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Review

# Mitochondrial stress: Balancing friend and foe

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ABSTRACT

Mitochondria are vital organelles of the aerobic eukaryotic cell. Their dysfunction associates with aging and widespread age-related diseases. To sustain mitochondrial integrity, the cell executes a distinct set of stress-induced protective responses.
The mitochondrial unfolded protein response (UPR$^{mt}$) is a response of the cell to mitochondrial damage. The transcription factor ATFS-1 triggers UPR$^{mt}$ effector gene expression in the nucleus. The selective exclusion of ATFS-1 from mitochondrial import by stress-induced alterations of the mitochondrial membrane potential is currently discussed as key activation mechanism. Surprisingly, UPR$^{mt}$ activation often coincides with a lifespan extension in *Caenorhabditis elegans* and the same has recently been reported for mammalian cells.
This review summarizes the current model of the UPR$^{mt}$, its inducers, and its crosstalk with other cellular stress responses. It focuses on the role of mitochondrial function as a regulator of aging and longevity.


## 1. Introduction

Despite their endosymbiotic origin, mitochondria have been integrated well into the hosts' cellular network and have developed to vital organelles of aerobic cells. Mitochondria are the prime cellular ATP suppliers. They functionally interact with other organelles like the endoplasmatic reticulum (ER), are embedded in many cellular signaling pathways including apoptosis, and play a central role in metabolism. Mitochondria communicate with the nucleus to modify gene expression as a response to altered metabolic demand and stress. It is therefore not surprising that the importance of mitochondria in the process of aging and longevity and a number of age-related diseases, such as Alzheimer's and Parkinson's Disease, begins to emerge [for review, see (Balaban et al., 2005; Ivanova and Yankova, 2013)].

To counteract stress and to protect cells from stress-induced damage, mitochondria elicit several responses to stress that may be executed sequentially (Fig. 1). An early response is the induction of the mitochondrial unfolded protein response (UPR$^{mt}$), which results in the activation of a signaling cascade to the nucleus and the expression of nuclearly encoded mitochondrial chaperone genes. The newly synthesized chaperones are then transported to the mitochondria where their function is required [for review, see (Haynes and Ron, 2010; Pellegrino et al., 2013)]. Activation of the mitochondrial fission/fusion and, in severe cases, their destruction by the autophagic machinery represent other responses to mitochondrial stress. By fusion of damaged mitochondrial sections with healthy organelles damage can be compensated. Alternatively, damaged mitochondrial sections are isolated via fission and removed by a variant of autophagy, termed mitophagy. If mitochondrial function cannot be restored, a death signal is released from the mitochondria to initiate the apoptotic removal of the entire cell [for review, see (Shutt and McBride, 2013; Youle and van der Bliek, 2012)]. This review, which accompanies a second review in the same issue of this journal, presents our current knowledge on the UPR$^{mt}$ that is mostly derived from experiments done in the nematode *Caenorhabditis elegans*.

## 2. The mitochondrial unfolded protein response

The existence of an UPR$^{mt}$ as a response to mitochondrial stress has initially been proposed by the Hoogenraad laboratory in mammalian cells (Martinus et al., 1996; Zhao et al., 2002). A related retrograde response in the nematode *C. elegans* was first identified by the Ron laboratory (Yoneda et al., 2004). Both groups showed that UPR$^{mt}$ signaling results in the induced expression of nuclear-encoded mitochondrial chaperone genes. Subsequent research in mammalian cells and *C. elegans* revealed mechanistic components of the UPR$^{mt}$ [(Aldridge et al., 2007; Benedetti et al., 2006; Haynes et al., 2007, 2010; Horibe and Hoogenraad, 2007; Zhao et al., 2002); for review, see (Haynes and Ron, 2010)]. An elegant model based on a novel mechanism of transcription factor activation was suggested (Nargund et al., 2012). According to this model, the bZip transcription factor ATFS-1 serves as a key regulator of the UPR$^{mt}$ (Haynes et al., 2010). Remarkably, it contains both a nuclear translocation signal and a mitochondrial targeting

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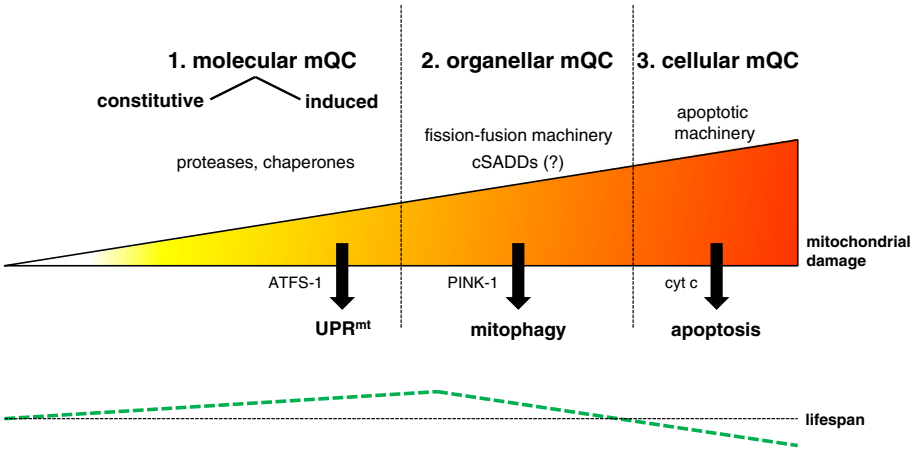


