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The mitochondrial unfolded protein response - synchronizing genomes

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Abstract

Maintenance of the mitochondrial proteome is performed primarily by chaperones, which fold and assemble proteins, and by proteases, which degrade excess damaged proteins. Upon various types of mitochondrial stress, triggered genetically or pharmacologically, dysfunction of the proteome is sensed and communicated to the nucleus, where an extensive transcriptional program, aimed to repair the damage, is activated. This feedback loop, termed the mitochondrial unfolded protein response (UPR^{mt}), synchronizes the activity of the mitochondrial and nuclear genomes and as such ensures the quality of the mitochondrial proteome. Here we review the recent advances in the UPR^{mt} field and discuss its induction, signaling, communication with the other mitochondrial and major cellular regulatory pathways and its potential implications on health and lifespan.

Keywords

mitochondria; aging; proteostasis

Introduction

Mitochondria supply cells with ATP, the cellular energy currency, and are essential for many other aspects of cellular homeostasis, thereby influencing not only cellular metabolism, but also organismal health and lifespan [1,2]. Inborn mitochondrial defects result in severe multisystem diseases and mitochondrial dysfunction also underlies several common metabolic and neurodegenerative diseases [3,4]. Mitochondrial unfolded protein response (UPR^{mt}) is an emerging adaptive stress response pathway, which ensures optimal quality and function of the mitochondrial proteome. UPR^{mt} internally surveys mitochondrial proteostasis and responds to stress signals by activating an intricate mitochondrial protein quality control (PQC) network [5-7]. Here we review the recent literature on mechanisms that trigger UPR^{mt} activation, its signaling pathways, crosstalk with other mitochondrial quality control systems and interactions with the wider network of cellular responses.

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Activation of UPR^{mt}

Mitochondria are evolutionarily derived from proteobacteria that evolved in symbiosis within eukaryotic cells [8]. Mitochondria contain multiple copies of the circular mitochondrial DNA (mtDNA), a vestige of the proteobacterial genome, which encodes 13 protein constituents of the multiprotein complexes of the electron transport chain (ETC). The remainder of the mitochondrial proteome (~1500 proteins) is transcribed from the nuclear DNA (nDNA). After translation in the cytoplasm, these nuclear encoded proteins are imported, folded and assembled within the mitochondria [9,10]. Four out of five ETC complexes contain proteins encoded in both genomes, requiring a robust synchrony between the mitochondrial and nuclear genome to warrant optimal mitochondrial function [11].

Proteostasis in the mitochondria is ensured by an elaborate protein quality control (PQC) network, composed of two main functional groups of proteins, chaperones and proteases [12,13]. Chaperones mtHsp70, Hsp60 and Hsp10 fold and assemble proteins that are imported into the mitochondria and refold damaged mitochondrial proteins. Excess proteins that are unassisted by chaperones are digested by ATP-dependent PQC proteases, specific for each mitochondrial compartment: the ClpXP and Lon proteases in the matrix, the i-AAA (Yme1L1) and m-AAA proteases (Afg3l2 and Spg7), acting in the intermembrane space (IMS) and matrix, respectively. Upon mitochondrial proteotoxic stress, these PQC chaperones and proteases are induced as a result of a retrograde mitochondria-to-nucleus signaling termed UPR^{mt}. Using mitochondrial chaperones and proteases as UPR^{mt} biomarkers, this PQC pathway has now been established in worms, flies, mammalian cell cultures and mice. Various conditions have been shown to trigger the UPR^{mt}, most of which interfere with the mitochondrial proteostasis either by disturbing the PQC system or by increasing the load of damaged, unfolded or unassembled proteins (table 1).

RNAi based downregulation of components of the mitochondrial protein handling machinery, such as the import proteins TIM-17 and TIM-23 [14,15], the inner membrane protein scaffold PHB-2 [16,17], the PQC protease SPG-7 [17,18] or the chaperone mtHsp70 [19] all induce UPR^{mt} in *C. elegans* or in mammals. Moreover, increasing the workload of PQC machinery by overexpression of aggregation-prone proteins, such as a mutant form of ornithine transcarbamylase (OTC- and EndoG, also activates UPR^{mt} in mammalian cells [19-21] and flies [22]. On a similar note, the treatment with the reactive oxygen species (ROS) generator paraquat, which increases the amount of damaged proteins, also induces UPR^{mt} in *C. elegans* [17,23]. Additionally, pathogenic bacteria can induce UPR^{mt} by production of toxins, which antagonize mitochondrial proteostasis [24,25].

Another way to induce UPR^{mt} is by manipulating ETC assembly either by the downregulation or inhibition of single (or groups of) ETC components, which are encoded by either mtDNA or nDNA [26]. This results in a mismatch between mtDNA and nDNA encoded ETC subunits, creating orphaned unassembled subunits, which stay associated with chaperones; this phenomenon is termed mitonuclear protein imbalance [26]. Thus downregulation of ETC subunits by *cco-1* (complex IV) RNAi [18,27], in *isp-1* (complex III) or *clk-1* (ubiquinone synthesis) mutant strains [27,28], or by using pharmacological ETC inhibitors, such as antimycin [23,24] and rotenone [23], activates UPR^{mt}. Additionally,

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downregulation of mitochondrial ribosomal proteins or treatment with the bacterial (also mitochondrial) translation inhibitors doxycycline or chloramphenicol [26], as well as mtDNA depletion induced by ethidium bromide [17] result in a mitonuclear protein imbalance and consequently induce UPR^{mt}.

