS-sensitive enzymes, higher sensitivity to oxidative stress, impaired mitochondrial respiration, and greater levels of DNA oxidative damage in both nucleus and mitochondria (*19*). Despite this, $Sod2^{+/-}$ mice

appear normal and have wild-type lifespan. Thus, mitochondrial damage induced by a decrease in antioxidant defenses is not sufficient to compromise longevity in mice.

Conceivably, impaired mitochondrial function does not shorten lifespan before reaching a critical threshold. This argument provides a potential rationale for why, despite the prime importance of mitochondria for many cellular functions, some mitochondrial defects, such as those mentioned above, are not associated with a shortened lifespan. However, even mice that have suffered severe and prolonged mitochondrial dysfunction are still able to live as long as controls when mitochondrial function is partially restored at mid-life (*20*). Mutant mice in which the *Mclk1* gene was globally deleted in two-month old adults (aog*Mclk1* KO) show a severe loss of UQ and impaired mitochondrial respiration. Heart, kidney and skeletal muscle have only 50% of the normal respiratory rate. The mutant mice died around 9 months with severe phenotypes, including small size, absence of fat, hair loss, low blood glucose, elevated lactate, low triglycerides, intervals of catatonia and, finally, severe neurological symptoms (*20*). UQ biosynthesis can be restored in the absence of the MCLK1-catalyzed step by treatment with an appropriate unnatural biosynthetic precursor, 2,4-dihydroxybenzoate (2,4-DHB)(*21*). Treatment of KO mice with 2,4-DHB shortly before death led to virtually full phenotypic recovery, with animals looking essentially wild-type, except for a small deficit in weight, despite only partial normalization of mitochondrial function. Furthermore, the treatment resulted in full restoration of a normal lifespan despite the fact that the mice had lived almost their entire lives with mitochondrial dysfunction (*20*). Thus, neither chronic nor acutely severe mitochondrial dysfunction is sufficient to produce irreversible phenotypes that limit lifespan.

Insights from invertebrate studies

Several studies with vertebrates described above fail to support a causal role for mitochondrial dysfunction in the aging process. In addition, recent work with invertebrate animal model systems suggest that mitochondrial dysfunction can in fact lead to the generation of intracellular signals that stimulate anti-aging processes. It is widely assumed that the mechanisms of aging are conserved and can be studied in model organisms, including the nematode *Caenorhabditis elegans* and the fruit fly *Drosophila melanogaster. clk-1* is the *C. elegans* orthologue of the mouse *Mclk1* gene discussed above. Twenty years ago, the *clk-1* mutant was the first long-lived mutants to be described in which increased longevity was associated with mitochondrial dysfunction (*22*). Several more long-lived *C. elegans* mutants were subsequently found to be associated with mitochondrial dysfunction (*23, 24*). In addition, it was found that using RNA interference (RNAi) to knock down *C. elegans* genes whose products function in mitochondria, in particular in the ETC, frequently resulted in increased lifespans (*25, 26*), a phenomenon that appears to be conserved in Drosophila (*27*), and possibly in mice (*18*). Interestingly, at least some of the mutations appear to increase lifespan by mechanisms that are distinct from those that underlie the effects of RNAi (*23*). However, whether distinct mechanisms are at work and even what mechanisms are engaged by RNAi knockdowns to induce longevity remains controversial.

Long-lived electron transport chain mutants

A point mutation in *C. elegans isp-1*, which encodes the iron sulfur protein from mitochondrial respiratory complex III, leads to mitochondrial dysfunction but also to a dramatically increased lifespan (*24*). Interestingly, the mitochondrial dysfunction in *isp-1* and other similar long-lived ETC mutants elevates mitochondrial superoxide generation (e.g. *nuo-6* (*23*)). This elevation appears to be necessary for extended longevity as it is suppressed by treatment with antioxidants.

A pro-longevity role for superoxide generation is further supported by the observation that treatment with very low concentration (0.1mM) of the mitochondrial superoxide generator paraquat (PQ) can dramatically increases the lifespan of wild type animals without impairing their mitochondrial function, but is without effect on the long-lived ETC mutants (*28*). Together with some of the findings in vertebrates reviewed above, this led to the proposal that the observed association of aging with increased mtROS generation does not indicate that ROS cause aging, but that mtROS are part of a stress response that combats the damage accumulation that accompanies aging (*29*) (BOX 2).