**Fig. 1.** Mitochondrial quality control mechanisms and their relation to lifespan. A constitutively active network of mitochondrial proteases and chaperones maintains organellar proteostasis. It is suggested that increasing mitochondrial damage triggers a hierarchical set of responses: (1) The UPR$^{mt}$, as the first line of defense, induces the transcription of nuclearly encoded protective genes, among them mitochondrial chaperones. (2) With the activation of the fission/fusion machinery damaged mitochondria can hyperfuse with healthy mitochondrial fractions to complement existing damage, or are separated from functional mitochondria by fission and degraded by mitophagy [for review, see (Youle and van der Bliek, 2012)]. Activation of cSADDs to engage organismal survival is another line of defense (Melo and Ruvkun, 2012). (3) Eventually, harsh mitochondrial damage results in cell death by the induction of apoptosis. Mild mitochondrial damage, which induces the UPR$^{mt}$, seems to correlate with lifespan extension. Notably, in some cases, induction of the UPR$^{mt}$ instead coincides with a reduction of lifespan (see Table 1).

sequence (MTS), a key finding leading to the revelation of its activation mechanism. Under basal conditions, ATFS-1 is by default imported into the mitochondria via the TIM/TOM import machinery, where it is proteolytically inactivated by the matrix resident Lon protease. Upon mitochondrial stress, ATFS-1 import is attenuated, which was proposed as a consequence of mitochondrial membrane potential ($\Delta\psi$) dissipation. As a result, ATFS-1 degradation is prevented, allowing its nuclear translocation where it activates target gene transcription. Truncation of its MTS suffices to promote nuclear localization of ATFS-1 (Nargund et al., 2012). With this observation, the requirement of the other identified components of the UPR$^{mt}$, such as HAF-1 (Haynes et al., 2010), in principle can be reduced to an accessory role (Nargund et al., 2012; Runkel et al., 2013). The caveat here is that, so far, no direct $\Delta\psi$ measurements have been correlated to ATFS-1/UPR$^{mt}$ induction. A decrease of $\Delta\psi$ (Senoo-Matsuda et al., 2003) as well as UPR$^{mt}$ induction (Durieux et al., 2011) have been observed in the *mev-1*(*kn1*) mutant. *mev-1* encodes cytochrome b, which is a subunit of complex II of the electron transport chain (ETC). Notably, the addition of the mitochondrial uncoupling agent 2,4-dinitrophenol (DNP) that should reduce $\Delta\psi$ did not promote UPR$^{mt}$ induction (Yoneda et al., 2004), which may indicate that the reduction of $\Delta\psi$ alone probably is not sufficient to activate ATFS-1. How mitochondria on the one hand prevent the mitochondrial import of ATFS-1 as a prerequisite of its activation, but allow import of UPR$^{mt}$ effector proteins (mitochondrial chaperones) is another open question. The import of ATFS-1 might be more sensitive towards attenuation of the mitochondrial import efficiency than the import of HSP-6/60, or accessory proteins may be involved in blocking ATFS-1 mitochondrial import, but not that of the chaperones.

Interestingly, the mitochondrial serine/threonine kinase PINK1 is also activated by $\Delta\psi$ dissipation. In a mechanism related to ATFS-1, PINK1, in the absence of stress is imported into mitochondria, where it is processed and, unlike ATFS-1, re-translocated to the cytoplasm for proteasomal degradation [(Yamano and Youle, 2013); for review, see (Matsuda et al., 2013)]. Following mitochondrial stress and $\Delta\psi$ reduction, PINK1 mitochondrial import and degradation are prevented, resulting in its accumulation at the mitochondrial outer membrane. There, PINK1 recruits the E3 ubiquitin ligase parkin and induces mitophagy (Bertolin et al., 2013; de Vries and Przedborski, 2013; Springer and Kahle, 2011).

It would be interesting to see whether ATFS-1 or PINK-1 import is gradually attenuated with decreasing $\Delta\psi$. If ATFS-1 import is more sensitive to $\Delta\psi$ alterations than PINK1, this could be a mechanism to coordinate UPR$^{mt}$ and mitophagy induction with increasing mitochondrial stress.

According to such a model, the TIM/TOM import complexes were the main sensors of mitochondrial stress and perturbations of the mitochondrial membrane potential.

## 3. Inducers of the UPR$^{mt}$

Mitochondrial proteostasis is sustained by resident chaperones and proteases which are commonly referred to as the mitochondrial quality control (mQC) machinery [for review, see (Anand et al., 2013; Gerdes et al., 2012)]. These include the conserved mitochondrial matrix chaperones mtHsp70 and Hsp60, as well as the Lon and ClpXP proteases (Baker et al., 2012; for review, see Baker and Haynes, 2011). Proteostasis is challenged by a number of impacts, mainly by the accumulation of misfolded proteins and by the excessive production of reactive oxygen species (ROS) that readily damage proteins and DNA. Since most protein complexes of the ETC are composed of a mixture of nuclearly and mitochondrially encoded proteins, correct folding of both has to be assured for ETC complex integrity. In *C. elegans*, UPR$^{mt}$ induction can be monitored with reporter transgenes in which the promoters of the mQC chaperone genes *hsp-6* (*mtHsp70*) or *hsp-60* (*Hsp60*) are fused to *gfp* (Yoneda et al., 2004). These reporter transgenes appear to have distinct sensitivities and can respond differently to damage (Runkel et al., 2013).