Similarly, the activation of mitochondrial biogenesis by resveratrol or rapamycin [26] also reduces the levels of mitochondrially encoded ETC subunits, triggering UPR^{mt}. Boosting NAD$^+$ levels by the NAD$^+$ precursor, nicotinamide riboside (NR), or by inhibiting NAD$^+$ consumption, as seen after treatment with PARP inhibitors [29], also enhances biogenesis, but the raise in NAD$^+$ levels specifically increases the transcription and translation of mtDNA-encoded ETC subunits [30], creating also a mitonuclear imbalance, which triggers the UPR^{mt}. In accordance with these findings related to mitochondrial biogenesis, UPR^{mt} can be activated in worms, only if the perturbation in mitochondrial proteostasis takes place during L3/L4 transition [31], which coincides with a major burst in mitochondrial biogenesis [32], further emphasizing its role in the induction of UPR^{mt}. The induction of UPR^{mt} during biogenesis is in most cases mediated by actvation of sirtuins, protein deacylases, which are major regulators of metabolism and aging [33], namely Sirt1 in mouse or sir-2.1 in worms [29,30,34,35]. In addition to Sirt1, recently, Sirt7 and its downstream target transcription factor GABPβ1 were shown to control the expression of multiple mitochondrial ribosomal proteins, responsible for mitochondrial translation [36]. Although the potential role of Sirt7 in UPR^{mt} induction has not yet been examined, given its major impact on mitochondrial ribosomal proteins [26], Sirt7 might be pivotal for mitochondrial proteostasis, while its deficiency could induce UPR^{mt}.

UPR^{mt} signaling

The first trigger for UPR^{mt} in *C. elegans* is the excess of damaged and unfolded proteins, which are digested by the CLPP-1 protease into small peptides [37], and then transported outside of the mitochondria by the transporter HAF-1 [38] (figure 1). The role of these peptides is unknown yet, but presumably they contribute to weaken mitochondrial import during stress, which on its turn is important for the nuclear translocation of the main UPR^{mt} transcriptional regulator ATFS-1 [15]. ATFS-1 is able to shuttle between mitochondria and nucleus due to presence of a mitochondrial targeting sequence (MTS) and a nuclear localization sequence (NLS). In normal conditions, ATFS-1 is imported and degraded by the Lon protease in the mitochondria, but upon mitochondrial stress ATFS-1 translocates into the nucleus [15]. Together with other transcriptional regulators UBL-5 [39] and DVE-1 [37], which also move into the nucleus in stress conditions, ATFS-1 then induces the transcription of UPR^{mt} targets in the worm. Of note, the Ubl5 protein levels also correlate tightly with UPR^{mt} effector chaperones and proteases in several tissues in the BXD mouse genetic reference population (GRP) and in humans, which indicates that presumably it is also involved in the initiation of the mammalian UPR^{mt} [18]. Interestingly, ATFS-1 does not have an unambiguous sequence homolog in mammals, making it doubtful whether an ATFS-1 counterpart and its shuttling mechanism are conserved in mammalian UPR^{mt}. ATFS-1 induces multiple genes with a pleiotropic outcome. It activates the transcription of mitochondrial chaperones and proteases, as well as that of detoxification enzymes to neutralize the generation of ROS, and of mitochondrial transporters, which presumably

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correct the import deficit after the resolution of the perturbation [15] (figure 2). ATFS-1 also induces glycolytic genes, which indicates that there is a concomitant transient shift in cellular ATP production from mitochondrial oxidative phosphorylation to cytoplasmic glycolysis during mitochondrial stress [15]. Such a metabolic shift, while maintaining cellular energy supply, avoids overtaxation of mitochondrial energy harvesting in stress situations.

How mitochondrial stress in mammals is sensed and triggers UPR^{mt}, and whether it involves the possible generation of peptides similarly as in *C. elegans*, is still unknown. In mammalian cells transfected with OTC- , the UPR^{mt} transcriptional response was shown to involve JNK2 phosphorylation, which triggers c-Jun to bind and activate the CHOP and C/EBPβ promoters [20,40] (figure 1). c-Jun was shown to be also required for UPR^{mt} induction in flies [41] and CHOP induction has been observed upon EndoG overexpression [21] or in complex IV deficient Surf–/– mice [42]. Consequently, UPR^{mt} target gene expression is coordinated by a dimer of the transcription factors CHOP and C/EBPβ, which binds target promoters on a specific CHOP binding site flanked by two UPR^{mt} response elements (MUREs) [43]. MUREs have been identified in the promoters of human mitochondrial PQC chaperones and proteases (HSP60, HSP10 and mtDnaJ, ClpP, YME1L1 and PMPCB), as well as in the enzymes NDUFB2, endonuclease G and thioredoxin 2 [43]. A recent transcriptomics and proteomics analysis revealed that UPR^{mt} effector proteins Hsp60, Hsp10, mtHsp70, ClpP, Lonp1 and Ubl5 form a tight coexpression network in mice GRPs and human populations, suggestive of their transcriptional control [18]. However, the fact that stronger correlations were observed on protein than on transcript level, indicates also importance of posttranslational mechanisms in UPR^{mt} regulation [18].

Evidence for conservation of UPR^{mt} pathway in mammals

Although the UPR^{mt} has been intensively investigated in yeast [16], worms [15,17,27,37-39,44], flies [22,41], and mammalian cells [17,20,26,28,29,45], it is not yet defined when and where UPR^{mt} occurs in intact mammals.

We previously demonstrated that mitonuclear protein imbalance, as seen upon reduced expression of *Mrps* and/or inhibition of mitochondrial translation, induces a robust UPR^{mt} in the BXD mouse strains, which translated in a significant lifespan extension [26]. As further proof of concept that similar mechanisms could activate UPR^{mt} across species, we recently showed that subtle variations in the expression of orthologs of two prototypical UPR^{mt} components—i.e. *cco-1*, a nuclear encoded component of ETC complex IV [27] and the protease *spg-7* [17]—whose loss-of-function trigger worm UPR^{mt}, also induce a UPR^{mt} signature in unchallenged mice from the BXD GRPs [18]. These robust correlations on a population levels are remarkable as they indicate that UPR^{mt} is a physiological pathway, which is not only activated by robust genetic or pharmacological perturbations, but has a role in subtle homeostatic processes [18], that can impact on lifespan [26]. The tight correlation and regulation of the UPR^{mt} was furthermore also conserved in several different human tissues, supporting the cross-species nature of UPR^{mt} [18].

