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Signaling the Mitochondrial Unfolded Protein Response

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Abstract

Mitochondria are compartmentalized organelles essential for numerous cellular functions including ATP generation, iron-sulfur cluster biogenesis, nucleotide and amino acid metabolism as well as apoptosis. To promote biogenesis and proper function, mitochondria have a dedicated repertoire of molecular chaperones to facilitate protein folding and quality control proteases to degrade those proteins that fail to fold correctly. Mitochondrial protein folding is challenged by the complex organelle architecture, the deleterious effects of electron transport chain-generated reactive oxygen species and the mitochondrial genome's susceptibility to acquiring mutations. In response to the accumulation of unfolded or misfolded proteins beyond the organelle's chaperone capacity, cells mount a mitochondrial unfolded protein response (UPR^{mt}). The UPR^{mt} is a mitochondria-to-nuclear signal transduction pathway resulting in the induction of mitochondrial protective genes including mitochondrial molecular chaperones and proteases to re-establish protein homeostasis within the mitochondrial protein-folding environment. Here, we review the current understanding of UPR^{mt} signal transduction and the impact of the UPR^{mt} on diseased cells.

1. Introduction

Mitochondrial protein homeostasis is maintained through proper folding and assembly of newly translated polypeptides, as well as efficient trafficking and turnover of those proteins that fail to fold correctly [1–3]. The load of unfolded proteins in mitochondria must precisely match the chaperone protein-folding capacity. If the chaperone capacity is exceeded, each organelle becomes susceptible to the deleterious effects of protein misfolding and aggregation. However, during stress cells employ strategies to protect the protein-folding environment including organelle-specific quality control proteases to degrade the unfolded or misfolded proteins [4] and mitochondrial unfolded protein responses to increase chaperone capacity and re-establish homeostasis within the mitochondrial protein-folding environment [5]. Several factors challenge the mitochondrial protein-folding environment including complexities in mitochondrial biogenesis, DNA and protein damaging reactive oxygen species (ROS) that are generated within mitochondria, as well as environmental factors such as changes in temperature and exposure to toxins [6].

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1.1 Complexities of mitochondrial biogenesis that threaten protein homeostasis

Mitochondria are double-membrane bound organelles composed of four compartments: the outer and inner membranes, the intermembrane space (IMS) and the matrix. Each compartment is a separate protein-folding environment, which must be maintained for efficient mitochondrial biogenesis and proper function. The mitochondrial proteome is composed of approximately 1200 proteins encoded by two separate genomes [7]. The mitochondrial genomes (mtDNA) are localized within the matrix and encode 13 essential components of the electron transport chain (ETC) and the ATP synthase as well as a number of mitochondrial-specific tRNAs. The remainder of the mitochondrial proteome is encoded by the nuclear genome, translated in the cytosol and imported into each mitochondrion [8, 9]. Because ETC complexes I, III, IV and the ATP synthase are composed of components encoded by both genomes, it is imperative that expression from both genomes be coordinated to prevent the accumulation of orphaned subunits.

The mitochondrial protein-folding environment can be disturbed by excessive ROS generated from the ETC primarily via the NADH-ubiquinone oxidoreductase (complex I) and the ubiquinol cytochrome c oxidoreductase (complex III) [10, 11], which directly perturb protein folding and structure. Additionally, mtDNA is prone to the accumulation of mutations, presumably because of its exposure to ROS and that it is not protected by histones [12, 13]. Mutations that reduce expression of ETC components or perturb their ability to fold, compromise assembly of the individual complexes putting stress on the protein-folding environment. Additionally, numerous toxins impair protein homeostasis such as paraquat, which causes high levels of ROS accumulation [14], and rotenone, which impairs complex I assembly and function [15].

1.2 Mitochondrial protein quality control: chaperones and proteases

To promote efficient mitochondrial protein folding and complex assembly, mitochondria have a dedicated repertoire of localized molecular chaperones located in both the IMS and matrix [1, 9]. The Hsp60 chaperonin is in the matrix and consists of both Hsp60 and Hsp10 subunits which form a barrel-shaped complex. Hsp60 primarily facilitates the folding of relatively small, soluble monomeric proteins [16–18]. mtHsp70 also resides in the matrix where it performs multiple functions. At the translocase of the inner membrane (TIM23) channel, mtHsp70 functions in the multi-subunit PAM (Presequence Translocase-Associated Motor) where it interacts with the translocating polypeptides to drive their movement through the import channel into the matrix [9, 19]. In a separate complex, mtHsp70 promotes protein folding and complex assembly of imported polypeptides while preventing aggregation [20–22]. Additionally, mtHsp70 is required for the biogenesis of iron-sulfur clusters within the matrix [23]. Mitochondria also contain an Hsp90 isoform known as TRAP-1 (TNF receptor-associated protein 1) that is thought to promote protein folding in a manner similar to the cytosolic isoforms of Hsp90 [24]. At present, no member of the Hsp60 or Hsp70 family of molecular chaperones has been observed in the IMS of mitochondria, however the Tim9-Tim10 complex promotes the import of highly hydrophobic membrane spanning proteins by preventing non-productive protein-protein interactions as the polypeptides traverse the IMS [25].

