The mitochondrial unfolded protein response: Signaling from the powerhouse

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Mitochondria are multifaceted and indispensable organelles required for cell performance. Accordingly, dysfunction to mitochondria can result in cellular decline and possibly the onset of disease. Cells use a variety of means to recover mitochondria and restore homeostasis, including the activation of retrograde pathways such as the mitochondrial unfolded protein response (UPRmt). In this Minireview, we will discuss how cells adapt to mitochondrial stress through UPRmt regulation. Furthermore, we will explore the current repertoire of biological functions that are associated with this essential stress-response pathway.

Mitochondria are double membrane organelles commonly associated with the production of cellular energy via oxidative phosphorylation (OXPHOS). Mitochondria are also required for the metabolism of nucleotides, amino acids, and lipids and have an essential function in regulating apoptosis. Maintaining mitochondrial integrity is therefore a key aspect in ensuring cellular and organismal viability. Consequently, a decline in mitochondrial function is frequently associated with the development of numerous diseases.

Mitochondria are dependent on a diverse compilation of proteins to carry out their vital functions. However, the mitochondrial proteome is faced with various challenges, most notably the partitioning of protein encoding genes between the mitochondrial and nuclear genomes. Remarkably, the human mitochondrial genome only encodes ~1% of the total mitochondrial proteome with the remaining proteins being encoded by nuclear genes. Sophisticated mechanisms have evolved to efficiently transfer nuclear-encoded mitochondrial proteins to their proper organelle destination following translation on cytosolic ribosomes. To accomplish this complex task, proteins are sorted to mitochondria via targeting sequences that form characteristic amphipathic helices composed of positively charged residues. Such mitochondrial targeting sequences (MTS) are recognized and translocated via the mitochondrial Tom–Tim complex to their respective sub-organelle compartment. The UPRmt is a mitochondrial stress signaling pathway discovered in mammalian cells, but much of what is known with regard to its regulation was discovered in C. elegans (Fig. 1A). Conditions that increase mitochondrial proteotoxicity such as mitochondrial DNA depletion, impaired mitochondrial protein quality control machinery, and OXPHOS perturbation elicit the UPRmt (7, 8). A number of UPRmt regulators have been discovered using genetic approaches, including the mitochondrial matrix protease ClpP, the ubiquitin-like protein UBL-5, the transcription factor DVE-1, the mitochondrial ABC transporter HAF-1, and the bZIP transcription factor ATFS-1 (9, 10). The current paradigm suggests that ClpP proteolytically degrades improperly folded mitochondrial proteins with a subsequent release of the resulting peptides from mitochondria via HAF-1 leading to UPRmt activation (9, 10). How mitochondrial peptidase extrusion affects the activity of the UPRmt is unclear, but it is clear that HAF-1 is not essential for UPRmt activation.

