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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.

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 et al., 2009; Li et al., 2009; Schriner et al., 2005; Treuting et al., 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 et al., 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 et al., 2007, 2011; Strong et al., 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 et al., 2010; Bjelakovic et al., 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

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higher levels are detrimental, resulting in frailty or premature death. Conditions suggested to promote longevity through reactive oxygen species include inhibition of glycolysis (Schulz et al., 2007), impaired insulin-like signaling (Zarse et al., 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 et al., 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 et al. (2012) and Yanos et al. (2012)). Both screens identified multiple RNAi clones corresponding to ETC components that increased lifespan when knockdown occurred during development but not adulthood (Dillin et al., 2002; Lee et al., 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 et al., 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 et al., 2013; de Jong et al., 2004; Felkai et al., 1999; Feng et al., 2001; Lakowski and Hekimi, 1996).

The mitochondrial unfolded protein response (UPR^{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 et al., 1996; Ryan and Hoogenraad, 2007; Zhao et al., 2002). Recent studies have identified a UPR^{mt} in *C. elegans* that appears similar to that of mammals (Benedetti et al., 2006; Durieux et al., 2011; Haynes et al., 2007; Haynes et al., 2010; Yoneda et al., 2004). Induction of the UPR^{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^{mt} using GFP reporters for *hsp-6* and *hsp-60* (Durieux et al., 2011; Yoneda et al., 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 et al., 2013; Shore et al., 2012; Yoneda et al., 2004).

The details of the *C. elegans* UPR^{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 et al., 2010), the CLPP-1 protease (Haynes et al., 2007), a ubiquitin-like protein UBL-5 (Benedetti et al., 2006), and two transcription factors, DVE-1, and ATFS-1 (ZC376.7) were shown to be necessary for induction of *hsp-60*$_p$::*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 et al., 2007, 2010; Nargund et al., 2012). More recently, a screen for RNAi clones that prevent induction of the UPR^{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 et al., 2013). About half of these were specific for paraquat induction of the *hsp-6*$_p$::*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 caused by paraquat (Runkel et al., 2013), or for induction of *hsp-60*$_p$::*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 et al., 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 et al., 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-6*$_p$::*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. (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 et al., 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 et al., 2008; Nijtmans et al., 2002; Tatsuta et al., 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 et al., 1997; Piper and Bringloe, 2002; Piper et al., 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-6*$_p$::*gfp* and *hsp-60*$_p$::*gfp* reporters in worms (Schleit et al., 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 et al., 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^{mt}. One interpretation of these data is that the UPR^{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^{mt} and result in a net shortening of lifespan, such as in the case of prohibitin deficiency. Interestingly, the combination of *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

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