In addition to molecular chaperones, mitochondria house several quality control proteases that recognize and degrade those proteins that fail to fold or assemble correctly. Both ClpXP and Lon are AAA proteases (ATPase Associated with diverse cellular Activities) located within the matrix that primarily degrade misfolded soluble proteins [3, 4]. Interestingly, the Lon protease has been shown to preferentially degrade oxidatively-damaged proteins including aconitase [26]. Both Paraplegin (encoded by the *SPG7* gene) and YME1L are AAA proteases that are anchored within the inner membrane with their active sites facing

the matrix and IMS, respectively. The primary role of YME1L and Paraplegin is to degrade misfolded or misassembled subunits of the ETC [27], although a recent role has been described for Paraplegin in mitochondrial ribosome biogenesis [28, 29]. OMI/Htra2 resides in the IMS where it has been suggested to recognize and degrade soluble proteins that fail to fold correctly [30].

Numerous disease scenarios in which mitochondrial protein homeostasis is compromised emphasize the importance of maintaining the mitochondrial protein-folding environment [13, 31, 32]. Both paraquat and rotenone, as well as the *Htra2*-deletion cause Parkinson's-like symptoms in mice [15, 33]. Mutations in the mitochondrial chaperonin Hsp60 and mitochondrial quality control protease Paraplegin cause the neurodegenerative disease spastic paraplegia [34]. In addition to a variety of diseases, loss of mitochondrial protein homeostasis has been closely associated with the aging process [6, 13].

2. Compartment-specific unfolded protein response pathways

The cytosol, endoplasmic reticulum and mitochondria are all exposed to nascent polypeptides, thus each compartment requires dedicated protein-folding machinery, which constitutes each organelle's protein-folding capacity. Stress occurs when the quantity of unfolded or misfolded proteins exceeds a compartment's protein-folding capacity, rendering the organelle susceptible to catastrophic damage. To adjust folding capacity, eukaryotic cells have evolved organelle-specific signaling pathways known as unfolded protein responses (UPRs).

The heat shock response protects the cytosolic protein-folding environment and is regulated by the transcription factor Heat Shock Factor 1 (HSF1) [35]. In the absence of stress, HSF1 associates with the cytosolic chaperones Hsp70 and Hsp90. However, when unfolded proteins accumulate beyond the cytosolic chaperone capacity, HSF1 dissociates allowing it to trimerize and interact with the promoters of genes that constitute the heat shock response [36]. HSF1 mediates the expression of a number of chaperone genes that localize to the cytosol and nucleus including Hsp70 and Hsp90. Additionally, HSF1 induces the expression of a number of components of the ubiquitin-proteasome system to degrade terminally misfolded proteins and reduce the burden on the cytosolic protein-folding machinery [36]. Conditions that activate the heat shock response include increased temperatures and exposure to toxins such as arsenite that perturb protein folding in the cytosol [37, 38].

The protein-folding environment of the ER is protected by a separate unfolded protein response (UPR^{ER}) [39]. The most conserved branch of the UPR^{ER} consists of the ER membrane spanning kinase Ire1 and the bZip transcription factor Xbp1. Ire1 monitors the protein-folding environment of the ER lumen and initiates URP^{ER} signaling by directly recognizing unfolded proteins [40]. If stress occurs in the ER, Ire1 oligomerizes [41] activating its cytosolic kinase domain. Once activated, the cytosolic domain of Ire1 splices an intron from the Xbp1 transcript [42] allowing translation of a functional bZip protein which traffics to the nucleus to induce the UPR^{ER}. The UPR^{ER} includes a number of ER-targeted protein-folding machineries including BiP, an ER-targeted Hsp70, protein disulfide isomerase and the glycosylation machinery [43]. Additionally, the UPR^{ER} activates expression of ERAD (ER-associated degradation) components which serve to recognize misfolded proteins, retrotranslocate them across the ER membrane to the cytosol where they are ubiquitylated and degraded by the proteasome [44].

In addition to increasing ER-specific protein folding and quality control machinery, a branch of the UPR^{ER} also briefly attenuates protein translation to reduce the load on ER folding capacity [39]. In response to ER stress, the ER membrane spanning kinase PERK

(Pancreatic enriched ER kinase) dimerizes and phosphorylates the alpha subunit of eIF2 (eukaryotic initiation factor), which serves to attenuate protein translation [45].

Conceptually similar to the UPR^{ER} and heat shock response, accumulating evidence supports the existence of a UPR^{mt}; a mitochondria-to-nucleus signal transduction pathway that senses unfolded protein stress within the organelle and transmits a signal out of mitochondria, through the cytosol to the nucleus where the up-regulation of genes encoding mitochondrial chaperones and quality control proteases takes place to re-establish mitochondrial protein homeostasis [5].