In addition to these data coming from holistic genetic approaches, recently also single gene perturbations in mice have been linked with UPR^{mt}. A UPR^{mt} signature was for instance

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detected in muscles of mtDNA *Deletor* and *Sco2*$^{KO/KI}$ mice, models of inherited mitochondrial myopathies [34,35]. Phenotypic analysis of *Surf1*$^{-/-}$ mice, deficient in ETC complex IV, also revealed activation of the UPR^{mt} markers Hsp60, ClpP, Lonp and Chop [42]. Furthermore, UPR^{mt} can also be induced pharmacologically in mice [30]. Like in worms, treatment with PARP inhibitors triggers a robust UPR^{mt} in mice as a consequence of a mitonuclear protein imbalance caused by the enhanced translation of the 13 mtDNA encoded ETC proteins [30]. These emerging data warrant further investigation of the eventual presence of UPR^{mt} in other mice models and in human patient biopsies.

UPR^{mt}-induced protective responses

Under stress, several lines of defense are activated by mitochondria. First, production and import of new mitochondrial proteins is temporarily blocked. Specific kinases, GCN-2 in the worm [44] and PKR in mammals [28], phosphorylate eIF2a, which leads to attenuation of global translation (figure 2). In *C. elegans*, reduction of mitochondrial import is important to initiate the UPR^{mt} transcriptional response [15]. Furthermore, specific reduction in mitochondrial import occurs also in mammalian cells upon UPR^{mt}, as the Yme1l1 protease selectively degrades the translocation pore component Tim17A [14]. The reduction of mitochondrial proteins and function during stress is consistent with the reallocation of ATP production to glycolysis in the cytoplasm [15].

In addition, several parallel protective responses are activated upon UPR^{mt}. SIR-2.1 in worms and mammalian Sirt3 were shown to regulate UPR^{mt} in part by deacetylating DAF-16 or its mammalian homolog Foxo3a, respectively, which then activates an antioxidant response [21,29] (figure 2). Another major oxidative stress response pathway, coordinated by Nrf2 (NFE2L2), was activated in complex IV deficient *Surf1*$^{-/-}$ mice [42]. Interestingly, the Nrf2 pathway is coordinated by c-Jun [46], which also regulates CHOP and C/EBPβ in the context of mammalian UPR^{mt}, as discussed above. On a similar note, in *C. elegans* treated with antimycin or *spg-7* RNAi to induce UPR^{mt}, pathogen defense and drug detoxification are enhanced [24,25]. The activation of these protective pathways allows the worm to recognize and avoid pathogens, which target mitochondria, and can increase its resistance to a wider network of stressors. For instance, worms and mammalian cells with an active UPR^{mt} are more resistant to ROS generator paraquat [14,29]. Additionally, worm gain-of-function mutants of ATFS-1 with constitutively activated UPR^{mt}, are resistant to statin (inhibitors of HMG-CoA reductase) toxicity [47].

Recent findings suggest that mitochondrial remodeling, namely fission and fusion, as well selective removal of terminally defective mitochondria by mitophagy, take place under stress conditions. Both increased fusion [29] and fission [19,22,26] have been detected under UPR^{mt}, which presumably depends on the type and strength of UPR^{mt} inducer and requires further studies. Increased mitophagy has been observed in mammalian cells and flies overexpressing mutant forms of EndoG [21] or OTC- [19,22,48], as well as upon RNAi inactivation of the ETC component ND75 [41]. In these systems mitophagy is potentially regulated by Foxo3a [21], AMPK [22] and secreted Insulin antagonizing peptide ImpL2, which non-autonomously repressed insulin signaling in distant tissues [41] (figure 2). Whether mitophagy is upregulated in UPR^{mt} inducing conditions in worms, has not been

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directly investigated, but seems also likely, as autophagy genes are among the ATFS-1 targets [49]. Interestingly, mitophagy and UPR^{mt} might share the same initial mitochondrial damage detection steps, as in worms, synthesis of ceramide, a sphingolipid which marks domains of mitochondrial dysfunction and induces mitophagy by anchoring autophagolysosomes to these domains [50], was required for UPR^{mt} activation [24]. Mitophagy is induced by PINK1, that accumulates on the depolarized outer mitochondrial membrane, and then recruits the E3 ubiquitin ligase Parkin, targeting mitochondria to autophagosomes [51]. Mitophagy induction might be altered in UPR^{mt} conditions, as upon OTC- expression in cells, PINK1 and Parkin accumulate on stressed, but not depolarized mitochondria [48]. This might be regulated at the level of PINK1 degradation in basal conditions, in which mitochondrial PQC proteases, namely Lonp1, seem to be also involved [52].

Both mitochondrial dynamics and mitophagy pathways contribute to reconstitution of cellular homeostasis in stress conditions, by redistribution and removal of the irreversibly damaged elements of mitochondrial network. Inability to induce sufficient levels of mitophagy, under strong mitochondrial stress and activation of UPR^{mt}, induces apoptosis and has negative systemic effects on whole organism physiology [21,22].