Mitochondrial UPR regulation

Caenorhabditis elegans

The UPRmt is a mitochondrial stress signaling pathway discovered in mammalian cells (6), but much of what is known with regard to its regulation was discovered in C. elegans (Fig. 1A). Conditions that increase mitochondrial proteotoxicity such as mitochondrial DNA depletion, impaired mitochondrial protein quality control machinery, and OXPHOS perturbation elicit the UPRmt (7, 8). A number of UPRmt regulators have been discovered using genetic approaches, including the mitochondrial matrix protease ClpP, the ubiquitin-like protein UBL-5, the transcription factor DVE-1, the mitochondrial ABC transporter HAF-1, and the bZIP transcription factor ATFS-1 (9, 10). The current paradigm suggests that ClpP proteolytically degrades improperly folded mitochondrial proteins with a subsequent release of the resulting peptides from mitochondria via HAF-1 leading to UPRmt activation (9, 10). How mitochondrial peptidase extrusion affects the activity of the UPRmt is unclear, but it is clear that HAF-1 is not essential for UPRmt activation.
Using a genetic approach, two metabolic pathways were identified as important regulators of the UPR\textsuperscript{mt} (18). First, knockdown of components in the sphingolipid biosynthesis pathway, including the enzymes serine palmitoyltransferase and ceramide synthase, results in UPR\textsuperscript{mt} attenuation that can be rescued with ceramide supplementation. Interestingly, ceramide was found to accumulate at mitochondria prior to UPR\textsuperscript{mt} activation (18). Also, inhibition of the sphingolipid biosynthesis pathway could not block UPR\textsuperscript{mt} activation from a gain-of-function ATFS-1 that is constitutively nuclear irrespective of mitochondrial stress (18, 19). Therefore, ceramide accumulation at mitochondria may be an early step in the activation of the UPR\textsuperscript{mt}. In the same genetic screen, knockdown of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase, a component of the mevalonate pathway, also blocked UPR\textsuperscript{mt} activation in the presence of mitochondrial stress (18). The mevalonate pathway is necessary for the synthesis of isoprenoids such as cholesterol. Interestingly, constitutive nuclear accumulation of ATFS-1 rescues the ill effects of statins, a class of cholesterol-lowering drugs that inhibit the mevalonate pathway (19) likely by increasing ubiquinone biosynthesis that is impaired by the statins. Intriguingly, statins block UPR\textsuperscript{mt} activation during mitochondrial stress suggesting that the side effects experienced while taking this drug may be due to an inability to sense and respond to conditions that perturb mitochondrial function (18). In addition to the synthesis of cholesterol, the mevalonate pathway is also important for the production of prenylated lipids such as farnesyl pyrophosphate that can be blocked with statin treatment (20). Protein prenylation involves the addition of the farnesyl moiety to client proteins (21), a reaction that is particularly crucial for the activity of small GTPases (22). Importantly, gain-of-function ATFS-1 animals are also resistant to the toxicity exerted by glitoxin (19), which blocks the addition of farnesyl to small GTPases through the inhibition of farnesyl transferase. This suggests that impaired prenylation may contribute to the negative effects of statins. Perhaps GTPase prenylation is required for UPR\textsuperscript{mt} activation that can be mitigated through constitutive activation of ATFS-1. In support of this possibility, the small GTPase Rheb is required for UPR\textsuperscript{mt} activity (9).

Recent observations suggest that chromatin remodeling is also critically required for UPR\textsuperscript{mt} activity. In the first study, the histone lysine demethylases JMJD-1.2 and JMJD-3.1 were discovered in a genetic screen for positive UPR\textsuperscript{mt} regulators (23). Both show specificity for histone H3K27me2/me3 and are required and sufficient for the UPR\textsuperscript{mt}. Consistent with mediating a mitochondrial protective transcriptional response, genes differentially expressed from JMJD-1.2 and JMJD-3.1 overexpression show significant overlap with gene expression profiles of mitochondrially stressed animals with perturbed OXPHOS (23). Analogous findings were also observed with the mammalian homologs of JMJD-1.2 (Phf8) and JMJD-3.1 (Jmjd3), suggesting a conserved mechanism of UPR\textsuperscript{mt} regulation (23). In the second study, the H3K9 methyltransferase MET-2 and the novel protein LIN-65 were found to positively regulate the UPR\textsuperscript{mt} (24). LIN-65 is initially cytosolic but localizes to the nucleus in the presence of mitochondrial stress in a MET-2-dependent manner (24), suggesting they are functionally
related. Indeed, both MET-2 and LIN-65 are required for the methylation of histone H3K9 that is needed for accumulation of the UPR\textsuperscript{mt} transcriptional regulator DVE-1 into discrete nuclear puncta, specifically to chromatin regions thought to represent sites of active gene expression (9, 24). These findings add an extra layer of complexity to the regulation of the UPR\textsuperscript{mt}, suggesting that epigenetic modifications are a necessary step for the activation of this mitochondrial recovery program.