3. Retrograde transcriptional responses from mitochondria suggested the presence of a UPR^{mt}

The survival of cells devoid of mtDNA (ρ^0 cells) suggested the activation of nuclear responses as compensation for severe mitochondrial dysfunction. Indeed, ρ^0 cells undergo a number of changes in nuclear gene expression including the induction of mitochondrial molecular chaperone and protease genes [46–49]. The absence of mtDNA places a considerable amount of stress on the mitochondrial protein-folding environment as those ETC components encoded by the nucleus are still imported into mitochondria but unable to assemble into stoichiometric complexes in the absence of their mtDNA-encoded binding partners. In addition to increasing the mitochondrial protein homeostasis machinery, cells down-regulate the expression of the ETC components in complex I, III and IV to protect the protein-folding environment but not the ATP synthase as it is required to maintain the membrane potential and cell viability [46]. ρ^0 cells also down-regulate numerous components required for protein translation [49], consistent with slowed import reducing the load on the mitochondrial protein-folding environment [50]. Interestingly, the lack of mtDNA also affects the expression of a number of cell cycle regulators such as p19, a cyclin dependent kinase inhibitor involved in cell cycle arrest at G1 [46], suggesting that the cell cycle is slowed during periods of mitochondrial dysfunction to prevent replication of cells with defective mitochondria.

Other forms of mitochondrial stress also elicit transcriptional programs consistent with the presence of a UPR^{mt}. For example, deletion of the yeast Paraplegin homolog Yta12, which encodes a mitochondrial quality control protease [4], results in mitochondrial protein aggregation and widespread changes in nuclear-encoded genes [51, 52]. Similarly, inhibition of mtHsp90 results in the up-regulation molecular chaperone genes as well as a number of genes involved in metabolism [53]. Together, these data suggest the presence of specific stress response pathways that protect against mitochondrial dysfunction.

Perhaps the best-characterized mitochondria-to-nuclear signal transduction pathway is the retrograde response (RTG), thoroughly characterized in yeast. The RTG pathway is activated during mitochondrial dysfunction including that caused by mtDNA depletion to increase activity of numerous metabolic pathways that compensate for the lack of mitochondrial activity. For example, a peroxisomal isoform of citrate synthase (*CIT2*) is induced to increase activity of the glyoxylate cycle, a variant of the tricarboxlic acid cycle induced in ρ^0 cells [54]. The RTG response is mediated by *RTG1* and *RTG3*, two basic helix-loop-helix leucine zipper (bHLH/Zip) transcription factors [55, 56]. When mitochondria are functional, RTG3 is hyperphosphorylated, sequestering both itself and RTG1 in the cytoplasm [56]. However, mitochondrial dysfunction causes RTG3 to be partially dephosphorylated allowing nuclear translocation of the RTG1/3 complex and the induction of the compensatory response.

While the RTG response is required for the induction of many genes required for metabolic adaptations, it does not regulate the expression of mitochondrial chaperone or protease genes during mitochondrial stress, suggesting an independent mechanism for UPR^{mt} activation. Here, we review the emerging data regarding the mechanisms of stress sensing and mitochondria-to-nucleus signal transduction.

4. Mitochondrial-to-nuclear UPR^{mt} signal transduction in mammalian cell culture

To demonstrate the presence of a UPR^{mt}, a terminally misfolded, mutant form of ornithine transcarbamylase (OTC) was targeted to the mitochondrial matrix. The presence of unfolded protein in the matrix resulted in the increased expression of several genes that promote mitochondrial protein homeostasis including *Hsp60*, *Hsp10*, *mtDnaJ* and *ClpP* (Figure 1) [57]. Importantly, expression of cytosolic and ER chaperone genes were unaffected, indicating specificity of the UPR^{mt}. The promoters of the *Hsp60*, *ClpP* and *mtDnaJ* genes were shown to contain a mitochondrial stress responsive element that corresponds to the CHOP (CCAAT/enhancer-binding protein (C/EBP)-homologous protein) transcription factor consensus binding site [57, 58]. Additionally, the *CHOP* gene itself is up-regulated during mitochondrial stress, further supporting a central role for CHOP in UPR^{mt} signaling [57].

However, it was somewhat surprising that CHOP was shown to induce a mitochondrial specific response considering CHOP is also known to be activated by ER stress, genotoxic stress, as well as arsenite exposure [59–61]. Interestingly, an AP-1 (activator protein-1) element within the CHOP promoter is necessary for mitochondrial stress-induced expression [62]. The transcription factor c-Jun is known to bind to the AP-1 consensus sequence upon activation by JNK (c-Jun N-terminal kinase) [63], suggesting that c-jun and JNK play a role upstream of CHOP in UPR^{mt} signaling. Indeed, increased mitochondrial unfolded protein stress stimulates the phosphorylation of JNK2, providing further support for a role of this kinase in CHOP activation [62] (Figure 1). Numerous downstream regulatory events in UPR^{mt} signaling have been documented including transcription factor activation and the resulting transcriptional outputs. However, several questions still remain including the mechanism cells use to sense unfolded proteins within the matrix and how the signal is transmitted across both mitochondrial membranes. Additionally, does the response, which has been studied in cell lines, occur in vivo and does CHOP inhibition impair mitochondrial chaperone induction?

