The mitochondrial unfolded protein response and increased longevity: Cause, consequence, or correlation?

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Abstract

The mitochondrial unfolded protein response is a conserved pathway that allows mitochondrial chaperones and other factors to be induced in response to mitochondrial dysfunction. Activation of this pathway has been proposed to underlie lifespan extension from knockdown or mutation of several nuclear encoded mitochondrial genes in Caenorhabditis elegans. In some cases, however, induction of the mitochondrial unfolded protein response is associated with a reduction of lifespan in both yeast and C. elegans. It also has yet to be demonstrated that induction of the mitochondrial unfolded protein response is sufficient to increase lifespan in the absence of overt mitochondrial dysfunction. In this perspective, we briefly review the evidence for and against a direct pro-longevity role of the mitochondrial unfolded protein response and suggest important areas of investigation for experimentally addressing this question.

Keywords

mitochondrial unfolded protein response; C. elegans; ATFS-1; prohibitins; HSP-6

1. Introduction

The idea that mitochondria play an important role in the basic biology of aging is well established. More than 50 years ago, Denham Harman proposed a theory of aging based on free radical chemistry that posited that aging is caused by free radicals produced within cells by “respiratory enzymes involved in the direct utilization of molecular oxygen” (Harman 1956). This idea has since been extended and modified in a variety of ways, but with the same central theme that oxidative byproducts of mitochondrial respiration create damage that accumulates with age and contributes to declines in cellular and tissue function. Many targets have been proposed for this damage, including mitochondrial DNA, nuclear DNA, lipids, and proteins.
A consequence of the free radical theory of aging has been the popularization of the idea that antioxidants should slow aging. The actual experimental evidence for this idea is mixed, however. In mammals, the best evidence that reducing mitochondrial oxidative stress can impact aging comes from studies of mice overexpressing transgenic catalase targeted specifically to mitochondria. These animals have extended lifespan and show improved healthspan by a variety of measures, including cardiac function, tumor burden, exercise tolerance, and inflammatory markers (Dai and others 2009; Li and others 2009; Schriner and others 2005; Treuting and others 2008). Other studies have failed to detect any link between oxidative stress and longevity, however, including lifespan analysis of several antioxidant deficient mouse lines that show no change in survival (Perez and others 2009). A series of survival studies by the National Institute on Aging Interventions Testing Program has also provided mixed results from dietary supplementation with different antioxidants. Most, including resveratrol, had no effect on lifespan, although aspirin and nordihydroguaiaretic modestly increased lifespan in male mice (Miller and others 2011; Miller and others 2007; Strong and others 2008). Epidemiological data in humans are also inconclusive, with some studies suggesting that antioxidant supplementation has no positive benefit on all-cause mortality and may even be harmful (Biesalski and others 2010; Bjelakovic and others 2007).

In recent years, a different view has gained popularity by proposing that, instead of being generally harmful, free radicals play an important role in cellular signaling which, under certain circumstances, is protective and even beneficial for longevity. For example, low doses (0.1 mM) of the superoxide generating compound paraquat have been shown to extend lifespan in Caenorhabditis elegans (Yang and Hekimi 2010), as has deletion of the mitochondrial superoxide dismutase gene sod-2 (Van Raamsdonk and Hekimi 2009). These and additional data in worms, along with complementary evidence in both yeast and flies, has led to the popularization of the “mitohormesis” model. This model suggests that reactive oxygen species produced by mitochondria result in an adaptive response that promotes cellular health and organismal longevity (reviewed in (Pan 2011; Ristow and Schmeisser 2011; Ristow and Zarse 2010)).