### 3.1. Interference with the mitochondrial quality control machinery

Mitochondrial protein stress occurs as soon as mQC capacity cannot cope anymore with unfolded or misfolded protein load, i.e. if the performance of mQC is reduced or if the concentration of un-/misfolded proteins increases. Accordingly, reduction of matrix mQC chaperone genes triggers UPR$^{mt}$ signaling in both mammalian cells and *C. elegans* (Yoneda et al., 2004; Zhao et al., 2002), indicating that mQC integrity or performance is monitored constantly. Knockdown of the matrix protease gene *spg-7* activates UPR$^{mt}$ as well (Yoneda et al., 2004). In contrast, the knockdown of the matrix mQC proteases ClpXP or Lon does not induce UPR$^{mt}$ signaling in *C. elegans* (Haynes et al., 2007; Nargund et al., 2012). To this end a mechanistic involvement of both proteases in the signaling response itself has been proposed [(Haynes et al., 2007; Nargund et al., 2012), as discussed above]. Although it is

intuitively assumed that decreased mQC capacity leads to an accumulation of mis-/unfolded proteins no direct experimental approaches have been developed to study this aspect.

### 3.2. Increase of reactive oxygen species (ROS)

Mainly because the way the ETC handles electron transfer is error prone, mitochondria are considered as the prime source of cellular ROS in aerobic eukaryotes [for review, see (Brand, 2010; Tahara et al., 2009; Turrens, 2003)]. Basally produced ROS are scavenged by buffering systems to prevent ROS-induced damage [for review, see (Sharma et al., 2012)]. Non-physiologically increased ROS levels, however, unleash protective responses, among them the UPR$^{mt}$, to reduce ROS-induced protein damage (Nystrom, 2005; Yang and Hekimi, 2010a).

Several ETC mutants with elevated ROS levels have been described in *C. elegans*. These include mutations in *mev-1* (cytochrome b in respiratory complex II) (Ishii et al., 1990, 1998; Senoo-Matsuda et al., 2001), *clk-1*, encoding a demethoxyubiquinone (DMQ) hydroxylase (Lee et al., 2010; Miyadera et al., 2001; Yang and Hekimi, 2010a), *isp-1*, encoding a Rieske iron sulfur protein (ISP) which is a subunit of complex III (Feng et al., 2001; Lee et al., 2010; Yang and Hekimi, 2010a), and *nuo-6*, the NDUFB4/B15 subunit of complex I (Yang and Hekimi, 2010b). These mutants constantly induce the UPR$^{mt}$ visualized by a constitutive expression of the *hsp-6*::*gfp* reporter (see Table 1). RNAi with *cco-1* (cytochrome c oxidase in complex IV) also triggers UPR$^{mt}$ (Durieux et al., 2011), as it was observed for the ETC inhibitors rotenone (targeting complex I) and antimycin A (targeting complex III) (Runkel et al., 2013; Shore et al., 2012) which are commonly used to elevate ROS levels [for review, see (Degli Esposti, 1998; Nistico et al., 2011)]. The ROS generator paraquat [for review, see (Nistico et al., 2011)] was shown to increase ROS levels in *C. elegans* (Yang and Hekimi, 2010a) and to induce the UPR$^{mt}$ (Runkel et al., 2013; Yoneda et al., 2004). In summary, increasing mitochondrial ROS levels trigger the UPR$^{mt}$, probably to react to ROS-induced protein damage. Whether UPR$^{mt}$ induction is a direct consequence of ROS accumulation or results from secondary ROS-induced protein damage remains to be addressed.

Notably, ROS play a bifacial role in the context of mitochondrial integrity. While ROS can serve as signaling molecules to promote mitochondrial protection (Baker et al., 2012) and increase lifespan (Yang and Hekimi, 2010a), detrimental effects of ROS resulting in molecular damage and a shortened lifespan have been clearly demonstrated. This bifacial role of ROS suggested the concept of mitohormesis, which postulates that low levels of ROS are beneficial, acting as signaling molecules or upregulate protective responses (Lee et al., 2010; Yang and Hekimi, 2010a,b), whereas at high concentrations the damaging aspect of ROS predominates [for review, see (Hekimi et al., 2011; Ristow and Schmeisser, 2011; Ristow and Zarse, 2010)].

### 3.3. Generation of a mitonuclear imbalance

About 99% of the mitochondrial proteome is encoded in the nuclear DNA (nDNA) (Meisinger et al., 2008), while the remaining 12 (*C. elegans*) to 13 (*Homo sapiens*) proteins are derived from the mitochondrial genome (mtDNA). As a consequence, with the exception of complex II, ETC complexes comprise both nDNA- and mtDNA-encoded proteins in a defined stoichiometric ratio. Houtkooper et al. recently suggested that a stoichiometric mismatch of nuclearly and mitochondrially encoded proteins, termed a “mitonuclear protein imbalance”, suffices to trigger the UPR$^{mt}$. Indeed, interference either with the translation of nDNA- (by rapamycin) or, alternatively, with mtDNA-encoded genes (by doxycycline or by the depletion of proteins of the mitochondrial ribosomes) causes a stoichiometric imbalance and induces the UPR$^{mt}$ (Houtkooper et al., 2013). This interpretation is interesting, although at the moment it seems to be rather suggestive. It remains elusive why rapamycin, a chemical inhibitor of TOR, seems to induce the UPR$^{mt}$ (Houtkooper et al., 2013), as it was shown before that RNAi against the *C. elegans* homologue of mTOR *let-363* and *rheb-1*(*Rheb1*) suppresses, rather than induces the UPR$^{mt}$ [(Baker et al., 2012), and our unpublished observations]. Whereas Houtkooper et al. propose that interference with cytosolic translation causes a mitonuclear imbalance and as a consequence mitochondrial stress, Baker et al. suggested from their observations that suppression of the cytosolic translation relieves the unfolded protein load in mitochondria and thus protects from stress (Baker et al., 2012). A comparable protective mechanism had been shown before for the unfolded protein response of the endoplasmic reticulum (UPR$^{ER}$).