Remarkably, mitochondrial damage originating from one tissue can induce a UPR\textsuperscript{mt} in distal tissues via cell non-autonomous signaling (Fig. 2) (25). For example, neuronal mitochondrial dysfunction can induce the UPR\textsuperscript{mt} in the intestine through the production of secreted "mitokines" (25). Presumably, sensory neurons that are in contact with the external environment housing potentially damaging toxins would be ideal to relay mitochondrial stress signals to tissues, like the intestine, that are likely to be impacted by toxin exposure during feeding. Two recent advancements have been made into the identity of the "mitokines" in animals: serotonin and the neuropeptide FLP-2.

Comparison of the UPR\textsuperscript{mt} and UPR\textsuperscript{ER}

Much like mitochondria, the endoplasmic reticulum (ER) is prone to the accumulation of misfolded proteins and similarly responds to increases in proteotoxicity through the transcriptional up-regulation of ER chaperones and proteases to restore protein homeostasis via the UPR\textsuperscript{ER} (33). Although conceptually similar to the UPR\textsuperscript{mt}, the UPR\textsuperscript{ER} possesses distinct signaling mechanisms that are involved in its regulation. There are three main regulatory pathways that govern the UPR\textsuperscript{ER}: the serine/threonine kinase/endonuclease IRE1; the bZIP transcription factor ATF6; and the kinase PERK. All three proteins are bound in an inactive state to the ER chaperone BiP and are released in increased expression of mitochondrial protein quality control genes in the nucleus (Fig. 1B) (6). This transcriptional response to mitochondrial dysfunction is thought to require the C/EBP transcription factor CHOP as the corresponding consensus binding site is present in multiple genes that are induced during the UPR\textsuperscript{mt} (28, 29). Consistently, CHOP is transcriptionally induced during the UPR\textsuperscript{mt} presumably by the transcription factor c-Jun (6, 28).

Inspired by the discovery of ATFS-1, Fiorese et al. (30) identified the bZIP transcription factor ATF5 as an additional regulator of the UPR\textsuperscript{mt} response. Mitochondrial import efficiency appears to regulate ATF5 in a manner akin to ATFS-1 because ATF5 also contains a presequence that directs its localization to mitochondria in the absence of mitochondrial stress (30). Similar to ATFS-1, ATF5 transcriptionally regulates mitochondrial protective gene expression that is necessary for cell growth during mitochondrial stress (30). Interestingly, ATF5 can induce a UPR\textsuperscript{mt} in C. elegans in the absence of ATFS-1 suggesting that it is a conserved mammalian homolog (30). However, there are still questions left unanswered with regard to ATF5 and the mammalian UPR\textsuperscript{mt}. First, is ATF5 proteolytically degraded in healthy mitochondria by LONP1 similar to ATFS-1? LONP1 expression is transcriptionally induced during the UPR\textsuperscript{mt} by ATF5 suggesting a functional relationship (30). Second, does ATF5 accumulation in the nucleus during the UPR\textsuperscript{mt} require ClpP-derived peptide efflux via the mammalian homolog of HAF-1 (TAP)? Finally, how are ATF5 and CHOP coordinated during the UPR\textsuperscript{mt}? Interestingly, the CHOP consensus sequence is present in some genes that are also regulated by ATF5 during the UPR\textsuperscript{mt} (28, 30) suggesting some degree of overlap in target gene regulation. Further work is therefore needed to better understand the specific mechanisms of ATF5 regulation during the UPR\textsuperscript{mt}.

The mitochondrial intermembrane space (IMS) contains its own suite of protein quality machinery and potentially distinct stress response. Perturbation to the IMS protein folding environment elicits a protective response through the phosphorylation of the estrogen receptor α by Akt (31). In a recent study, proteotoxicity to the mitochondrial matrix and IMS activated the deacetylase SirT3 to promote mitochondrial recovery through the activation of anti-oxidant machinery and the stimulation of mitophagy (32). Presumably, the SirT3 response during mitochondrial stress is mediated by the transcription factor FOXO3A that accumulates in the nucleus following SirT3-mediated deacetylation (32).