UPR^{mt} systemic effects on aging

Disruption of almost any subunit of the ETC paradoxically extends lifespan in yeast, worms, flies and mice [53-55]. The lifespan extension is associated with typical phenotypes, such as delayed development, small size and reduced fertility. Interference with ETC has hormetic effects on longevity, demonstrated by RNAi dilution experiments: moderate knockdown extends lifespan, while too low and too strong knockdowns either do not have an effect or reduce lifespan, respectively [56]. Moreover, there are specific spatio-temporal restrictions, as selective interference with ETC only in neurons and intestine during larval stages increases worm longevity [27,31]. UPR^{mt} is almost invariably present [57], follows the same spatio-temporal specifications [27], and is required for lifespan extension in worms with ETC problems [26,27,44,58]. In flies, disruption of the complex I component ND75 in the muscle by low-levels of RNAi, for a defined time period in the adult stage, activated UPR^{mt} and increased lifespan [41]. In line with this, the reduced expression of Mrps5, a mitochondrial ribosomal protein, which regulates the translation of mtDNA encoded ETC genes, induces a mitonuclear imbalance resulting in UPR^{mt}, which correlates with increased lifespan in the BXD mouse GRP [26]. This effect on lifespan in the BXD strains was all the more striking as it was not linked to loss of gene function, but just due to a subtle variation in Mrps5 expression levels. The positive effects of UPR^{mt} on lifespan are also exemplified in worms [59] and flies [41] with forced overexpression of UPR^{mt} effector chaperones.

Despite this rather convincing evidence linking UPR^{mt} activation and longevity obtained in several independent laboratories and across multiple species (worm, fly, mice), not all UPR^{mt} inductions may be beneficial [22,60]. This is not too surprising given the hormetic nature of UPR^{mt}, with a clear dose effect relationship and with well-defined spatio-temporal frames. If the level of mitochondrial stress is too high, the protective effects of UPR^{mt} may

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hence be insufficient to counteract the damage, making a beneficial adaptive response become maladaptive.

Conclusions and perspectives

First described ~20 years ago, the UPR^{mt} is now emerging as an important regulator of mitochondrial health, interacting with other mitochondrial quality control systems, such as the oxidative stress response, mitochondrial biogenesis and mitophagy. Although some specific UPR^{mt} regulators and pathways have been described in invertebrates, our knowledge of the exact molecular machinery of the UPR^{mt} is still evolving and incomplete. Further studies defining the UPR^{mt} sensors, signal transduction pathways and effectors, particularly in mammals are hence required. Also how the UPR^{mt} intersects with other cellular signaling pathways, such as those controlled by sirtuins, AMPK or insulin, requires further investigation. The fact that a UPR^{mt} signal is present in unchallenged mouse and human populations across multiple tissues [18] is an important step towards ascertaining its importance in mammals. It furthermore suggests that this pathway not only has a role in stress defense but also in homeostasis, where UPR^{mt} could synchronize mitochondrial and nuclear genomes at the proteome level. We hope that better understanding of UPR^{mt} may one day help translate the benefits of the UPR^{mt} into therapies for rare inherited and common age-related related diseases with mitochondrial dysfunction.

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References and recommended reading

1. Dillin A, Gottschling DE, Nystrom T. The good and the bad of being connected: the integrons of aging. Curr Opin Cell Biol. 2014; 26:107–112. [PubMed: 24529252]
2. Houtkooper RH, Williams RW, Auwerx J. Metabolic networks of longevity. Cell. 2010; 142:9–14. [PubMed: 20603007]
3. Andreux PA, Houtkooper RH, Auwerx J. Pharmacological approaches to restore mitochondrial function. Nat Rev Drug Discov. 2013; 12:465–483. [PubMed: 23666487]
4. Nunnari J, Suomalainen A. Mitochondria: in sickness and in health. Cell. 2012; 148:1145–1159. [PubMed: 22424226]
5. Jensen MB, Jasper H. Mitochondrial Proteostasis in the Control of Aging and Longevity. Cell Metab. 2014; 20:214–225. [PubMed: 24930971]
6. Haynes CM, Ron D. The mitochondrial UPR - protecting organelle protein homeostasis. J Cell Sci. 2010; 123:3849–3855. [PubMed: 21048161]
7. Jovaisaite V, Mouchiroud L, Auwerx J. The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. J Exp Biol. 2014; 217:137–143. [PubMed: 24353213]
8. Gray MW, Burger G, Lang BF. The origin and early evolution of mitochondria. Genome Biol. 2001; 2:REVIEWS1018. [PubMed: 11423013]
9. Pagliarini DJ, Calvo SE, Chang B, Sheth SA, Vafai SB, Ong SE, Walford GA, Sugiana C, Boneh A, Chen WK, et al. A mitochondrial protein compendium elucidates complex I disease biology. Cell. 2008; 134:112–123. [PubMed: 18614015]
10. Neupert W, Herrmann JM. Translocation of proteins into mitochondria. Annu Rev Biochem. 2007; 76:723–749. [PubMed: 17263664]