Recent findings have indicated the presence of a separate UPR^{mt} signaling pathway that specifically responds to unfolded protein stress within the IMS (Figure 1) [30, 64]. Expression of mutant EndoG that localizes to the IMS causes AKT phosphorylation and activation of the nuclear hormone receptor estrogen receptor alpha (ERα) [64], consequently resulting in the increased expression of the IMS-localized quality control protease HtrA2 and the transcription factor NRF1, which is involved in mitochondrial biogenesis [64]. UPR^{mt} regulation by the estrogen receptor is consistent with its documented role in promoting mitochondrial fitness [65]. In addition to HtrA2, activity of the proteasome is also increased in response to IMS stress which is hypothesized to prevent the accumulation of misfolded IMS proteins by ubiquitylating them prior to import [64]. The early studies on the IMS-specific UPR^{mt} provide a framework for signal transduction but several similar questions to those of the matrix-specific response remain to be addressed.

5. Mitochondrial-to-nuclear UPR^{mt} signal transduction in *C. elegans*

To identify additional UPR^{mt} signaling components and dissect the signaling mechanisms we established a genetically tractable model system using *C. elegans*. Mitochondrial chaperone gene expression is monitored in vivo using UPR^{mt} reporter worms which harbor promoters of the mitochondrial chaperone genes *Hsp60* or *mtHsp70* driving expression of GFP [52]. Similar to mammalian cells, treatment of *C. elegans* with ethidium bromide, a chemical reagent known to reduce mtDNA transcription and replication [66], causes increased expression of the mitochondrial chaperone reporters as well as endogenous mitochondrial chaperone genes [52]. Additionally, knockdown of the mitochondrial quality control protease SPG-7/Paraplegin and mitochondrial chaperone genes activates mitochondrial chaperone gene expression, indicating that perturbations in the mitochondrial protein-folding environment activate this transcriptional response.

Using the UPR^{mt} reporter worms, we performed a genome-wide RNAi-based screen to identify a number of components required for UPR^{mt} signal transduction that constitute a signaling pathway connecting the mitochondrial matrix to the nucleus [67–69]. The current data suggest the following model for how stress is sensed in the mitochondrial matrix and transmitted to the nucleus. As unfolded proteins exceed the matrix chaperone capacity, they are degraded by a quality control protease into peptides, which are pumped across the inner membrane by a peptide transporter. Peptide efflux leads to the activation of a bZip transcription factor, which accumulates in the nucleus to activate mitochondrial chaperone gene induction (Figure 2) [5, 69]. The data that support this model are described in the following sections, however it should be noted that perturbations that affect the mitochondrial protein-folding environment are likely to also affect diverse aspects of mitochondrial biology and potentially unidentified signaling mechanisms.

5.1 Initiation of UPR^{mt} signaling

Knockdown of the mitochondrial quality control protease ClpP abolishes induction of mitochondrial chaperone genes during mitochondrial stress [68], which has more recently been observed in mammalian cells [70]. Consistent with a role in mitochondrial protection, worms with reduced ClpP activity develop much slower in the presence of mitochondrial stress [68]. The localization of ClpP within the mitochondrial matrix, the same compartment where the stress originates, suggests an upstream function for the quality control protease in UPR^{mt} signaling.

ClpP recognizes and degrades misfolded proteins into peptides of approximately 8–20 residues [71]. Interestingly, peptides that accumulate in the mitochondrial matrix are extruded into the IMS via an ABC (ATP Binding Cassette) transporter; Mdl1 in yeast [72] and HAF-1 in *C. elegans* [69] suggesting ClpP-derived peptides may act as signaling components in the UPR^{mt}. Indeed, deletion of the ATP-dependent peptide transporter HAF-1 attenuates UPR^{mt} activation during mitochondrial stress [69]. HAF-1 is localized within the mitochondrial inner membrane and is essential for survival under conditions of protein misfolding [69] similar to ClpP. Additionally, ATP-dependent efflux of peptides from mitochondria is reduced following loss of *clpp-1* and *haf-1*, suggesting a role of ClpP upstream of HAF-1 [69].

5.2 UPR^{mt} signaling requires the transcription factor ATFS-1

The requirement for ClpP and HAF-1 suggest a means to sense unfolded protein stress and transmit the signal to the cytoplasm, however the downstream transcription factor was unknown. We recently identified the bZip transcription factor ATFS-1 (**A**ctivating **T**ranscription **F**actor associated with **S**tress, previously known as ZC376.7) as being

required for UPR^{mt} signaling and acting downstream of HAF-1 [69]. Similar to inhibition of ClpP and HAF-1, worms lacking ATFS-1 develop much more slowly in the presence of mitochondrial stress consistent with a role in mitochondrial protection. Furthermore, during mitochondrial stress, ATFS-1 accumulates in the nucleus in a HAF-1-dependent manner, demonstrating that matrix protein degradation and peptide efflux act upstream of ATFS-1 [69]. The mechanism by which the efflux of mitochondrial-derived peptides influences ATFS-1 remains to be determined. However, it is conceivable that the released peptides are recognized by specialized receptors or perhaps the rate of peptide efflux determines downstream activation of ATFS-1.