The mitochondrial imbalance model is further supported by the observation that interference with individual subunits of all ETC complexes generally triggers the UPR$^{mt}$, but not when the complex II component *mev-1* is depleted (Houtkooper et al., 2013). Houtkooper et al. suggest that *mev-1* depletion might be an inefficient inducer of UPR$^{mt}$, because all complex II components are encoded by nDNA, and so no mitonuclear imbalance would be achieved. This view is contradicted by the observation of UPR$^{mt}$ induction by *mev-1* RNAi (our lab, unpublished) as well as in the mutant *mev-1*(*kn1*) (Durieux et al., 2011).

In summary, more data have to be acquired to finally integrate all observations made and to pin down the eventual UPR$^{mt}$ trigger(s), such as increased ROS and/or unfolded proteins, a mitonuclear imbalance, or a decrease in the mitochondrial membrane potential $\Delta\psi$.

## 4. The crosstalk of the UPR$^{mt}$ with other stress responses

### 4.1. Cellular surveillance activated detoxification and defenses (cSADDs)

Interference with mitochondrial function not only triggers UPR$^{mt}$ signaling, but also initiates another set of cellular defense responses, the cellular surveillance-activated detoxification and defenses (cSADDs) (Melo and Ruvkun, 2012). Based on this model, *C. elegans* constantly surveys miscellaneous essential cellular functions and interprets their disruption as a putative pathogen attack. Consequently cSADDs can be triggered by several toxins and also by RNAi targeting key functions of the cell (Melo and Ruvkun, 2012) to activate anti-microbial effector genes and a mechanism of avoiding (potentially toxic) food. Notably, RNA interference of *spg-7* did trigger the ATFS-1 dependent expression not only of mitochondrial-protective genes (UPR$^{mt}$), but also of genes which are linked to antimicrobial defense (cSADDs) (Nargund et al., 2012).

Crosstalk between cSADDs and UPR$^{mt}$ is further supported by an UPR$^{mt}$ suppressor screen performed in our lab (Runkel et al., 2013). UPR$^{mt}$ was induced with comparably low concentrations of paraquat that, in contrast to *spg-7* RNAi, did not induce cSADDs. Surprisingly, most UPR$^{mt}$ suppressors that we identified by RNAi down-regulation are also cSADD activators, suggesting that in this experimental setup cSADD activation correlates with UPR$^{mt}$ suppression. We have proposed that, under conditions at which mild mitochondrial stress (low paraquat concentrations) is paired with severe non-mitochondrial stress induced by RNAi suppressing the UPR$^{mt}$, cSADDs are executed at the expense of UPR$^{mt}$ — possibly to spare resources for the antimicrobial defense. If, on the other hand, cSADDs are activated by severe mitochondrial stress (e.g. by *spg-7* RNAi), UPR$^{mt}$ would be promoted instead, to allow protective mechanisms to focus on mitochondria. Consistent with this view, severe perturbations of mitochondrial functions, like those induced by *spg-7* and *cco-1* RNAi or higher concentrations of paraquat, induce both cSADDs (Melo and Ruvkun, 2012) and UPR$^{mt}$ (Durieux et al., 2011; Melo and Ruvkun, 2012). This suggests that cSADDs and the UPR$^{mt}$ may be coordinated and their crosstalk allows distinct responses to insults of different impacts. Therefore, an inhibitory interference resulting from low mitochondrial stress may be turned into an activating signal as a consequence of stronger mitochondrial defects.

**Table 1**
Inducers of the UPR$^{mt}$ in *C. elegans*. *C. elegans* genes, which induce the UPR$^{mt}$ when depleted, were collected without bias from the literature and compared to the GenAge database (Build 16) (Tacutu et al., 2013). 58 of the 99 genes (59%) have been linked to extended lifespan, whereas only 7 genes (7%) have been linked to a shortened lifespan. This corresponds to a 25-fold enrichment of longevity promoting genes compared to the total genome, whereas lifespan decreasing genes are enriched only fivefold. The basis of this calculation is that GenAge reports an increased lifespan for 473 genes and a reduced lifespan for 226 genes compared to a total number of protein encoding genes of 20,537 in Wormpep234. *hsp-6*, one of only two genes for which both lifespan extending and reducing phenotypes have been reported, is discussed in more details in the text. UPR$^{mt}$ induction by the *mev-1*(*kn1*) mutant has been published in Durieux et al. (2011) and was observed by us with RNAi (unpublished), whereas no effect was reported by Houtkooper et al. (2013).