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11. Lane N. Mitonuclear match: optimizing fitness and fertility over generations drives ageing within generations. Bioessays. 2011; 33:860–869. [PubMed: 21922504]
12. Baker BM, Haynes CM. Mitochondrial protein quality control during biogenesis and aging. Trends Biochem Sci. 2011; 36:254–261. [PubMed: 21353780]
13. Tatsuta T, Langer T. Quality control of mitochondria: protection against neurodegeneration and ageing. EMBO J. 2008; 27:306–314. [PubMed: 18216873]
14••. Rainbolt TK, Atanassova N, Genereux JC, Wiseman RL. Stress-regulated translational attenuation adapts mitochondrial protein import through Tim17A degradation. Cell Metab. 2013; 18:908–919. [PubMed: 24315374] [This study shows that mitochondrial import during stress conditions is perturbed by selective degradation of translocase subunit Tim17A, which promotes mitochondrial proteostasis.]
15••. Nargund AM, Pellegrino MW, Fiorese CJ, Baker BM, Haynes CM. Mitochondrial import efficiency of ATFS-1 regulates mitochondrial UPR activation. Science. 2012; 337:587–590. [PubMed: 22700657] [This work describes the molecular mechanism of initiation of UPR^{mt} transcriptional program by ATFS-1, which is dependent on the mitochondrial import efficiency.]
16. Schleit J, Johnson SC, Bennett CF, Simko M, Trongtham N, Castanza A, Hsieh EJ, Moller RM, Wasko BM, Delaney JR, et al. Molecular mechanisms underlying genotype-dependent responses to dietary restriction. Aging Cell. 2013; 12:1050–1061. [PubMed: 23837470]
17. Yoneda T, Benedetti C, Urano F, Clark SG, Harding HP, Ron D. Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. J Cell Sci. 2004; 117:4055–4066. [PubMed: 15280428]
18••. Wu Y, Williams Evan G, Dubuis S, Mottis A, Jovaisaite V, Houten Sander M, Argmann Carmen A, Faridi P, Wolski W, Kutalik Z, et al. Multilayered Genetic and Omics Dissection of Mitochondrial Activity in a Mouse Reference Population. Cell. 2014; 158:1415–1430. [PubMed: 25215496] [Demonstration of the conservation of the UPR^{mt} effector coexpression network in mice and human populations and of a role of the UPR^{mt} in homeostasis, beyond its function in stress response.]
19. Burbulla LF, Fitzgerald JC, Stegen K, Westermeier J, Thost AK, Kato H, Mokranjac D, Sauerwald J, Martins LM, Woitalla D, et al. Mitochondrial proteolytic stress induced by loss of mortalin function is rescued by Parkin and PINK1. Cell Death Dis. 2014; 5:e1180. [PubMed: 24743735]
20. Zhao Q, Wang J, Levichkin IV, Stasinopoulos S, Ryan MT, Hoogenraad NJ. A mitochondrial specific stress response in mammalian cells. EMBO J. 2002; 21:4411–4419. [PubMed: 12198143]
21. Papa L, Germain D. SirT3 regulates the mitochondrial unfolded protein response. Mol Cell Biol. 2014; 34:699–710. [PubMed: 24324009]
22. Pimenta de Castro I, Costa AC, Lam D, Tufi R, Fedele V, Moisoi N, Dinsdale D, Deas E, Loh SH, Martins LM. Genetic analysis of mitochondrial protein misfolding in Drosophila melanogaster. Cell Death Differ. 2012; 19:1308–1316. [PubMed: 22301916]
23••. Runkel ED, Liu S, Baumeister R, Schulze E. Surveillance-Activated Defenses Block the ROS-Induced Mitochondrial Unfolded Protein Response. PLoS Genet. 2013; 9:e1003346. [PubMed: 23516373] [Demonstration of the conservation of the UPR^{mt} effector coexpression network in mice and human populations and of a role of the UPR^{mt} in homeostasis, beyond its function in stress response.]
24••. Liu Y, Samuel BS, Breen PC, Ruvkun G. Caenorhabditis elegans pathways that surveil and defend mitochondria. Nature. 2014; 508:406–410. [PubMed: 24695221] [This paper establishes that UPR^{mt} is an important mitochondrial defense against drugs and pathogens, for which ceramide and mevalonate synthesis pathways are required.]
25••. Pellegrino MW, Nargund AM, Kirienko NV, Gillis R, Fiorese CJ, Haynes CM. Mitochondrial UPR-regulated innate immunity provides resistance to pathogen infection. Nature. 2014 In press. [This work further describes the role of UPR^{mt} in innate immunity.]
26••. Houtkooper RH, Mouchiroud L, Ryu D, Moullan N, Katsyuba E, Knott G, Williams RW, Auwerx J. Mitonuclear protein imbalance as a conserved longevity mechanism. Nature. 2013; 497:451–457. [PubMed: 23698443] [This study defines mitonuclear imbalance as the cause for UPR^{mt} induction by multiple genetic and pharmacological perturbations. It also shows that mitochondrial ribosomal proteins are conserved regulators of UPR^{mt} and longevity in mice genetic reference population and in worms.]

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27••. Durieux J, Wolff S, Dillin A. The cell-non-autonomous nature of electron transport chain-mediated longevity. Cell. 2011; 144:79–91. [PubMed: 21215371] [Landmark study that describes the role of UPR^{mt} in ETC-mediated longevity, its cell non-autonomous aspects and demonstrates the involvement of so-called "mitokines".]

28. Rath E, Berger E, Messlik A, Nunes T, Liu B, Kim SC, Hoogenraad N, Sans M, Sartor RB, Haller D. Induction of dsRNA-activated protein kinase links mitochondrial unfolded protein response to the pathogenesis of intestinal inflammation. Gut. 2012; 61:1269–1278. [PubMed: 21997551]

29••. Mouchiroud L, Houtkooper RH, Moullan N, Katsyuba E, Ryu D, Canto C, Mottis A, Jo YS, Viswanathan M, Schoonjans K, et al. The NAD(+)/Sirtuin Pathway Modulates Longevity through Activation of Mitochondrial UPR and FOXO Signaling. Cell. 2013; 154:430–441. [PubMed: 23870130] [This report reveals that Sirtuin 1 activation by increased NAD^+ levels induces mitonuclear imbalance, UPR^{mt} and induction of antioxidant defense by FOXO transcription factor *daf-16*.]

30•. Pirinen E, Canto C, Jo YS, Morato L, Zhang H, Menzies KJ, Williams EG, Mouchiroud L, Moullan N, Hagberg C, et al. Pharmacological Inhibition of poly(ADP-ribose) polymerases improves fitness and mitochondrial function in skeletal muscle. Cell Metab. 2014; 19:1034–1041. [PubMed: 24814482] [This study shows that PARP inhibitors increase NAD^+ levels, mitochondrial protein translation, UPR^{mt} and have a beneficial impact on muscle function in mice.]