In particular, the mitohormesis model proposes that the degree of mitochondrial dysfunction and reactive oxygen species production is important for the ultimate effect on longevity and healthspan. Specifically, low levels of potentially damaging reactive oxygen species activate beneficial cellular stress responses and signaling pathways, while higher levels are detrimental, resulting in frailty or premature death. Conditions suggested to promote longevity through reactive oxygen species include inhibition of glycolysis (Schulz and others 2007), impaired insulin-like signaling (Zarse and others 2012), and mutations in mitochondrial electron transport chain (ETC) components (Yang and Hekimi 2010), among others. Despite the appeal of this model and some experimental support, there is limited direct evidence correlating the amount of oxidative stress with longevity. For example, there is increased oxidative damage in several of the mitochondrial mutants in C. elegans, but in some cases, such as in the complex I mutant gas-1(fc21) and complex III mutant isp-1(qm150), similar levels of oxidative damage result in vastly different effects on lifespan (Dingley and others 2010). In order to fully understand the relationship between mitochondrial damage and longevity, multiple parameters of mitochondrial health including...
mitochondrial membrane potential, oxidative damage of different substrates, and unfolded protein load need to be assessed.

2. The mitochondrial unfolded protein response and lifespan extension

This idea that mitochondrial dysfunction could promote longevity rather than limit it first gained prominence from parallel genome-wide RNAi screens carried out in C. elegans (reviewed in (Hwang and others 2012; Yanos and others 2012)). Both screens identified multiple RNAi clones corresponding to ETC components that increased lifespan when knockdown occurred during development but not adulthood (Dillin and others 2002; Lee and others 2003). Subsequently, it was shown that this window of opportunity where ETC knockdown can robustly promote longevity occurs during the L3/L4 larval stage of development, and that the effect on lifespan is highly sensitive to the degree of mitochondrial knockdown (Rea and others 2007). In addition to RNAi knockdown, a few mutations that perturb mitochondrial function and extend lifespan have also been identified. These include mutation of the gene encoding a coenzyme Q biosynthetic enzyme, clk-1, the Rieske iron-sulfur protein gene isp-1, or the thiamine pyrophosphokinase gene tpk-1 (Butler and others 2013; de Jong and others 2004; Felkai and others 1999; Feng and others 2001; Lakowski and Hekimi 1996).

The mitochondrial unfolded protein response (UPR\text{mt}) is a stress response first identified from human cells in culture where it was observed that several mitochondrial chaperones and heat shock proteins are induced in response to ethidium bromide treatment or expression of an unstable mitochondrially localized enzyme (Martinus and others 1996; Ryan and Hoogenraad 2007; Zhao and others 2002). Recent studies have identified a UPR\text{mt} in C. elegans that appears similar to that of mammals (Benedetti and others 2006; Durieux and others 2011; Haynes and others 2007; Haynes and others 2010; Yoneda and others 2004). Induction of the UPR\text{mt} in C. elegans results in transcriptional up-regulation of the mitochondrial chaperone genes hsp-6 and hsp-60, and RNAi knockdown of a subset of ETC components has been shown to induce the UPR\text{mt} using GFP reporters for hsp-6 and hsp-60 (Durieux and others 2011; Yoneda and others 2004). Inducing mitochondrial stress through treatment of worms with chemicals that impair mitochondrial function, including ethidium bromide, paraquat, antimycin A, and rotenone, is also sufficient to induce the reporter (Runkel and others 2013; Shore and others 2012; Yoneda and others 2004).