**Mammals**

The UPR\textsuperscript{mt} was first discovered in mammalian cultured cells expressing a misfolded mitochondrial protein that resulted in
the presence of ER stress. Active IRE1 results in preferential mRNA splicing of the bZIP transcription factor XBP1 resulting in its activation and subsequent transcriptional up-regulation of proteostasis genes. ATF6 also positively regulates the expression of proteostasis genes following its activation by proteolytic cleavage.

In addition to transcriptionally regulating the expression of ER protective genes, the UPRER helps restore ER homeostasis by attenuating protein translation through PERK-dependent phosphorylation of the translation initiation factor eIF2α (34). PERK is among four kinases that constitute the integrated stress response (ISR) that diminishes protein translation in response to various cellular stresses (35). Interestingly, the ISR kinase GCN2 is activated by increased ROS during mitochondrial stress and helps restore mitochondrial homeostasis by lowering cytosolic protein translation via phosphorylation of eIF2α, thus reducing the load of incoming unfolded mitochondrial proteins (36). Although general protein translation is attenuated during the ISR, mRNAs containing upstream open reading frames (uORFs) are favorably translated. Intriguingly, ATF5 is among the uORF containing mRNAs that are preferentially translated following eIF2α phosphorylation during ER stress (37). Whether mitochondrial stress results in a similar preferential translation of ATF5 is still unknown.

It is noteworthy that the UPRmt and UPRER share some common regulatory proteins, suggesting a possible intersection in the regulatory inputs of both stress responses. For example, CHOP is also activated during ER stress downstream of the bZIP transcription factor ATF4 (38). In this context CHOP assumes a pro-apoptotic role by transcriptionally regulating ATF5, but whether a similar mode of regulation occurs during UPRmt activation is not known (39). In contrast to the pro-apoptotic role of CHOP and ATF5 during ER stress, these transcription factors adopt a pro-survival function during mitochondrial stress through the regulation of mitochondrial protective gene expression (6, 30). Possibly, CHOP and ATF5 undergo stress-specific post-translational modifications or heterodimerization that dictate their particular function.

Physiological roles of the UPRmt

Protection against pathogen infection

Mitochondria are desirable targets for bacterial pathogens during infection. For example, various pathogens manipulate mitochondrial-dependent cell death pathways to promote their own survival (40). Mitochondria also play an important role in regulating the innate immune response (41) that can be compromised during infection by certain pathogens (42). As such, bacterial pathogens have evolved various means to exploit mitochondrial function, including the precise targeting of protein virulence factors directly to mitochondria (43).

Host resistance to infection is dependent on innate and adaptive immune responses that limit the colonization of pathogenic microbes. In contrast, host tolerance during infection is defined as the ability to withstand the damage caused by harmful pathogens and possibly the self-damage inflicted by the host resulting from the inflammatory response (44). The UPRmt supports host tolerance by regulating mitochondrial recovery while additionally promoting host resistance through the transcriptional regulation of various innate immunity genes such as secreted anti-microbial peptides and lysozymes (Fig. 3A) (45). Various microbes such as the opportunistic pathogen Pseudomonas aeruginosa can activate the UPRmt through the production of mitochondrial toxins (18, 45). Consistent with a role in protecting the host from infection, ATFS-1 is required and sufficient for survival during P. aeruginosa infection (45, 46). Interestingly, the mitochondrial chaperone HSP-60 that is transcriptionally up-regulated during the UPRmt by ATFS-1 (7, 10, 11) promotes host resistance during infection by enhancing the PMK-1/p38 MAPK innate immunity pathway through direct binding and stabilization of SEK-1/MAPK kinase 3 (46).