31. Dillin A, Hsu AL, Arantes-Oliveira N, Lehrer-Graiwer J, Hsin H, Fraser AG, Kamath RS, Ahringer J, Kenyon C. Rates of behavior and aging specified by mitochondrial function during development. Science. 2002; 298:2398–2401. [PubMed: 12471266]

32. Tsang WY, Lemire BD. Mitochondrial genome content is regulated during nematode development. Biochem Biophys Res Commun. 2002; 291:8–16. [PubMed: 11829454]

33. Houtkooper RH, Pirinen E, Auwerx J. Sirtuins as regulators of metabolism and healthspan. Nat Rev Mol Cell Biol. 2012; 13:225–238. [PubMed: 22395773]

34. Khan NA, Auranen M, Paetau I, Pirinen E, Euro L, Forsstrom S, Pasila L, Velagapudi V, Carroll CJ, Auwerx J, et al. Effective treatment of mitochondrial myopathy by nicotinamide riboside, a vitamin B3. EMBO Mol Med. 2014; 6:721–731. [PubMed: 24711540]

35. Cerutti R, Pirinen E, Lamperti C, Marchet S, Sauve AA, Li W, Leoni V, Schon EA, Dantzer F, Auwerx J, et al. NAD(+)-dependent activation of Sirt1 corrects the phenotype in a mouse model of mitochondrial disease. Cell Metab. 2014; 19:1042–1049. [PubMed: 24814483]

36. Ryu D, Jo YS, Lo Sasso G, Stein S, Zhang H, Perino A, Lee JU, Zeviani M, Romand R, Hottiger MO, et al. A SIRT7-Dependent Acetylation Switch of GABPbeta1 Controls Mitochondrial Function. Cell Metab. 2014

37. Haynes CM, Petrova K, Benedetti C, Yang Y, Ron D. ClpP mediates activation of a mitochondrial unfolded protein response in C. elegans. Dev Cell. 2007; 13:467–480. [PubMed: 17925224]

38. Haynes CM, Yang Y, Blais SP, Neubert TA, Ron D. The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376.7 in C. elegans. Mol Cell. 2010; 37:529–540. [PubMed: 20188671]

39. Benedetti C, Haynes CM, Yang Y, Harding HP, Ron D. Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. Genetics. 2006; 174:229–239. [PubMed: 16816413]

40. Horibe T, Hoogenraad NJ. The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response. PLoS One. 2007; 2:e835. [PubMed: 17848986]

41••. Owusu-Ansah E, Song W, Perrimon N. Muscle mitohormesis promotes longevity via systemic repression of insulin signaling. Cell. 2013; 155:699–712. [PubMed: 24243023] [This work demonstrates that mild interference with the ETC in fly muscle extends lifespan through induction of UPR^{mt} and the insulin antagonizing peptide ImpL2 (a mitokine), which non-autonomously represses insulin signaling and increases mitophagy. Furthermore, it shows that forced expression of UPR^{mt} genes increases lifespan.]

42. Pulliam DA, Deepa SS, Liu Y, Hill S, Lin AL, Bhattacharya A, Shi Y, Sloane L, Viscomi C, Zeviani M, et al. Complex IV-deficient Surf1−/− mice initiate mitochondrial stress responses. Biochem J. 2014; 462:359–371. [PubMed: 24911525]

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43. Aldridge JE, Horibe T, Hoogenraad NJ. Discovery of genes activated by the mitochondrial unfolded protein response (mtUPR) and cognate promoter elements. PLoS One. 2007; 2:e874. [PubMed: 17849004]

44••. Baker BM, Nargund AM, Sun T, Haynes CM. Protective coupling of mitochondrial function and protein synthesis via the eIF2alpha kinase GCN-2. PLoS Genet. 2012; 8:e1002760. [PubMed: 22719267] [This work highlights GCN-2 mediated inhibition of translation in the cytoplasm in mitochondrial stress conditions.]

45. Martinus RD, Garth GP, Webster TL, Cartwright P, Naylor DJ, Hoj PB, Hoogenraad NJ. Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. Eur J Biochem. 1996; 240:98–103. [PubMed: 8797841]

46. Jeyapaul J, Jaiswal AK. Nrf2 and c-Jun regulation of antioxidant response element (ARE)-mediated expression and induction of gamma-glutamylcysteine synthetase heavy subunit gene. Biochem Pharmacol. 2000; 59:1433–1439. [PubMed: 10751553]

47. Rauthan M, Ranji P, Aguilera Pradenas N, Pitot C, Pilon M. The mitochondrial unfolded protein response activator ATFS-1 protects cells from inhibition of the mevalonate pathway. Proc Natl Acad Sci U S A. 2013; 110:5981–5986. [PubMed: 23530189]

48. Jin SM, Youle RJ. The accumulation of misfolded proteins in the mitochondrial matrix is sensed by PINK1 to induce PARK2/Parkin-mediated mitophagy of polarized mitochondria. Autophagy. 2013; 9:1750–1757. [PubMed: 24149988]

49. Guo B, Huang X, Zhang P, Qi L, Liang Q, Zhang X, Huang J, Fang B, Hou W, Han J, et al. Genome-wide screen identifies signaling pathways that regulate autophagy during Caenorhabditis elegans development. EMBO Rep. 2014; 15:705–713. [PubMed: 24764321]

50. Sentelle RD, Senkal CE, Jiang W, Ponnusamy S, Gencer S, Selvam SP, Ramshesh VK, Peterson YK, Lemasters JJ, Szulc ZM, et al. Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. Nat Chem Biol. 2012; 8:831–838. [PubMed: 22922758]

51. Kroemer G, Marino G, Levine B. Autophagy and the integrated stress response. Mol Cell. 2010; 40:280–293. [PubMed: 20965422]

52. Thomas RE, Andrews LA, Burman JL, Lin WY, Pallanck LJ. PINK1-Parkin pathway activity is regulated by degradation of PINK1 in the mitochondrial matrix. PLoS Genet. 2014; 10:e1004279. [PubMed: 24874806]

53. Wong A, Boutis P, Hekimi S. Mutations in the clk-1 gene of Caenorhabditis elegans affect developmental and behavioral timing. Genetics. 1995; 139:1247–1259. [PubMed: 7768437]