The details of the C. elegans UPR\text{mt} are still being worked out, with several factors having been identified as necessary for full induction in response to different forms of mitochondrial stress. The HAF-1 peptide exporter (Haynes and others 2010), the CLPP-1 protease (Haynes and others 2007), a ubiquitin-like protein UBL-5 (Benedetti and others 2006), and two transcription factors, DVE-1, and ATFS-1 (ZC376.7) were shown to be necessary for induction of hsp-60\text{p::gfp} in an uncharacterized mutant (referred to as zc32) showing constitutive activation of the reporter and for larval development in animals with high levels of mitochondrial stress (Haynes and others 2007; Haynes and others 2010; Nargund and others 2012). More recently, a screen for RNAi clones that prevent induction of the UPR\text{mt} following treatment with paraquat identified ATFS-1 along with 54 additional factors, including two vacuolar ATPase subunits, proteasomal regulatory subunits, cytosolic
chaperonins, and several ribosomal protein genes (Runkel and others 2013). About half of these were specific for paraquat induction of the hsp-6ₚ::gfp reporter, while RNAi knockdown of the others also prevented induction of this reporter in zc32 animals. Further characterization of these factors will be important to determine which specifically respond to mitochondrial stress and also to which types of mitochondrial stress. For example, it has been recently shown that HAF-1 is not required for induction of caused by paraquat (Runkel and others 2013), or for induction of hsp-60ₚ::gfp by high dose ethidium bromide treatment or RNAi knockdown of several mitochondrial factors, including cco-1, spg-7, tim-23, and tomm-40 (Nargund and others 2012). Therefore, it is possible that many identified UPR^{mt} factors are specific to a subset of mitochondrial stress conditions, such as the zc32 mutation or paraquat, and play a less general role in the UPR^{mt} than currently assumed.

The UPR^{mt} was first implicated in aging by Durieux et al. (Durieux and others 2011), who reported that lifespan extension from mutations in isp-1 or clk-1 could be suppressed by RNAi knockdown of ubl-5, dve-1, hsp-6, hsp-60, or clpp-1. This study also showed that neuronal knockdown of the cytochrome c oxidase subunit gene, cco-1, was sufficient to induce the hsp-6ₚ::gfp reporter in the intestine, suggesting that a signal is transduced from neurons to peripheral cells in response to mitochondrial stress. Based on these and other observations, Durieux et al. (Durieux and others 2011) proposed that the UPR^{mt} is a “potent transducer of the ETC longevity pathway”. Further support for this model was provided by a subsequent study reporting that ubl-5(RNAi) can attenuate lifespan extension from knockdown of cco-1 or mrps-5, which encodes a mitochondrial ribosomal protein (Houtkooper and others 2013). There are significant limitations in the experimental validation of the UPR^{mt}-longevity model, however, which is discussed further in Section 4 below.

3. The mitochondrial unfolded protein response and lifespan reduction

The mitochondrial prohibitins (PHB1 and PHB2) are a highly conserved protein pair that form a ring-like structure in the mitochondrial inner membrane and influence mitochondrial respiration, mitochondrial fusion, and mitochondrial protein quality control (Arnold and Langer 2002; Merkwirth and others 2008; Nijtmans and others 2002; Tatsuta and others 2005). Prohibitin deficiency induced by RNAi knockdown results in reduced lifespan in C. elegans and deletion of either prohibitin gene, PHB1 or PHB2, shortens replicative lifespan in the budding yeast Saccharomyces cerevisiae (Artal-Sanz and Tavernarakis 2009; Coates and others 1997; Piper and Bringloe 2002; Piper and others 2002). A recent study showed that, in addition to shortening lifespan, prohibitin deficiency also causes enrichment of several UPR^{mt} components in mitochondria of yeast cells and increased expression of the hsp-6ₚ::gfp and hsp-60ₚ::gfp reporters in worms (Schleit and others 2013). Both the reduction in lifespan and apparent induction of the UPR^{mt} were suppressed in each organism by reducing cytoplasmic translation, which was accomplished by dietary restriction or by inhibition of components of the mechanistic target of rapamycin (mTOR) pathway (Schleit and others 2013).