The coupling of host tolerance and resistance to one stress-response pathway is an efficient means of protecting the host during infection considering that mitochondria are targeted by a number of pathogens (18, 43, 45). Furthermore, the UPRmt may be regarded as an additional sensor of infection from the damage that is inflicted by harmful microbes targeting mitochondrial function. It is unclear whether all metazoans couple the UPRmt to the regulation of innate immunity during infection. However, the homolog of ATFS-1 in the whiteleg shrimp induces the expression of antimicrobial peptides and is required for survival during pathogen infection suggesting that UPRmt-mediated regulation of host resistance may be conserved (47).

Accumulation of deleterious mitochondrial genomes

Deletions in mitochondrial DNA (mtDNA) occur as an organism ages and contributes to various diseases (48). However, each cell houses hundreds of mitochondrial genomes as a mixture of wild-type and mutant forms, a phenomenon known as heteroplasmy (49), and accordingly the number of deleterious mitochondrial genomes only needs to exceed a certain threshold to be pathogenic (typically comprising more than 60% of the total number of mitochondrial genomes) (49). The mechanism that regulates the number of deleterious mitochondrial genomes has remained elusive until the discoveries made by
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two recent independent studies (50, 51). Here, a C. elegans heteroplasmic strain harboring mtDNA with a 3.1-kb deletion (ΔmtDNA (52)) activated the UPR<sup>mt</sup> as a result of reduced OXPHOS activity (50, 51). Surprisingly, loss of the UPR<sup>mt</sup> through ATFS-1 inhibition reduced the number of ΔmtDNA copies, whereas constitutive activation of ATFS-1 promoted the accumulation of ΔmtDNA, further reducing OXPHOS efficiency and causing impaired development (50, 51).

How does ATFS-1 promote the accumulation of deleterious ΔmtDNA? Potentially, the UPR<sup>mt</sup> limits mitophagy by promoting mitochondrial health, but ATFS-1 appears to preferentially promote ΔmtDNA accumulation independent from mitophagy (50, 51). ATFS-1 does promote the expression of genes involved in mitochondrial biogenesis, including regulators of mtDNA replication such as mtDNA polymerase and TFAM (51) as well as transcriptionally regulating genes involved in mitochondrial dynamics (7, 51). Reducing regulators of mtDNA replication and mitochondrial dynamics attenuate the increased ΔmtDNA number that is observed in constitutively active ATFS-1 animals suggesting that both are involved in the ATFS-1-dependent propagation of ΔmtDNA (51). Together, these studies reveal an unexpected toxic consequence of the UPR<sup>mt</sup> mitochondrial recovery program that expands ΔmtDNA through the stimulation of mitochondrial biogenesis (Fig. 3B).

**Metabolic adaptations**

Paradoxically, in addition to nuclear accumulation, ATFS-1 also accumulates in mitochondria during stress despite reduced mitochondrial import efficiency (7, 11). Multiple isoforms of ATFS-1 are present in mitochondria during the UPR<sup>mt</sup>, but the exact identity of these isoforms is still unknown. However, two splice variants of ATFS-1 exist with a difference of just 16 amino acids (11). Although both the short and long ATFS-1 isoforms contain the MTS, it is not known which isoform accumulates in mitochondria during stress. Within mitochondria ATFS-1 binds the D-loop of mtDNA, an A-T-rich region residing in the control region adjacent to transcriptional regulatory elements (11). Here, ATFS-1 limits the abundance of OXPHOS transcripts encoded by the mitochondrial genome. ATFS-1 regulates mitochondrial OXPHOS transcript numbers potentially through transcriptional repression via its association with the D-loop region; however, its effects on transcript stability have yet to be studied. Interestingly, ATFS-1 also negatively regulates nuclear-encoded OXPHOS gene transcript abundance through direct or indirect binding of their gene promoters (11). Thus, ATFS-1 appears to transcriptionally attenuate or limit OXPHOS biogenesis until the protein folding capacity is restored as a means of promoting mitochondrial recovery. In addition to repressing the abundance of OXPHOS transcripts, ATFS-1 also transcriptionally induces the expression of genes involved in glycolysis (7, 11). By positively regulating glycolysis gene expression, ATFS-1 presumably ensures adequate ATP levels in lieu of reduced OXPHOS activity in an effort to promote mitochondrial repair and cell survival (Fig. 3C).