5.3 The UPR^{mt} requires a second transcriptional complex consisting of DVE-1 and UBL-5

The homeobox transcription factor DVE-1 and the ubiquitin-like protein UBL-5 are also required for transcriptional up-regulation of mitochondrial molecular chaperone genes [68]. DVE-1 is localized within nuclei of all cells but undergoes a nuclear re-distribution when the mitochondrial protein-folding environment is perturbed, at which time it binds to the promoters of mitochondrial molecular chaperone genes [68]. ClpP functions upstream of DVE-1 as ClpP inhibition impairs the nuclear redistribution of DVE-1 during stress [68]. Interestingly, the transporter HAF-1 is unnecessary for DVE-1 nuclear redistribution [69], suggesting a separate means of activation. During mitochondrial stress, *ubl-5* expression is up-regulated in a DVE-1 and HAF-1-dependent manner and forms a complex with DVE-1 that is required for UPR^{mt} activation [68]. Interestingly, we demonstrated that the mammalian orthologues of DVE-1 and UBL-5 (SatB2 and Ubl5) are also able to form a complex suggesting a similar role in mammalian cells [68]. However, it has yet to be determined if SatB2 is required for UPR^{mt} signaling in mammalian systems.

The current model of UPR^{mt} signal transduction suggests similarities and differences with the well-characterized UPR^{ER} (Section 2). While both culminate in the transcriptional induction of compartment-specific protein folding machinery by organelle-responsive transcription factors, the means by which unfolded proteins are detected and the signal transmitted to the respective transcription factors are different. The different signaling mechanisms appear to stem from differences in ER and mitochondrial architecture. The luminal domain of the ER-localized membrane spanning kinase, Ire1, directly senses unfolded proteins within the ER lumen [40]) and transmits the signal to the cytosolic domain of Ire1, which directly activates the transcription factor Xbp1 [39]. Mitochondria have separate stress responses that respond to perturbations in the matrix [52, 57] or IMS [64]. The current model for UPR^{mt} signaling suggests that unfolded or misfolded proteins are detected in the matrix by the quality control protease ClpP, which degrades them to peptides. The peptides are then pumped across the inner membrane leading to the activation of ATFS-1 through an unknown mechanism [69]. As data emerges on the variety of UPR^{mt} signaling mechanisms it will be interesting to compare and contrast them to the UPR^{ER}. For example, a separate branch of the UPR^{ER} attenuates protein synthesis to protect the protein-folding environment within the ER lumen when the load of unfolded proteins exceeds the capacity of ER chaperones. A conceptually similar response has not been identified in the UPR^{mt} despite pharmacological inhibition of translation being protective against mitochondrial dysfunction [50].

6. The relationship between the UPR^{mt} and other mitochondrial stress response pathways

Recent studies have indicated that severely damaged or energetically dead mitochondria are cleared from the cell via autophagic degradation through a pathway known as mitophagy [73–75]. The kinase PINK1 accumulates specifically on the outer membrane of

mitochondria in which the membrane potential has been completely dissipated [76]. PINK1 then recruits Parkin and the downstream autophagy machinery that directs the defective organelles to lysosomes for degradation [77]. Interestingly, similar stresses that ultimately result in mitophagy also activate the UPR^{mt}. For example, depletion of mitochondrial DNA, expression of mutant components of the ETC [78] as well as exposure to paraquat strongly activate both the UPR^{mt} as well as the mitophagy pathway [74]. Because the UPR^{mt} promotes total cellular mitochondrial function by up-regulating protective components to re-establish organellar homeostasis while mitophagy eliminates severely defective or dead organelles, we hypothesize that the UPR^{mt} is activated prior to the mitophagy pathway. If the organelle cannot maintain a membrane potential despite UPR^{mt} activation the defective organelle enters the mitophagy pathway (Figure 3). Ultimately, if mitochondrial damage becomes too pervasive, the cell undergoes apoptosis. As data emerge on all three pathways, it will be of interest to determine how the pathways integrate to protect mitochondrial, cellular, tissue and ultimately organismal health.