Thus, contrary to the model that the UPR^{mt} promotes longevity, in the case of prohibitin deficiency at least, induction of the UPR^{mt} is associated with reduced lifespan, and
interventions that suppress this reduced lifespan also suppress the UPR\textsuperscript{mt}. One interpretation of these data is that the UPR\textsuperscript{mt} itself plays no beneficial role in enhancing longevity and may actually limit lifespan under some conditions. An alternative possibility is that in some cases the negative consequences of mitochondrial stress can offset any benefits from induction of the UPR\textsuperscript{mt} and result in a net shortening of lifespan, such as in the case of prohibitin deficiency. Interestingly, the combination of \textit{phb-2}(RNAi) with the long-lived insulin/insulin-like growth factor (IGF) receptor DAF-2 mutant results in a large increase in lifespan (Artal-Sanz and Tavernarakis 2009). Thus, inhibition of the insulin-like signaling may attenuate the high levels of mitochondrial proteotoxic stress caused by \textit{phb-2}(RNAi) allowing lifespan extension through the UPR\textsuperscript{mt}. This explanation appears overly simplistic, however, as \textit{phb-2}(RNAi) also extends the lifespans of \textit{gas-1}, \textit{mev-1}, \textit{isp-1}, and \textit{clk-1} mitochondrial mutant animals (Artal-Sanz and Tavernarakis 2009), which already possess increased levels of oxidative damage and mitochondrial dysfunction (Dingley and others 2010; Kayser and others 2004; Yang and Hekimi 2010). In order to understand the relationship between prohibitins, the UPR\textsuperscript{mt}, and longevity, a greater understanding of the biology of the prohibitins and the mitochondrial defects associated with prohibitin deficiency will be required.

In addition to the prohibitin mutants, there are other cases where mitochondrial dysfunction is associated with reduced lifespan in \textit{C. elegans}. This includes mutation of the cytochrome b gene \textit{mev-1} (Ishii and others 1998) or the NDUF52 homologue \textit{gas-1} (Kayser and others 2004). Likewise, RNAi knockdown of some mitochondrial genes can shorten lifespan when knockdown exceeds a certain threshold (Rea and others 2007). For example RNAi knockdown of the mitochondrial ATPase subunit gene \textit{atp-3} extends lifespan when it is diluted 1:10 but shortens lifespan when undiluted (Rea and others 2007). In general, for \textit{atp-3}(RNAi) and other mitochondrial RNAi treatments, the level of knockdown correlates with \textit{hsp-6}\textsubscript{p}::\textit{gfp} induction (Ventura and Rea 2007). However, the level of UPR\textsuperscript{mt} activation does not necessarily correlate with lifespan extension. In addition, the differential lifespan effects caused by distinct mitochondrial perturbations may be caused by the specific deficiency rather than level of UPR\textsuperscript{mt} activation. This may be one reason that the UPR\textsuperscript{mt} is not correlated with longevity, especially when mitochondrial dysfunction is elevated past a certain threshold.

4. Experimental deficiencies in the UPR\textsuperscript{mt}-longevity model

In addition to examples where UPR\textsuperscript{mt} induction is not associated with longer lifespan, it is still unclear whether the UPR\textsuperscript{mt} is either necessary or sufficient to account for lifespan extension in response to mitochondrial mutation or knockdown of ETC genes. As described above, the experimental evidence suggesting that the UPR\textsuperscript{mt} is necessary for lifespan extension arises from two studies reporting that RNAi knockdown of different UPR\textsuperscript{mt} factors can suppress lifespan extension in response to certain forms of mitochondrial dysfunction (Table 1). One of these reports also argues that the degree of induction of the UPR\textsuperscript{mt} correlates with the magnitude of lifespan extension following RNAi knockdown of different mitochondrial ribosomal protein genes (Houtkooper and others 2013). They show that GFP expression in animals expressing the \textit{hsp-6}\textsubscript{p}::\textit{gfp} reporter is highly correlated with the percent lifespan extension from mitochondrial ribosomal protein RNAi across 8 different
RNAi clones that induce hsp-6::gfp five to fifteen-fold. This is difficult to interpret, however, given that they also report in the same figure that knockdown of the UPR^mt^ factor haf-1 prevents lifespan extension in mrps-5(RNAi) animals, even though the hsp-6::gfp reporter is still induced about 15-fold.