| Gene | Cosmid | UniProt | Lifespan | References |
|---|---|---|---|---|
| | B0205.6 | O61741 | | Melo and Ruvkun (2012) |
| *mrpl-47* | B0261.4 | Q2L6V2 | ↑ | Shore et al. (2012) and Yoneda et al. (2004) |
| *egl-8* | B0348.4 | Q95X30 | ↑ | Melo and Ruvkun (2012) |
| *eat-6* | B0365.3 | P90735 | ↑ | Melo and Ruvkun (2012) |
| *nhx-2* | B0495.4 | Q09432 | ↑ | Melo and Ruvkun (2012) |
| *mrps-30* | B0511.8 | O61818 | ↑ | Yoneda et al. (2004) |
| | C01A2.3 | O02207 | | Yoneda et al. (2004) |
| *atp-5* | C06H2.1 | Q17763 | ↑ | Shore et al. (2012) |
| *nuo-1* | C09H10.3 | Q17880 | ↑ | Shore et al. (2012) |
| *ril-2* | C14C10.3 | Q17973 | ↑ | Shore et al. (2012) |
| | C18E9.4 | Q18095 | ↑ | Shore et al. (2012) |
| *iars-2* | C25A1.7 | E2JL04 | | Yoneda et al. (2004) |
| | C33F10.12 | Q18390 | ↑ | Shore et al. (2012) |
| *atp-2* | C34E10.6 | P46561 | ↑ | Melo and Ruvkun (2012) and Shore et al. (2012) |
| *hsp-6* | C37H5.8 | P11141 | ↑ ↓ | Kimura et al. (2007), Ventura and Rea (2007), Yokoyama et al. (2002), and Yoneda et al. (2004) |
| *trim-9* | C39F7.2 | Q9UAQ3 | ↑ | Hamilton et al. (2005) |
| *tufm-2* | C43E11.4 | G5EDF5 | | Yoneda et al. (2004) |
| *sams-1* | C49F5.1 | O17680 | ↑ | Melo and Ruvkun (2012) and Shore et al. (2012) |
| *ril-1* | C53A5.1 | O17694 | ↑ | Shore et al. (2012) |
| *asg-2* | C53B7.4 | Q18803 | ↑ | Hamilton et al. (2005) and Shore et al. (2012) |
| *cyc-1* | C54G4.8 | Q18853 | ↑ | Shore et al. (2012), Ventura and Rea (2007), and Yoneda et al. (2004) |
| *rpl-24* | D1007.12 | O01868 | | Yoneda et al. (2004) |
| *rps-10* | D1007.6 | O01869 | ↑ | Yoneda et al. (2004) |
| *eat-3* | D2013.5 | Q18965 | ↑ | Our unpublished results |
| | D2030.4 | P90789 | ↑ | Hamilton et al. (2005), Rea et al. (2007), Shore et al. (2012), Ventura and Rea (2007), and Yoneda et al. (2004) |
| *mrps-5* | E02A10.1 | Q93425 | ↑ | Houtkooper et al. (2013) |
| *tkt-1* | F01G10.1 | O17759 | ↑ | Melo and Ruvkun (2012) |
| *flr-1* | F02D10.5 | G5EGI5 | | Melo and Ruvkun (2012) |
| *asb-2* | F02E8.1 | Q19126 | ↑ | Shore et al. (2012) |
| *pcyt-1* | F08C6.2 | P49583 | | Melo and Ruvkun (2012) |
| | F15D3.7 | Q9XVQ2 | | Melo and Ruvkun (2012) |
| *nduf-6* | F22D6.4 | Q19724 | | Yoneda et al. (2004) |
| *mrpl-54* | F25H5.6 | O17839 | | Yoneda et al. (2004) |
| | F26E4.6 | O18687 | ↑ | Hamilton et al. (2005), Shore et al. (2012), Ventura and Rea (2007), and Yoneda et al. (2004) |
| *cco-1* | F26E4.9 | P90849 | ↑ | Durieux et al. (2011), Hamilton et al. (2005), Melo and Ruvkun (2012), Shore et al. (2012), Ventura and Rea (2007), and Yoneda et al. (2004) |
| *atp-3* | F27C1.7 | P91283 | ↑ | Shore et al. (2012) and Ventura and Rea (2007) |
| | F27D4.1 | Q93615 | | Yoneda et al. (2004) |
| *itr-1* | F33D4.2 | Q9Y0A1 | ↑ | Melo and Ruvkun (2012) |
| *mrpl-1* | F33D4.5 | Q7KPW7 | ↑ | Houtkooper et al. (2013) |
| *elo-5* | F41H10.7 | Q20300 | ↓ | Melo and Ruvkun (2012) |
| *isp-1* | F42G8.12 | O44512 | ↑ | Cristina et al. (2009) |
| *idha-1* | F43G9.1 | Q93714 | ↑ | Shore et al. (2012) and Yoneda et al. (2004)) |
| *vha-17* | F49C12.13 | Q20591 | | Melo and Ruvkun (2012) |
| | F52A8.5 | Q20634 | | Yoneda et al. (2004) |
| *aco-2* | F54H12.1 | P34455 | ↑ | Hamilton et al. (2005) and Shore et al. (2012) |
| *mrpl-2* | F56B3.8 | O45110 | ↑ | Houtkooper et al. (2013) |
| *gsp-2* | F56C9.1 | P48727 | | Melo and Ruvkun (2012) |
| *frh-1* | F59G1.7 | Q9TY03 | ↑ ↓ | Rea et al. (2007) and Ventura and Rea (2007) |
| | F59C6.5 | Q93831 | ↑ | Shore et al. (2012) and Yoneda et al. (2004) |
| | H28O16.1 | Q9XXK1 | ↑ | Melo and Ruvkun (2012), Shore et al. (2012), and Yoneda et al. (2004) |
| *col-101* | K02D7.3 | O45105 | | Melo and Ruvkun (2012) |
| *nuo-4* | K04G7.4 | Q21233 | ↑ | Hamilton et al. (2005) and Shore et al. (2012) |
| *asg-1* | K07A12.3 | P90921 | ↑ | Yoneda et al. (2004) |
| *fah-1* | K10C2.4 | Q94272 | | Melo and Ruvkun (2012) |
| | K11B4.1 | G5ECC9 | ↑ | Yoneda et al. (2004) |
| *dld-1* | LLC1.3a | O17953 | ↑ | Melo and Ruvkun (2012) |
| *lin-61* | R06C7.7 | B2D6M2 | | Yoneda et al. (2004) |
| *mrps-6* | R12E2.12 | O61791 | | Yoneda et al. (2004) |
| | R53.4 | Q22021 | ↑ | Ventura and Rea (2007) |
| *pdha-1* | T05H10.6 | P52899 | | Melo and Ruvkun (2012) |
| *atp-4* | T05H4.12 | O16517 | ↑ | Shore et al. (2012) |
| *cchl-1* | T06D8.6 | P53703 | ↑ | Shore et al. (2012) |
| *mev-1* | T07C4.7 | P41956 | ↓ | Durieux et al. (2011) |
| | T08B1.1 | O44557 | | Melo and Ruvkun (2012) |