7. Perspective: the UPR^{mt} and the Regulation of Lifespan

The relationship between mitochondrial health and organismal life span has been the focus of much attention in recent years with numerous studies reporting a decline in mitochondrial function with age [12]. Therefore it is somewhat surprising that in worms, flies and mice, mutations that cause ETC dysfunction extend lifespan as much as 50% [79–82]. Many groups had hypothesized that the extension in lifespan was due to the activation of a cyto-protective compensatory response. Recently, it has been shown that ETC mutations that extend lifespan also activate the UPR^{mt} [83]. Impressively, the UPR^{mt} was required for the lifespan extension observed in the ETC mutants [83], supporting a role for the maintenance of the mitochondrial protein-folding environment in lifespan determination [6]. However, it has yet to be determined if HAF-1 or ATFS-1 are required for lifespan extension in the ETC mutants.

While the UPR^{mt} is necessary for lifespan extension, it has yet to be determined if it is sufficient. It will be interesting to determine if UPR^{mt} activation, perhaps by over-expression of ATFS-1, is capable of extending lifespan independent of mitochondrial dysfunction. Additional signaling pathways also contribute to the lifespan extension of ETC mutants including CEH-23 [84] and Hif-1 [85] indicating that multiple compensatory responses contribute to lifespan extension in the ETC mutants.

8. Perspective: the UPR^{mt} in cancer therapeutics

Mounting evidence suggests that cancer cells are exposed to higher levels of mitochondrial stress than normal cells, suggesting a dependence on cellular pathways and components that protect the mitochondrial protein-folding environment [86]. Cells within the tumor interior are exposed to hypoxic conditions, which inhibit protein folding in the IMS and cause remodeling of mitochondrial structure and function [87]. Additionally, cancer cells accumulate mtDNA mutations at relatively high rates [88] and mtDNA depletion has been associated with cancer progression [89]. Similarly, reduced expression of mitochondrial ETC components, including those of complexes I and III, have been observed in tumors [90–92]. While there is little doubt that cancer cells incur greater mitochondrial damage than normal cells, it is unclear whether the damage-associated alterations in mitochondrial function provides an advantage or disadvantage. Regardless, these observations suggest a role for the UPR^{mt} to protect cancer cell mitochondrial function.

For example, Hsp60 expression is increased in a number of cancers including tumors of the digestive, reproductive, nervous systems [93–96]. Elevated levels of Hsp60 likely promote efficient protein folding within the stressed folding environment, although alternative

functions of Hsp60 have also been suggested consistent with forced overexpression of Hsp60 causing transformation of embryonic fibroblasts [96]. Hsp60 has been shown to prevent apoptosis by stabilizing the anti-apoptotic protein Survivin [95] as well as by inhibiting the pro-apoptotic proteins Bax and Bak [97], although it is unclear where in the cell this interaction occurs. Consistent with a prominent role for Hsp60 in cancer cell survival, reducing Hsp60 levels by RNAi reduces the oncogenic properties of multiple tumor cell lines while having minimal effects on normal cells [53, 95], demonstrating the attractiveness of this chaperone in cancer target drug development.

Similarly, the Hsp90-related, mitochondrial localized TNF receptor-associated protein-1 (TRAP-1) chaperone is also highly expressed in a number of cancerous tissues [98]. Similar to Hsp60, TRAP-1 prevents apoptotic cell death via an inhibitory interaction with the immunophilin chaperone Cyclophilin D [99]. Recently, the compound Gamitrinib was shown to specifically inhibit the mitochondrial pool of Hsp90 chaperones and activate a UPR^{mt} [53, 100]. Interestingly, Gamitrinib treatment results in the specific death of tumor cells, suggesting a role for TRAP-1 in the function of cancer cell mitochondria. Gamitrinib-activated treatment also caused increased sensitivity to apoptosis [53] opening future possibilities for combined cancer therapies that target the UPR^{mt} and apoptotic pathways simultaneously as a means to enhance treatment.

Summary and future directions

The UPR^{mt} is the collective cellular response to increased levels of mitochondrial unfolded or misfolded proteins through the increased transcription of nuclear-encoded genes that act to promote mitochondrial protein folding. While models detailing the UPR^{mt} signal transduction pathways have been proposed (Figures 1 & 2), many unresolved questions remain. Of particular interest is how extruded peptides from mitochondria activate the transcription factor ATFS-1. In the yeast retrograde pathway, the phosphorylation state of the transcription factor *RTG3* determines its activity [56]. ATFS-1 has a serine-rich domain suggesting it may be regulated in a similar manner where mitochondrial peptide efflux may affect phosphorylation of ATFS-1 to impact its nuclear localization, although a putative kinase or phosphatase has not been identified. A second unresolved issue is the relationship between the transcription factors DVE-1 and ATFS-1. While both transcription factors are required for stress-induced expression of *mtHsp70* and *Hsp60*, the contribution of each is unclear. Conceivably, each regulates a subset of genes or alternatively, both are required for the induction of every UPR^{mt} gene. It will be important to identify the entire transcriptional output of each transcription factor and the promoter elements with which ATFS-1 and DVE-1 interact. Evidence from mammalian systems indicates that the DVE-1 orthologue, SatB2, acts as a more general regulator of transcription as it binds throughout the genome to AT rich sequences functioning as a nuclear scaffolding to affect global chromatin organization [101]. Potentially, DVE-1 is required for nuclear remodeling allowing ATFS-1 to directly interact with the promoters of the UPR^{mt} genes.