The observation that haf-1(RNAi) only modestly impairs induction of the hsp-6::gfp reporter but does prevent lifespan extension following mrps-5 knockdown (Houtkooper and others 2013) is particularly relevant, given that the other similar published experiments fail to show that the UPR^mt^ is attenuated or blocked upon knockdown of UPR^mt^ factors. For example, the studies reporting that ubl-5(RNAi) prevents lifespan extension in isp-1(qm150) or clk-1(e2519) animals did not also provide corresponding evidence that ubl-5(RNAi) prevented induction of the UPR^mt^ in those cases. These data are further complicated by the fact that any role for UBL-5 as a regulator of the UPR^mt^ may be non-specific, as the yeast ortholog of UBL-5 (Hub1) is localized to spliceosomes where it functions as a determinant of alternative pre-mRNA splicing (Mishra and others 2011). A related weakness is the assumption that the hsp-6::gfp reporter is a faithful reporter of endogenous activation or repression of the UPR^mt^. Several additional UPR^mt^ targets have been identified in mammalian cells (Aldridge and others 2007) and, in a few cases, validated in C. elegans (Nargund and others 2012). In addition, the UPR^mt^ transcription factor ATFS-1 has been shown to regulate numerous genes beyond hsp-6 and other mitochondrial chaperones, including a large set of detoxification and metabolic genes (Nargund and others 2012). Despite this wealth of information, the majority of studies linking the UPR^mt^ to aging in C. elegans have failed to quantify the expression of endogenous UPR^mt^ targets in the context of genetic experiments interpreted to support the UPR^mt^ longevity model.

Recent studies of the ATFS-1 transcription factor suggest a potential path toward addressing the question of whether the UPR^mt^ plays a direct role in aging. ATFS-1 contains a mitochondrial targeting sequence that causes it to be imported into the mitochondria where it is degraded (Nargund and others 2012). Upon induction of the UPR^mt^, import of ATFS-1 into the mitochondria is impaired, causing ATFS-1 to relocalize to the nucleus where it is involved in regulation of numerous UPR^mt^ target genes. Deletion or RNAi knockdown of atfs-1 prevents induction of the hsp-60::gfp reporter in response to treatment with paraquat or RNAi knockdown of the mitochondrial metalloprotease gene spg-7, as well as preventing induction of at least three endogenous UPR^mt^ genes, dnj-10, tim-23, and gpd-2 in response to spg-7(RNAi) (Nargund and others 2012). Thus, ATFS-1 appears to be more specifically and directly required for induction of the UPR^mt^ than other factors such as HAF-1 and UBL-5. It would be informative to know, therefore, whether deletion of atfs-1 prevents lifespan extension in response to different forms of mitochondrial stress such as RNAi knockdown of ETC components and mutation of isp-1 or clk-1.

ATFS-1 also provides an opportunity to directly test whether induction of the UPR^mt^ is sufficient to extend lifespan in the absence of overt mitochondrial dysfunction. Deletion of the mitochondrial targeting sequence of atfs-1 results in a constitutively active UPR^mt^, presumably because ATFS-1 is no longer targeted for mitochondrial degradation (Nargund and others 2012). Based on the UPR^mt^ longevity model, this should result in increased lifespan. Initial experiments using gain-of-function mutations in the atfs-1 mitochondrial
targeting domain suggest that this may not be the case, however. Several point mutations within the mitochondrial targeting sequence of *atfs-1* were isolated from a screen for enhanced resistance to statins (Rauthan and others 2013). Two of these alleles, *atfs-1(et15)* and *atfs-1(et17)*, were shown to result in constitutive activation of the *hsp-60p::gfp* reporter, but an initial lifespan study failed to detect increased longevity (Rauthan and others 2013). One possibility is that ATFS-1 normally functions in the mitochondria, and reducing its mitochondrial import could lead to mitochondrial dysfunction and prevent lifespan extension. However, mitochondrial respiration is not perturbed in *atfs-1(et15)* animals arguing against a direct role in oxidative metabolism (Rauthan and others 2013). Therefore, if these alleles indeed generally activate the UPR\textsubscript{mt} but do not increase lifespan, this would argue against the hypothesis that the UPR\textsubscript{mt} directly promotes longevity and greatly weaken the UPR\textsubscript{mt}-longevity model.