54. Munkacsy E, Rea SL. The paradox of mitochondrial dysfunction and extended longevity. Exp Gerontol. 2014; 56:221–233. [PubMed: 24699406]

55. Pulliam DA, Bhattacharya A, Van Remmen H. Mitochondrial Dysfunction in Aging and Longevity: A Causal or Protective Role? Antioxid Redox Signal. 2012

56. Rea SL, Ventura N, Johnson TE. Relationship between mitochondrial electron transport chain dysfunction, development, and life extension in Caenorhabditis elegans. PLoS Biol. 2007; 5:e259. [PubMed: 17914900]

57. Ventura N, Rea SL. Caenorhabditis elegans mitochondrial mutants as an investigative tool to study human neurodegenerative diseases associated with mitochondrial dysfunction. Biotechnol J. 2007; 2:584–595. [PubMed: 17443764]

58. Schieber M, Chandel NS. TOR signaling couples oxygen sensing to lifespan in C. elegans. Cell Reports. 2014 In press.

59. Yokoyama K, Fukumoto K, Murakami T, Harada S, Hosono R, Wadhwa R, Mitsui Y, Ohkuma S. Extended longevity of Caenorhabditis elegans by knocking in extra copies of hsp70F, a homolog of mot-2 (mortalin)/mthsp70/Grp75. FEBS Lett. 2002; 516:53–57. [PubMed: 11959102]

60. Bennett CF, Vander Wende H, Simko M, Klum S, Barfield S, Choi H, Pineda VV, Kaeberlein M. Activation of the mitochondrial unfolded protein response does not predict longevity in Caenorhabditis elegans. Nat Commun. 2014; 5:3483. [PubMed: 24662282]

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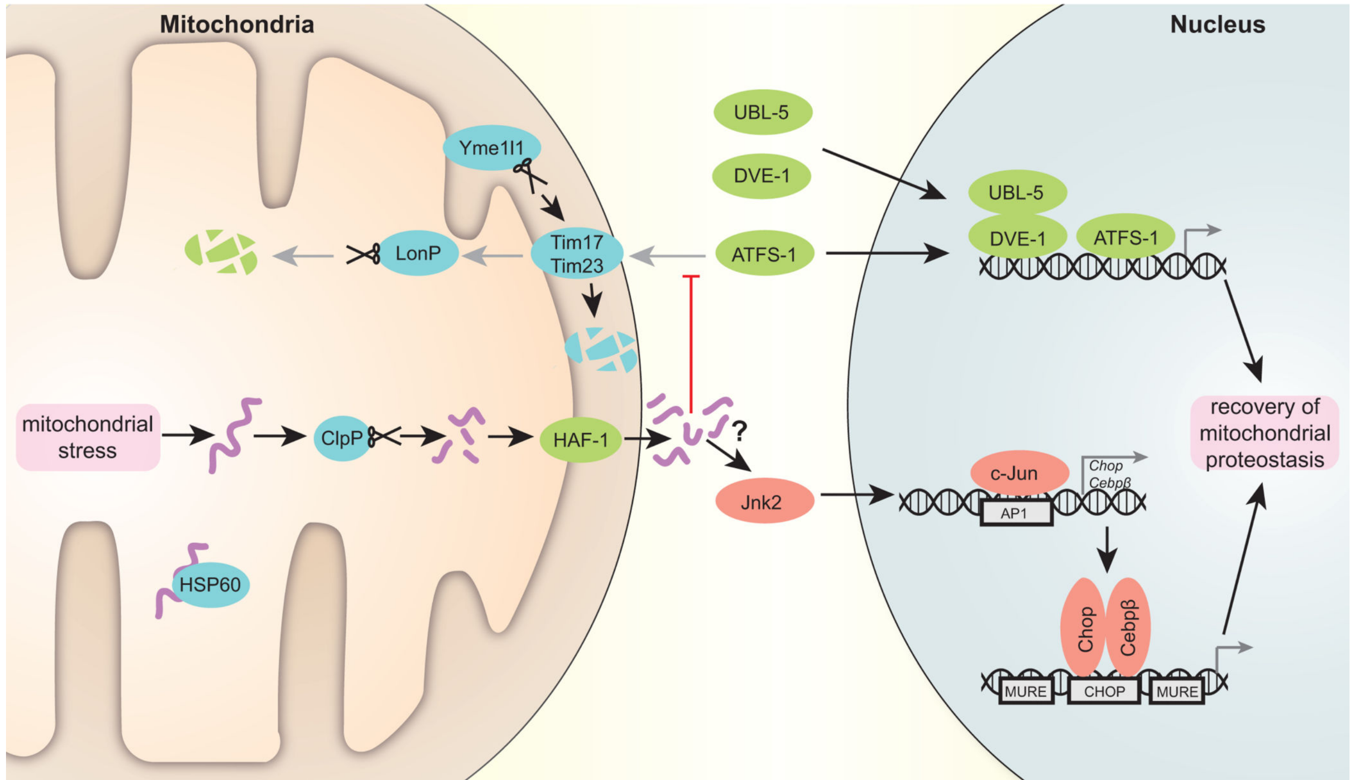

Figure 1. Scheme depicting the transcriptional regulation of the UPR^{mt}

Accumulating unfolded proteins, unassisted by chaperone Hsp60 in stressed mitochondria, are digested by the protease Clpp. The resulting peptides are transported through the double mitochondrial membrane into the cytosol. These peptides presumably stop mitochondrial import, which is also negatively affected by specific degradation of Tim17 component of the translocation pore by protease Yme1l1. As a result, *C. elegans* transcription factor ATFS-1, which in normal conditions is translocated to mitochondria and degraded by protease LonP, moves into the nucleus together with UBL-5 and DVE-1 to activate a reparative transcriptional program. In mammals, Jnk2 triggers c-Jun binding to AP1 sites, leading to the activation of Chop and Cebpβ transcription. Subsequently, Chop and Cebpβ dimers bind to CHOP sites flanked by MUREs and induce UPR^{mt} target gene transcription. Proteins characterized in *C. elegans* are marked in green, fly and mammalian system proteins in red and proteins conserved in all the systems are noted in blue (mouse nomenclature is used).