Many components involved in UPR^{mt} signaling have been identified in mammalian systems as well. It will be important to understand the degree of conservation between the two systems. ClpP is required for both the worm and mammalian UPR^{mt} but the component(s) that connect ClpP to the downstream transcription factors are currently unclear. The mammalian homologues of HAF-1 and ATFS-1 have yet to be identified although CHOP is a potential orthologue of ATFS-1 as both are bZip proteins. Additionally, the role of the mammalian UPR^{mt} has primarily been explored in cell culture. It will be interesting to examine its role in vivo to address the role of mitochondrial protective mechanisms in diseases associated with mitochondrial dysfunction including neurodegeneration and cancer [31, 32, 53]. Further dissection of the UPR^{mt} pathway will yield a better understanding of

how cells cope with mitochondrial dysfunction and allow for the discovery of new targets to modulate mitochondrial protein-folding capacity to promote cell survival or death.

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Highlights

- Mitochondrial protein homeostasis is maintained by localized chaperones and proteases
- Mitochondrial protein folding capacity can be adjusted by the mitochondrial UPRs
- Mitochondrial UPRs are mitochondrial-to-nuclear stress signaling pathways
- Mitochondrial dysfunction and stress responses are linked to aging and cancer

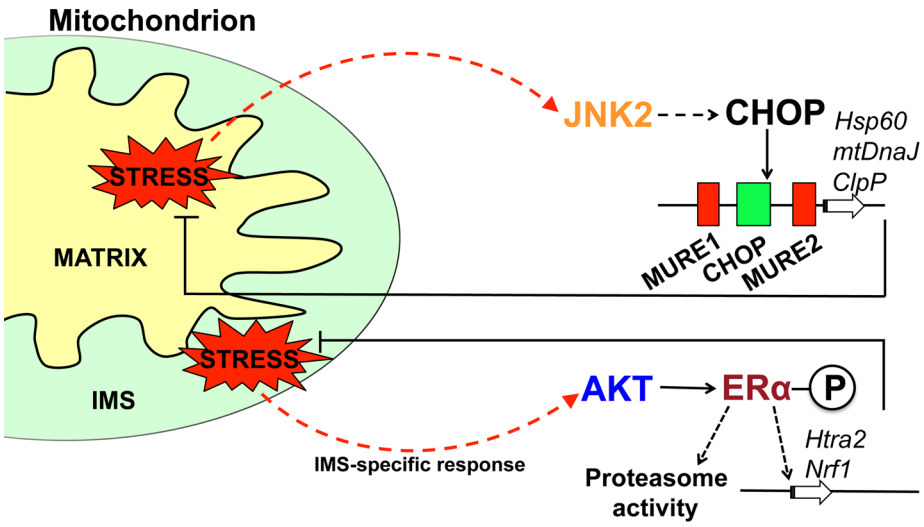

Figure 1. Model of the mammalian UPR^{mt} signaling pathway

Activation of the UPR^{mt} in mammalian cells occurs in response to stress originating from the mitochondrial matrix or the intermembrane space (IMS), each having distinct signal transduction pathways and transcriptional responses. Accumulation of unfolded proteins within the matrix stimulates the transcriptional up-regulation of the transcription factor CHOP via JNK2 and c-Jun [57, 62]. CHOP subsequently activates the transcription of genes including the quality control protease *ClpP* and the chaperonin *Hsp60* [58]. Alternatively, accumulating unfolded proteins in the IMS causes activation of the kinase AKT and phosphorylation of the estrogen receptor (ERα) leading to the transcriptional up-regulation of the IMS protease *Htra2* and the transcription factor NRF1 [64].