Whether the UPR\textsubscript{mt} directly promotes longevity or not, this pathway clearly plays a central role in coping with mitochondria proteotoxic stress. Going forward, it is important to gain more detailed mechanistic insight into the various forms of mitochondrial stress and the multiple outputs that can be potentially engaged by the cell to respond to that stress. Induction of mitochondrial chaperones is clearly a robust component of the UPR\textsubscript{mt}, but it is not the only component. In addition to the diverse transcriptional response regulated by ATFS-1, there is an equally relevant translational response mediated by GCN-2 in *C. elegans* (Baker and others 2012). New evidence also suggests important links between mitochondrial dysfunction and SIRT3 (Karamanlidis and others 2013; Papa and Germain 2013), mTOR (Johnson and others 2013), the PINK1/PARKIN pathway (Jin and Youle 2013; Pimenta de Castro and others 2012), and several additional factors that have been proposed to mediate lifespan extension following mitochondrial stress. In particular, there is evidence that several transcription factors, including HIF-1 (Lee and others 2010), CEH-23 ( Walter and others 2011), and TAF-4 (Khan and others 2013), can be induced by ETC inhibition and may promote longevity in response. In the case of HIF-1, this is further supported by the fact that activation of HIF-1 in the absence of mitochondrial stress is sufficient to extend lifespan in *C. elegans* (Leiser and others 2011; Mehta and others 2009; Muller and others 2009; Zhang and others 2009). Understanding how mitochondrial dysfunction triggers these factors and what the mechanisms are that determine the ‘tipping point’ between mitohormesis and pathology will be essential for understanding the relationship between mitochondrial function, aging, and disease.

### 5. Conclusion

The UPR\textsubscript{mt} is a highly conserved response to mitochondrial stress that may also modulate aging. Several interventions that extend lifespan in *C. elegans* by impairing mitochondrial function also induce fluorescent reporters of mitochondrial chaperones involved in the UPR\textsubscript{mt}. It has been proposed that induction of the UPR\textsubscript{mt} plays a direct, causal role in this lifespan extension; however, there are significant weaknesses in this model that require further experimental validation. Given the central importance of mitochondria in aging and age-associated diseases, clarifying the impact of the UPR\textsubscript{mt} on this process is necessary for the field to move forward.
Acknowledgments

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References


Highlights

1. The UPR\textsuperscript{mt} is a conserved response to mitochondrial stress
2. Several perturbations that induce the UPR\textsuperscript{mt} extend lifespan in \textit{C. elegans}
3. In some cases, the UPR\textsuperscript{mt} is associated with shortened or normal lifespan
4. The UPR\textsuperscript{mt} is proposed to promote longevity in \textit{C. elegans}
5. Key experimental support of the UPR\textsuperscript{mt} longevity model is lacking
Several genetic interventions have been shown to reduce mitochondrial function, increase lifespan, and induce the \textit{hsp-6\textsubscript{p}}::\textit{gfp} reporter. Data has been presented in a few cases suggesting that attenuating the UPR\textsubscript{mt} also blocks lifespan extension in this context. Cases where the “UPR\textsubscript{mt} attenuating intervention” prevented lifespan extension non-specifically in \textit{daf-2} and \textit{eat-2} mutants are not shown. “Not done” means this control was not provided in the report, and “-” indicates this could not be determined because of the missing control.

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<th>Lifespan extending/ \textit{hsp-6\textsubscript{p}}::\textit{gfp} -inducing intervention</th>
<th>UPR\textsubscript{mt} Attenuating Intervention</th>
<th>Effect on lifespan extension</th>
<th>Effect on \textit{hsp-6\textsubscript{p}}::\textit{gfp}</th>
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