(continued on next page)

**Table 1** (*continued*)

| Gene | Cosmid | UniProt | Lifespan | References |
|---|---|---|---|---|
| *mrpl-23* | T08B2.8 | Q9GYS9 | | Yoneda et al. (2004) |
| | T09B4.9 | O02161 | | Yoneda et al. (2004) |
| | T10B5.4 | O76405 | | Melo and Ruvkun (2012) |
| *nuo-2* | T10E9.7 | O01602 | ↑ | Shore et al. (2012) and Yoneda et al. (2004) |
| *dnj-21* | T19B4.4 | P91454 | | Yoneda et al. (2004) |
| | T20H4.5 | Q22619 | ↑ | Hamilton et al. (2005) and Shore et al. (2012) |
| *mrpl-50* | T21B10.1 | Q22623 | ↑ | Shore et al. (2012) |
| *mrps-2* | T23B12.3 | O17004 | | Billing et al. (2012) |
| *phb-2* | T24H7.1 | P50093 | ↓ | Schleit et al. (2013) |
| | T25B9.10 | G5EBY9 | | Melo and Ruvkun (2012) |
| | T25B9.9 | Q17761 | | Melo and Ruvkun (2012) |
| *fat-6* | VZK822L.1 | G5EGN2 | | Melo and Ruvkun (2012) |
| *nuo-6* | W01A8.4 | Q23098 | | Yoneda et al. (2004) |
| *dlst-1* | W02F12.5 | O45148 | | Melo and Ruvkun (2012) |
| | W09C5.8 | Q9U329 | ↑ | Hamilton et al. (2005), Shore et al. (2012), Ventura and Rea (2007), and Yoneda et al. (2004) |
| *mecr-1* | W09H1.5 | O45903 | ↑ | Gurvitz (2009) |
| *pif-1* | Y18H1A.6 | G5EFV7 | | Yoneda et al. (2004) |
| *hsp-60* | Y22D7AL.5 | P50140 | | Melo and Ruvkun (2012) and Yoneda et al. (2004) |
| *cco-2* | Y37D8A.14 | P55954 | ↑ | Shore et al. (2012) |
| *mrps-10* | Y37D8A.18 | Q9XWV5 | ↑ | Billing et al. (2012) |
| *phb-1* | Y37E3.9 | Q9BKU4 | ↓ | Schleit et al. (2013) |
| *nuo-5* | Y45G12B.1 | Q9N4Y8 | ↑ | Shore et al. (2012) |
| *spg-7* | Y47G6A.10 | Q9N3T5 | ↑ | Shore et al. (2012) and Yoneda et al. (2004) |
| *mrpl-37* | Y48E1B.5 | O18199 | ↑ | Houtkooper et al. (2013) |
| | Y50D7A.3 | Q9N3L4 | | Melo and Ruvkun (2012) |
| | Y53F4B.23 | Q9NA97 | ↑ | Hamilton et al. (2005) |
| | Y56A3A.19 | Q9U241 | ↑ | Shore et al. (2012) |
| *nuo-3* | Y57G11C.12 | A5Z2X2 | ↑ | Shore et al. (2012) |
| *pbo-1* | Y71H2AL.1 | Q9N2Y1 | | Melo and Ruvkun (2012) |
| *nlp-40* | Y74C9A.2 | Q9N4D8 | | Melo and Ruvkun (2012) |
| | Y75B8A.33 | Q9XW60 | ↑ | Hamilton et al. (2005) |
| *mrpl-15* | Y92H12BR.8 | Q9BPN6 | | Yoneda et al. (2004) |
| *clk-1* | ZC395.2 | P48376 | ↑ ↓ | Cristina et al. (2009) and Baker et al. (2012) |
| *gdh-1* | ZK829.4 | Q23621 | | Melo and Ruvkun (2012) |
| *lpd-5* | ZK973.10 | Q9N4L8 | ↑ | Yoneda et al. (2004) |

↑ increased lifespan.
↓ decreased lifespan.