Recent findings suggest that one of the principal mechanisms by which mtROS act as pro-longevity signaling molecules is by acting through the intrinsic apoptosis signaling pathway, without inducing cell death, but by activating a specific pattern of changes in gene expression (*30*)(Fig. 2A). The intrinsic pathway of apoptosis is physically associated with mitochondria and is sensitive to mtROS in vertebrates, where it is contributing to homeostasis by eliminating unwanted or dysfunctional cells. It was found that in the soma of *C. elegans* the signaling pathway that triggers apoptosis can be used in two ways: either to stimulate apoptosis when it is triggered by expression of EGL-1, or to stimulate greater survival when it is triggered by expression of CED-13 and mtROS. mtROS presumably act on CED-9 (Bcl2-like), which is tethered to the mitochondrial outer membrane, or CED-4 (Apaf1-like), which is bound by CED-9 (Fig. 2A). The expression of the *ced-13* gene is known to be controlled by the p53 *C. elegans* homologue CEP-1, which also affects mtROS pro-longevity signaling (*31, 32*). Yeast studies have suggested a different signaling process of mtROS affecting longevity, in which inhibition of TORC1 (target of rapamycin complex 1) extends lifespan in part by increasing respiration and superoxide

production that trigger epigenetic changes in nuclear DNA resulting in a noncanonical activation of DNA damage pathways (*33*).

Altered energy metabolism in the long-lived ETC mutants (low oxygen consumption and ATP levels) might also play a role in their longevity as mtROS in these mutants alters ATP-dependent behaviors and developmental processes, possibly by redirecting ATP use toward protective processes (*30*). This is consistent with the findings that the metabolic regulators AMP kinase and HIF-1 were found to modulate the effects of the mtROS pathway (*34, 35*).

Activating the mitochondrial unfolded protein response

The UPR^{mt} allows cells to cope with the presence of unfolded or misfolded proteins in mitochondria by conveying a stress signal to the nucleus and up-regulating mitochondrial chaperones and proteases (*36*). It has been proposed that UPR^{mt} activation promotes longevity and is responsible for the lifespan extension induced by resveratrol and rapamycin treatments (*17*), as well as for the lifespan extension that follows the mitochondrial dysfunction induced by RNAi knockdown of ETC components (*23, 37*) or components of the mitochondrial translation machinery (*18*)(Fig. 2B). However, the mechanism induced by RNAi and by the long-lived ETC point mutations appears to be fully distinct, in particular as their effects on lifespan are additive (*23*).

However, despite these exciting new findings, whether activation of the UPR^{mt} is sufficient for lifespan extension remains an open debate. Indeed, activation of the UPR^{mt} can be uncoupled from lifespan extension in *C. elegans* (*38*). For example, gain-of-function alleles of *atfs-1*, which encodes the nuclear transcription factor that turns on the UPR^{mt} by sensing mitochondrial stress,

do not lengthen lifespan. Furthermore, loss of function *atfs-1* alleles cannot always prevent the longevity of RNAi knockdown of ETC subunits.

Even though the ATFS-1-dependent UPR^{mt} and other mechanisms of mitochondrial protein homeostasis (*39*), might not confer longevity by themselves, they might be needed to permit for lifespan extension in long-lived mutants with stressed mitochondria, such as *clk-1* and *isp-1* (*37, 39*). Indeed, the extended lifespans of the mutants were abolished when the activation of these pathways, which shield mitochondria from the consequences of dysfunction, was prevented, as this resulted in very severe synthetic phenotypes.

In summary, it is clear that there are two distinct mechanisms of lifespan extension by dysfunctional mitochondria: one mechanism induced by point mutations in ETC subunits that also increases mtROS generation and engages the apoptotic pathway and one mechanism induced by RNAi knockdown of ETC components and components of the mitochondrial translation machinery. The role of the mechanisms of mitochondrial protein homeostasis in either mechanism is less clear, but these protective mechanisms might be facilitators for any lifespan extensions induced by mitochondrial stress (Fig.2).

Conclusions

Studies in both vertebrates and invertebrates clearly demonstrate the intimate connection between mitochondria and longevity. On one hand, there is no doubt that mitochondria wear down with age. However, by itself this functional decline appears to be insufficient to cause aging. On the other hand, deviations from normal mitochondrial states can elicit responses that are protective and pro-longevity in nature. This points to unexpectedly complex links between mitochondria and

longevity.