**Hematopoietic stem cell maintenance**

Quiescent stem cells have relatively little mitochondrial activity that increases during differentiation (53). In the study by Mohrin et al. (54), the histone deacetylase SIRT7 was found to reduce mitochondrial translation by complexing with the central metabolic regulator of mitochondrial gene expression NRF1 and repressing transcription of mitochondrial ribosomes and translation factors. Loss of SIRT7 consequently results in enhanced mitochondrial translation and biogenesis leading to the activation of the UPR<sup>mt</sup>. Interestingly, inhibiting SIRT7 in hematopoietic stem cells (HSC) enhances mitochondrial biogenesis and activates the UPR<sup>mt</sup>, thus reducing quiescence and the ability to differentiate. Consistent with a role in maintaining stem cell quiescence, SIRT7 levels are elevated during the quiescent stage and low in aged stem cell populations (54). Consequently, increasing SIRT7 levels in aged stem cells decrease mitochondrial proteotoxicity and improve their differentiation potential. Although it is still unclear whether specific loss of UPR<sup>mt</sup> regulators impacts HSC maintenance, this study suggests the importance of mitochondrial protein homeostasis in stem cell vitality (Fig. 3D).

**The future of the UPR<sup>mt</sup>**

A decline in the mitochondrial function likely impacts a wide range of cellular activities because of the essential nature of the organelle. Consequently, mitochondrial stress presumably activates parallel cytoprotective pathways. For instance, mitochondrial stress results in the activation of multiple signaling modules in addition to the UPR<sup>mt</sup>, including the hypoxia-response transcription factor HIF-1, the AMP-activated kinase pathway, and the transcription factors TAF-4 and CEH-23 (55–57). As well, increased mitochondrial ROS can activate an oxidative stress response regulated by the transcription factor SKN-1 (58). Intriguingly, ATFS-1 regulates the expression of skn-1 as part of the UPR<sup>mt</sup> transcriptional response (9). Finally, accumulation of mitochondrial precursor proteins in the cytosol as a result of impaired mitochondrial import elicits a separate stress-response pathway involving reduced protein translation and increased proteasome activity (12, 14). How the UPR<sup>mt</sup> integrates with these and other cellular stress pathways is therefore an area worthy of further investigation.

Also, as the UPR<sup>mt</sup> is an important regulator of mitochondrial recovery, it may be an attractive therapeutic target for those diseases that result from mitochondrial dysfunction. The therapeutic potential of the UPR<sup>mt</sup> is particularly promising in the field of cancer research. The relationship between mitochondrial dysfunction and cancer was first proposed based on the observation by Otto Warburg that cancer cells utilize glycolysis in the presence of oxygen presumably due to a defect in mitochondrial respiration and function (59). Although this remains true with certain cancers, it can by no means be generalized. Indeed, certain cancers display normal or even enhanced mitochondrial function (60) suggesting a considerable heterogeneity in cancer subtypes. It is clear though that some cancer cells express higher levels of mitochondrial chaperones indicative of an activated UPR<sup>mt</sup> (30, 61–63). The UPR<sup>mt</sup> in this context could help support tumor survival from the mitochondrial dysfunction that may arise from genetic mutations (64) or through the increase in mitochondrial biogenesis used to support tumor growth and proliferation (65). Interestingly, inhibiting mitochondrial chaperones or the
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UPR\textsuperscript{mt} regulator ATF5 increases tumor cell death while having little to no effect on healthy cells (30, 63, 66), supporting the targeting of the UPR\textsuperscript{mt} as an anti-cancer therapy. How the UPR\textsuperscript{mt} might be pursued for other mitochondrially related pathologies such as neurological disease and diabetes is still relatively unexplored and is likely to be an exciting avenue of research in the field.

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