### 4.2. The UPR$^{ER}$

Endoplasmic reticulum (ER) and mitochondria functionally and physically interact with each other [for review, see (Rowland and Voeltz, 2012)], but, so far, stress responses of the ER and mitochondria seem to function distinctly. Recently, we revealed that the conserved phosphoinositide-4-kinase encoding gene *pifk-1* is essential for the paraquat-induced UPR$^{mt}$ (Runkel et al., 2013). Interestingly, *pifk-1* downregulation also abrogated expression of the UPR$^{ER}$ reporter *Phsp-4*::*gfp* upon tunicamycin exposure, indicating that it is required for both organellar stress responses. Further research will be required to reveal mechanistic details of how PIFK-1 may exert its role in the unfolded protein responses of mitochondria and ER, or might even connect both.

## 5. Mitochondrial quality as a regulator of lifespan

Mitochondrial function declines during the aging process. This is indicated by the downregulation of numerous mitochondrial genes, including ETC genes and other genes encoding factors involved in mitochondrial metabolism (Landis et al., 2012). In addition, a continuous, age dependent accumulation of mitochondrial DNA mutations was also observed (Linnane et al., 1989). A further characteristic aspect of aged mitochondria is their compromised proteostasis. This is indicated by the observation that overexpression of the sole mitochondrial HSP70 chaperone (mtHsp70) is sufficient to elongate replicative cellular lifespan in mammalian cells (Kaul et al., 1998; Kaula et al., 2000) and it is also sufficient to elongate the lifespan of *C. elegans* (Yokoyama et al., 2002), whereas depletion of this protein causes a progeria-like phenotype (Kimura et al., 2007). Although metabolically active healthy mitochondria are a continuous source of ROS [for review, see (Balaban et al., 2005)], recent research revealed numerous arguments against the concept of the free radical theory of aging [for review, see (Doonan et al., 2008)] that proposes the accumulation of ROS-damaged macromolecules as a central source of aging [for review, see (Harman, 1972)]. However, because of the complexity of mitochondrial metabolism, many signals that can potentially influence the cellular or organismal aging process emerge from mitochondria, including ROS.

One such response is the UPR$^{mt}$. Already more than a decade ago it was recognized that a reduced function of ETC components in *C. elegans* results in a dramatic increase in lifespan (Dillin et al., 2002b). In a follow-up analysis, it was shown that neuronal depletion of the ETC component CCO-1 was sufficient to induce the UPR$^{mt}$ in the intestine (Durieux et al., 2011) and to promote longevity. This suggested the existence of a systemic cell non-autonomous signal which was named “mitokine”. No molecular identification of this predicted signal has been reported so far.

In order to assess the general role of the UPR$^{mt}$ in the promotion of longevity we compiled an unbiased collection of 99 genes whose depletion induces the UPR$^{mt}$ in *C. elegans*. According to this phenotype we call these genes UPR$^{mt}$ inducers, although their wild type function is to repress this response. Of these 99 genes 58 can be linked to lifespan extension, whereas only 7 genes have been linked to a reduced lifespan. This corresponds to a 25-fold enrichment of longevity associated genes compared to the total genome, whereas genes associated with a shortened lifespan are only fivefold enriched (Table 1). Because the UPR$^{mt}$ inducer genes encode predominantly mitochondrial proteins, it is important to investigate, if the high enrichment of longevity associated

genes is merely a general property of mitochondrial proteins, or if it is a specific trait of the UPR$^{mt}$ inducers. Therefore we analyzed a set of 1111 genes identified by protein mass-spectrometry analysis of mitochondrial preparations (Li et al., 2009). Of these, 122 genes are associated with an extension of lifespan. This corresponds to a 4.7-fold enrichment of longevity related genes compared to the total genome. A substantial portion of these genes (44 of 122) are UPR$^{mt}$ inducers. The higher enrichment of longevity related genes in the UPR$^{mt}$ inducing subset clearly suggests a mechanistic role of the UPR$^{mt}$ in the elongation of the nematode's lifespan. UPR$^{mt}$ might be the dominant mechanism of mitochondrially promoted longevity in *C. elegans*, because the number of UPR$^{mt}$ inducer genes is yet underestimated, since no genome wide screen has been performed so far.