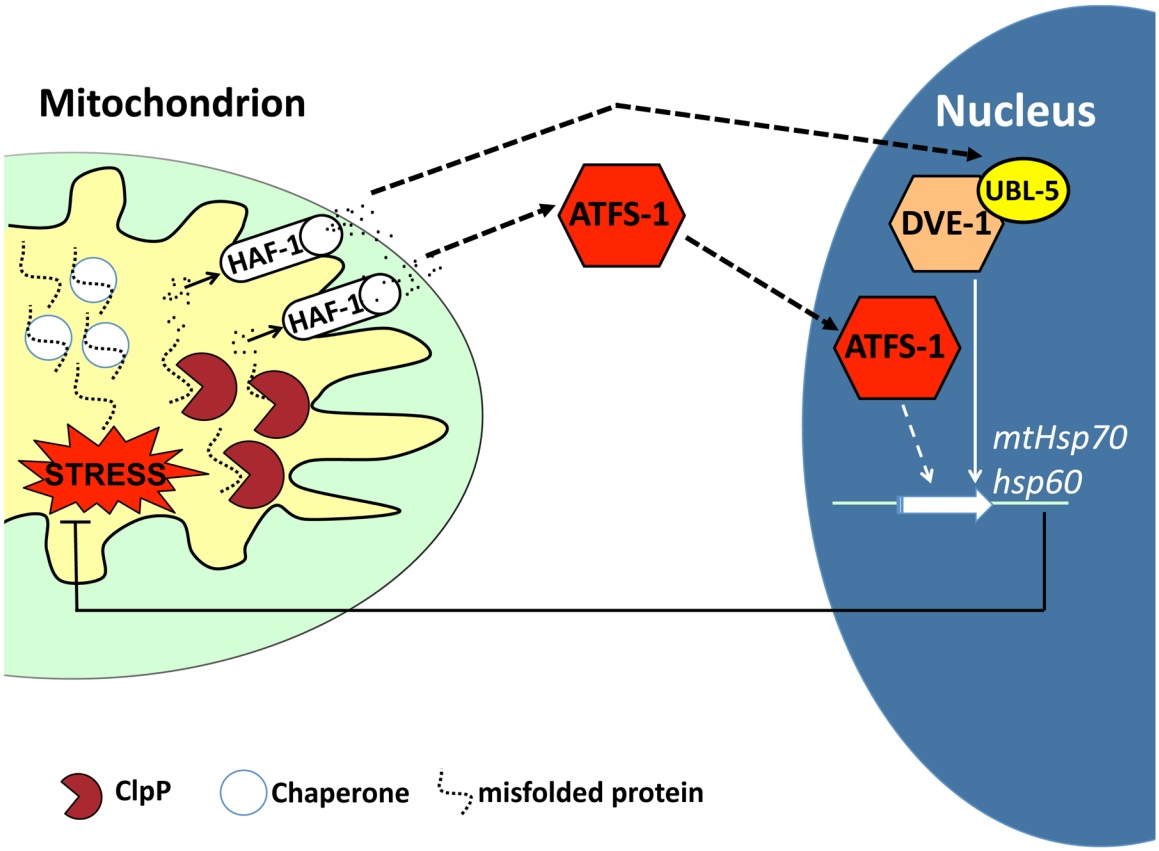

Figure 2. Model of the C. elegans UPR^{mt} signaling pathway

UPR^{mt} signaling is initiated as unfolded proteins accumulate beyond the resident chaperone folding capacity. To prevent protein aggregation, unfolded or misfolded proteins are degraded to peptides by the quality control protease ClpP in the mitochondrial matrix [68]. HAF-1-mediated peptide efflux leads to activation of the bZip transcription factor ATFS-1 that accumulates in the nucleus. HAF-1-mediated peptide efflux is also required for the accumulation of the ubiquitin-like protein UBL-5 which complexes with the transcription factor DVE-1 [69]. ATFS-1 and DVE-1/UBL-5 then cooperatively induce the expression of mitochondrial chaperone genes including *Hsp60* and *mtHsp70* in order to restore protein homeostasis.

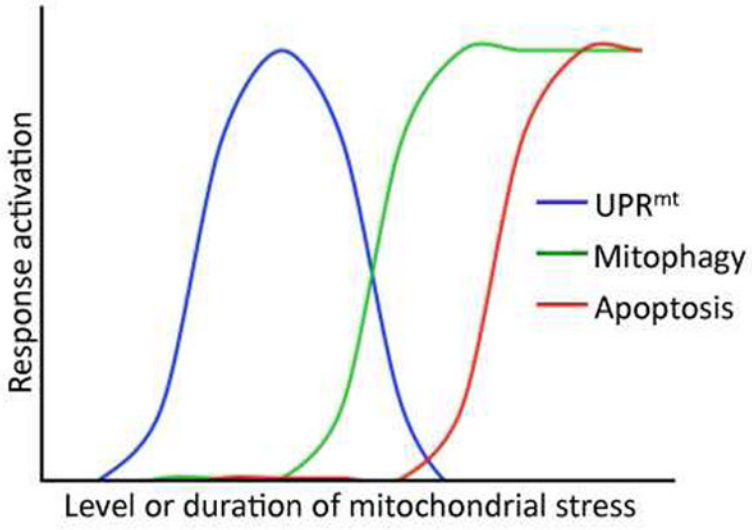

Figure 3. Proposed relationship between the UPR^{mt}, mitophagy and apoptosis during mitochondrial stress

Mitochondrial stress and dysfunction result in the activation of at least three cellular responses including the UPR^{mt}, mitophagy and apoptosis. We propose the depicted relationship between the three pathways as a function of mitochondrial stress level or the duration of mitochondrial stress. Because the UPR^{mt} is a mitochondrial protective response activated to re-establish homeostasis within stressed organelles and mitophagy removes severely defective mitochondria with a completely dissipated inner membrane potential, we propose the UPR^{mt} is activated at lower levels of stress or prior to the induction of mitophagy. As stress in individual mitochondrion exceeds the cytoprotective capacity of the UPR^{mt}, mitophagy eliminates the dead organelles. However, if the total cellular mitochondrial damage becomes too great the cell may undergo apoptosis.