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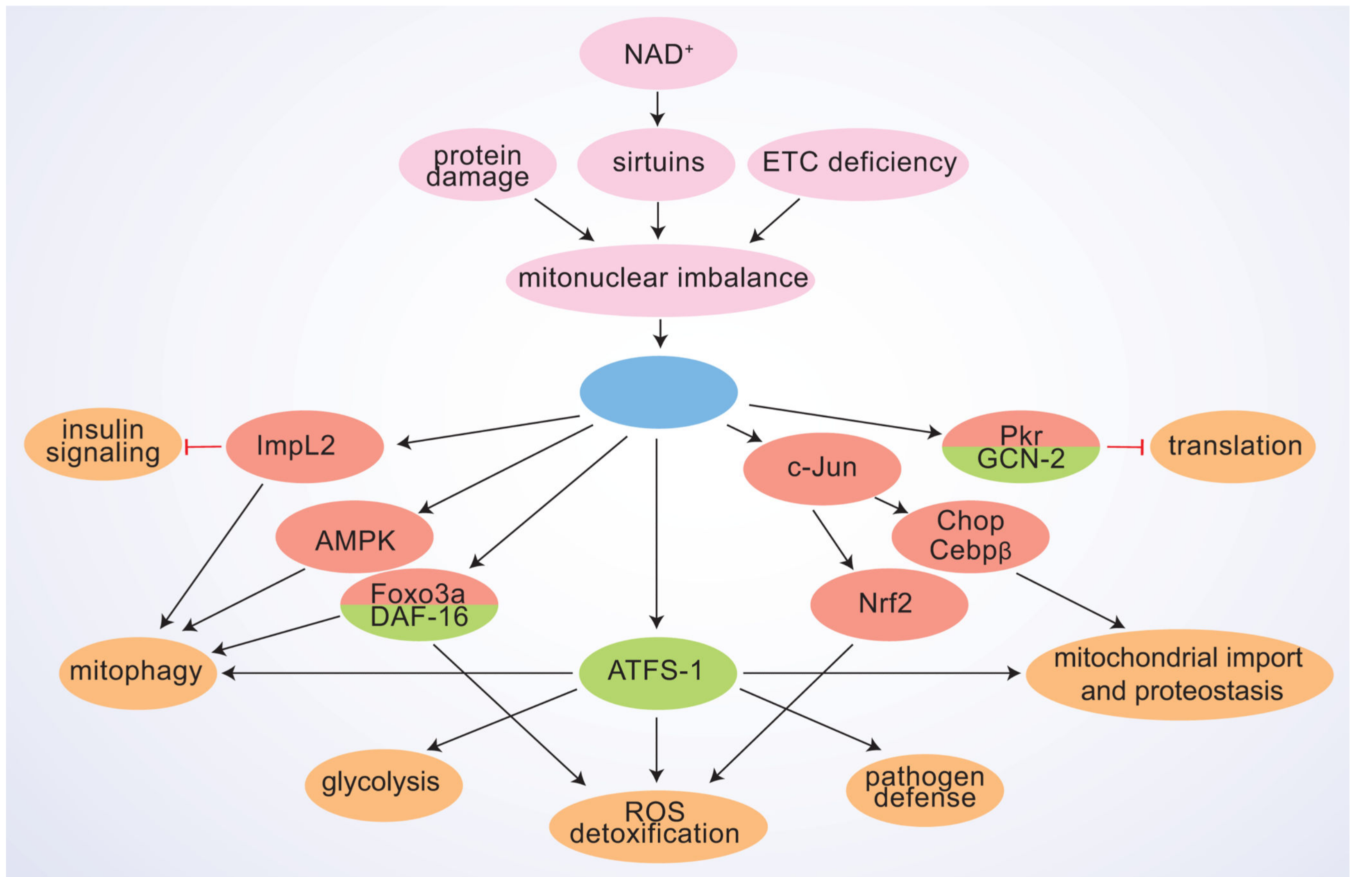

Figure 2. The pleiotropic effects of UPR^{mt}

A scheme summarizing the principle UPR^{mt} sensor/activator signals and the downstream interacting pathways, with their respective cellular effects. Proteins characterized in the fly and/or mammalian systems are marked in red and those studied in *C. elegans* in green.

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Table 1

UPR^{mt} inducing manipulations

	Genetic	**Pharmacological**
protein damage	aggregation prone OTC- [20,22], EndoG [21] overexpression	ROS generator paraquat [23], toxins, produced by pathogenic bacteria [24,25]
interference with PQC	knockdown of *Hspa9* [19], *hsp-60* [17], *dnj-21* [17], *spg-7* [17]	
interference with mitochondrial import and architecture	RNAi of *tim-17, tim-23* (RNAi) [14,15], *phb-2* [16,17]	arsenic (III) [14]
mtDNA depletion	RNAi of mtDNA helicase *pif-1* [17], Deletor mice [34]	ethidium bromide [17,45]
interference with mitochondrial translation	downregulation of various cytosolic and mitochondrial ribosomal proteins [17,26]	bacterial and mitochondrial translation inhibitors doxycycline and chloramphenicol [26]
loss of ETC subunits	*cco-1* RNAi [27], *isp-1* (qm150) [44], *clk-1* (qm30) [44] alleles, RNAi of ND75 [41], *Surf1*$^{-/-}$ mice [42]	ETC inhibitors antimycin [23,24], rotenone [23]
sirtuin activation and mitochondrial biogenesis	*sir-2.1* overexpression [29]	PARP inhibitors MRL45696 [30] and AZD2281 [29], NAD^{+} precursor NR [29], rapamycin [26]