Using the BXD reference population of mice for QTL mapping, a gene on chromosome 2, encoding ribosomal protein S5 (Mrps5), was recently identified to affect longevity (Houtkooper et al., 2013). Decreased *Mrps5* expression correlates with an increase of lifespan in mice and in *C. elegans* (*mrps-5*) and it also induces UPR$^{mt}$. Interference with the UPR$^{mt}$ by RNAi with *haf-1* or *ubl-5* partially suppressed the longevity promoting effect. Since in several experiments HAF-1 was shown to be non-essential for UPR$^{mt}$ signaling, it would have been more appropriate to show the contribution of UPR$^{mt}$ by suppressing longevity via an *atfs-1* loss of function mutant or *atfs-1*(RNAi) downregulation, because the transcription factor ATFS-1 is currently the only essential protein known in UPR$^{mt}$ signaling (Nargund et al., 2012; Runkel et al., 2013). Houtkooper et al. also proposed a correlation of UPR$^{mt}$ and longevity by an experiment in which they applied the antibiotic doxycycline and showed that this pharmacological treatment was sufficient to extend lifespan. Doxycycline, as they argued, inhibits mitochondrial translation when administered in sufficiently high concentrations and is a potent activator of the UPR$^{mt}$ (Houtkooper et al., 2013). This work provides the first example of a genetic longevity trait in mammals that can be linked to UPR$^{mt}$. Moreover, it shows for the first time a positive correlation between the level of UPR$^{mt}$ and the extension of lifespan, although the mechanism of the connection between a "mitonuclear imbalance" and lifespan extension remains elusive. Several inducers of UPR$^{mt}$, among them the complex I inhibitor paraquat, and overexpression of *hsp-6* do not obviously generate such a mitonuclear protein imbalance, but nevertheless elongate *C. elegans* lifespan (Lee et al., 2010; Yang and Hekimi, 2010a; Yokoyama et al., 2002). Houtkooper et al. also used the TOR inhibitor rapamycin, an established promoter of longevity (Harrison et al., 2009), and claim that it functions by inducing a mitonuclear protein imbalance. In mammalian cells, TOR kinase is seen as a promoter of mitochondrial biogenesis, a regulator of mitochondrial metabolism, and as an inhibitor of mitophagy [for review, see (Groenewoud and Zwartkruis, 2013)], but not as a regulator of mammalian mitochondrial protein stress compensation. Therefore further experiments are required to prove that rapamycin mediated lifespan extension is caused by UPR$^{mt}$ induction, particularly because on the contrary TOR signaling has been described as a requirement for UPR$^{mt}$ induction (Baker et al., 2012), as discussed above.

UPR$^{mt}$ mediated elongation of lifespan in *C. elegans* surprisingly requires its induction in larval stages, whereas induction of the UPR$^{mt}$ in adult animals has no effect on lifespan (Durieux et al., 2011; Houtkooper et al., 2013). This differs remarkably from the elongation of lifespan obtained after reduction of insulin/IGF like signaling, which in *C. elegans* can be triggered either during larval stages or during adulthood (Dillin et al., 2002a). UPR$^{mt}$ mediated elongation of lifespan was suggested to be independent of FoxO/DAF-16 (Durieux et al., 2011; Houtkooper et al., 2013). However, in another report, an UPR$^{mt}$ inducing mutation in the *C. elegans* gene encoding the mitochondrial protein prohibitin resulted in an elongation of lifespan only when combined with one out of a number of additional mutations. One of them was in *daf-2*, encoding the insulin/IGF receptor (Artal-Sanz and Tavernarakis, 2009). In this case the prohibitin mutation additionally prolonged the already elongated lifespan of the *daf-2* mutant in a *daf-16* (FOXO) dependent manner. Interestingly, a related result has been obtained in yeast, where the short-lived phenotype of a prohibitin mutant was suppressed by dietary restriction (Schleit et al., 2013). From proteomic analyses the same report also revealed an increase of the nuclear-encoded mitochondrial stress proteins Hsp60 (Cpn60), Hsp10 (Cpn10), and mtHsp70 (Ssc1) in a prohibitin deletion mutant. This is the first description of an UPR$^{mt}$-like mitochondrial stress pathway in yeast. In *C. elegans* a lifespan-extending concerted activation of UPR$^{mt}$ and FoxO signaling by the NAD+/Sirtuin pathway has been proposed (Mouchiroud et al., 2013). Unfortunately, despite the many connections of the UPR$^{mt}$ to the elongation of lifespan no studies involving loss of function mutants of the UPR$^{mt}$ master regulator transcription factor ATFS-1 in *C. elegans* have been published so far. Such studies are essential for a confirmation of the role of the UPR$^{mt}$ in the regulation of lifespan. However, UPR$^{mt}$ alone might not be sufficient to promote a long lifespan, because three gain of function alleles of the UPR$^{mt}$ master regulator ATFS-1 are short-lived (Rauthan et al., 2013). We conclude that either a co-activation of other processes or a specific timing of UPR$^{mt}$ activation is required to contribute to longevity.

How could the UPR$^{mt}$ achieve increased lifespan? One possibility would be a change in mitochondrial metabolism. Mitochondrial metabolites, including ROS, are potential signaling molecules and could influence cellular processes outside the mitochondria and even exert a systemic hormone-like function. Unfortunately, so far no metabolomic studies addressing this aspect are available. A second possibility is that UPR$^{mt}$ activates one of the known longevity pathways. A good candidate for such a scenario would be the phase II detoxification master regulator Nrf2/SKN-1, a transcription factor which can mediate longevity, and which is up-regulated in UPR$^{mt}$ (Nargund et al., 2012; Tullet et al., 2008). A third possibility is that the benefit of UPR$^{mt}$ mediated mitochondrial stress compensation is, in certain situations, sufficient to provide a lifespan benefit. This scenario would involve an improvement of the folding environment for mitochondrial proteins by increasing concentrations of chaperones, higher ROS detoxification capacity, and increased rates of mitochondrial fission and mitophagy. According to recent results, all these processes are upregulated upon *spg-7* RNAi-induced UPR$^{mt}$ (Nargund et al., 2012).

Additional research involving cytological studies, metabolomics, in vivo protein folding assays, and a thorough genetic analysis of interactions with other established life-elongating pathways is required to place mitochondrial maintenance in the already complex picture of genetically defined determinants of longevity. However, this might be a worthwhile undertaking, as mitochondrial biology could turn out to be the so far missing link between varying conditions of longevity, and a key to understanding age-dependent diseases.

## Conflict of interest

The authors have no conflicts of interests.

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