BOX 1. Benefits of preserving or boosting mitochondrial function

PGC-1 is a transcription co-activator of mitochondrial biogenesis and oxidative metabolism. Overexpression of PGC-1α has been used to preserve or boost mitochondrial function. PGC-1 can also regulate the expression of ROS-defense enzymes. In mice, increased muscle PGC-1α leads to preservation of muscle function during aging (*40*). In fruit flies, overexpression of PGC-1α in intestinal stem and progenitor cells leads to a longer lifespan (*41*). The mechanisms underpinning these effects are uncertain. NAD+ is a coenzyme for many reactions in OXPHOS and the TCA cycle. NAD+ levels decline with age. Boosting NAD+ levels has been shown to increase lifespan in *C. elegans* and improve health indicators in old mice (*17, 42*). The effects are mediated by sirtuins and are associated with more and healthier mitochondria.

BOX 2. ROS in other mechanisms of lifespan extension

ROS acting as beneficially signals have been linked to the longevity linked to disturbed insulin/IGF-1 signalling in *C. elegans* (*43*). Mitochondria have frequently been suggested to be implicated in the longevity from caloric restriction (CR). In *C. elegans*, a CR regime imposed by inhibition of glycolysis via 2-deoxy-D-glucose promotes ROS formation and subsequent longevity (*44*). However, mtROS generated by paraquat can further extend the lifespan of the eating-defective mutant *eat-2*, suggesting that the mechanism of CR is distinct from the lifespan extension initiated by mtROS (*28*). The transcription factor SKN-1/Nrf, which is crucial to the response to oxidative stress in vertebrates and invertebrates has also recently been implicated in linking mitochondrial function to CR (*45*).

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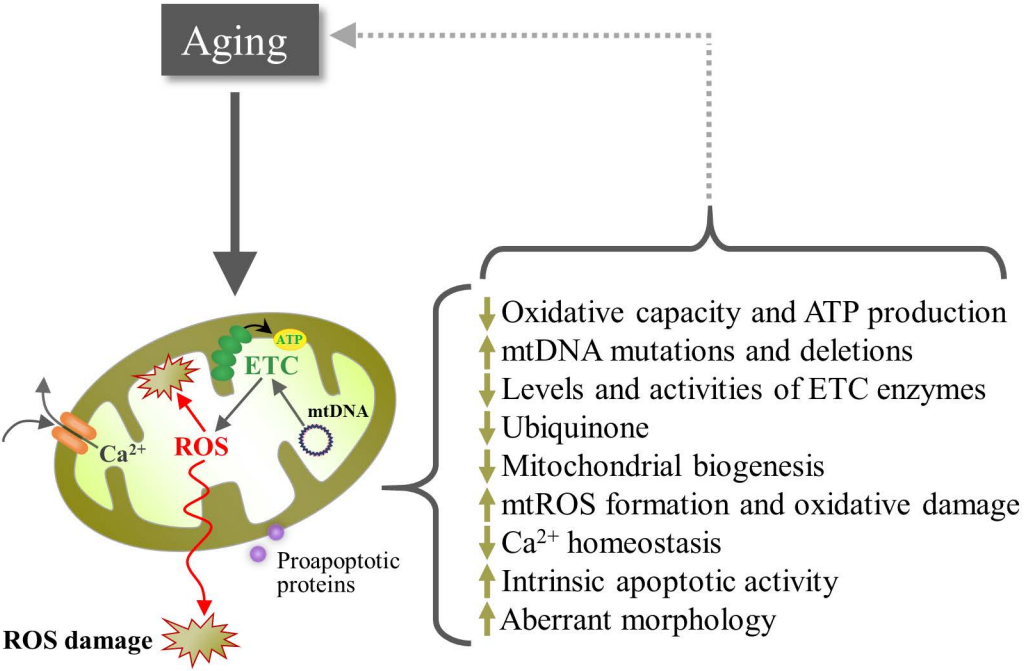
FIGURE LEGENDS

Fig. 1. Various mitochondrial defects are found to accompany aging. Whether they have a causal effect remains unclear.

Fig. 2. Pro-longevity responses to mitochondrial stress. **A)** In *C. elegans*, pro-longevity mtROS signaling acts through the intrinsic apoptosis pathway. This is further modulated by key stress response pathways. **B)** The UPR^{mt} has been suggested to link mitochondrial stress to lifespan extension, but it is still uncertain whether its activation is sufficient on its own.

Age-dependent gradual mitochondrial dysfunction



Pro-longevity responses to mitochondrial stress