



Signaling and Regulation of the Mitochondrial Unfolded Protein Response

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The mitochondrial proteome encompasses more than a thousand proteins, which are encoded by the mitochondrial and nuclear genomes. Mitochondrial biogenesis and network health relies on maintenance of protein import pathways and the protein-folding environment. Cell-extrinsic or -intrinsic stressors that challenge mitochondrial proteostasis negatively affect organellar function. During conditions of stress, cells use impaired protein import as a sensor for mitochondrial dysfunction to activate a stress response called the mitochondrial unfolded protein response (UPR^{mt}). UPR^{mt} activation leads to an adaptive transcriptional program that promotes mitochondrial recovery, metabolic adaptations, and innate immunity. In this review, we discuss the regulation of UPR^{mt} activation as well as its role in maintaining mitochondrial homeostasis in physiological and pathological scenarios.

Mitochondria are double-membrane-bound organelles of endosymbiotic origin (Gray 2012). Often, they are described as the “powerhouse of the cell” for their bioenergetic function of cellular ATP generation by oxidative phosphorylation (OXPHOS). In addition, mitochondria are required for innate immunity, maintaining calcium homeostasis, synthesis of numerous cofactors, including heme and iron-sulfur clusters, as well as regulating apoptosis (Vandecasteele et al. 2001; Wang and Youle 2009; Stehling and Lill 2013; Weinberg et al. 2015). Mitochondria also act as platforms for metabolic pathways, including the TCA cycle, the urea cycle, β -oxidation, and lipid synthesis. The functional versatility and adaptability of the organelle depends on the concerted action

by numerous proteins, protein complexes, and stress-response pathways.

Mitochondria are composed of more than 1000 proteins. Only 13 proteins are encoded by the mitochondrial genome (mitochondrial DNA [mtDNA]); all of which are components of the respiratory chain or ATP synthase (Anderson et al. 1981). All other mitochondrial proteins are encoded by nuclear genes, synthesized on cytosolic ribosomes, and subsequently imported into mitochondria via the TIM/TOM complexes (translocase of inner and outer membrane) (Chacinska et al. 2009). Given the high-protein concentration within mitochondria, coupled with complications deriving from the endosymbiotic origin of the organelle, maintenance of organellar protein homeostasis is

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challenging. Perturbations to proteostasis are caused by localized reactive oxygen species (ROS) production during normal and aberrant OXPHOS as well as uncoordinated expression of mitochondrial proteins encoded by either the mtDNA or nuclear genome (Curtis et al. 2012). Additionally, mutations in mtDNA and accumulation of damaged mtDNA adversely affect mitochondrial proteostasis (Moehle et al. 2018). Ultimately, such perturbations impair cellular health and are implicated in aging and several age-related diseases. For example, multiple studies have shown that mice harboring a mutation that annuls the proofreading activity of the mtDNA polymerase POLG γ have increased mtDNA mutations concomitant with an accelerated aging phenotype (Trifunovic et al. 2004; Kujoth et al. 2005). Thus, understanding pathways in place to maintain mitochondrial proteostasis and function not only provide insights into disease etiology but may also lead to new therapeutic approaches.

MITOCHONDRIAL BIOGENESIS, PROTEIN IMPORT, AND PROTEOSTASIS

Mitochondrial biogenesis is an elaborate process involving membrane synthesis, mtDNA replication, as well as import and trafficking of those proteins synthesized in the cytosol to the appropriate intramitochondrial compartments (outer membrane, intermembrane space, inner membrane, or matrix). Here, we focus on the trafficking of proteins to the matrix, which typically requires a mitochondrial targeting sequence (MTS) on the amino terminus of the protein to be imported. The MTS is a positively charged, amphipathic ~25–35 amino acid sequence that first interacts with the TOM complex before import (Mayer et al. 1995; Meisinger et al. 2001; Saitoh et al. 2007). On passing through the TOM complex in an unfolded state, precursor proteins translocate through the TIM23 complex, which requires the inner membrane potential generated by a functional respiratory chain (Martin et al. 1991). Finally, preproteins translocate into the mitochondrial matrix, which also requires the matrix-localized molecular chaperone mtHsp70 (Mapa et al. 2010). mtHsp70 interacts

directly with the TIM23 complex via cochaperones and facilitates import by either actively pulling proteins into the matrix or passively trapping preproteins to prevent retrograde diffusion out of the matrix (Gaume et al. 1998; Voisine et al. 1999; D'Silva et al. 2003). Once in the matrix, MTS is cleaved by the mitochondrial processing peptidase (MPP). Thus, mtHsp70 plays a dual role during protein import, both at the import channel and in the matrix to assist in proper protein handling. In yeast, it has been shown that the distinct functional roles of mtHsp70 in protein import and protein folding in the matrix can be attributed to its differential interactions with cochaperones forming two different complexes (Horst et al. 1997). At the import complex, mtHsp70 associates with Tim44 of the TIM23 complex, whereas in the matrix it interacts with Mdj1, the DnaJ homolog (Horst et al. 1997).

Chaperone-assisted protein folding following import is key to maintaining proteostasis in the matrix. In addition to mtHsp70, the chaperonin Hsp60 also interacts with imported proteins in the matrix to facilitate protein folding (Cheng et al. 1989). Hsp60s in cooperation with Hsp10 undergoes ATP-dependent conformational changes to form a protective socket that allows substrate proteins to fold within them by preventing contact with other surrounding proteins. Hsp60 also plays a protective role under conditions of heat stress by preventing aggregate formation (Bender et al. 2011).

Moreover, mitochondrial-localized quality-control proteases maintain proteostasis by degrading damaged or terminally misfolded proteins. The AAA proteases LON and ClpXP are largely responsible for removing damaged proteins from the mitochondrial matrix (Tatsuta and Langer 2009). Also, paraplegin and YMEL1 primarily prevent misfolding of respiratory chain complex proteins on or near the mitochondrial inner membrane (Leonhard et al. 1996).

In addition to OXPHOS activity and mitochondrial proteostasis, numerous additional factors in multiple cellular compartments contribute to the regulation and efficiency of mitochondrial protein import. For example,

phosphorylation of an MTS can reduce import efficiency (Colombo et al. 2005; Boopathi et al. 2008; Rao et al. 2011). In the cytosol, protein import can be regulated by the binding of protein partners or metabolites to precursor proteins (Vongsamphanh et al. 2001; Munakata et al. 2004). For instance, import of the mitochondrial protein 5-aminolevulinatase is impaired on heme binding in the cytosol (Dailey et al. 2005). Folding of the precursor proteins before interacting with the TOM channel can inhibit entry (Hill et al. 1998; Truscott et al. 2001; Strobel et al. 2002). As the majority of protein import requires the TOM complex, mechanisms that modulate the activity of the import machinery influence the entry of precursor proteins (Harbauer et al. 2014). Studies in yeast have shown that casein kinases (CKs) and protein kinase A (PKA) can phosphorylate TOM to modulate protein import. CKs phosphorylate Tom22 to promote import, whereas PKA phosphorylates Tom40, Tom22, as well as Tom70 to impair protein import (Schmidt et al. 2011; Rao et al. 2012; Gerbeth et al. 2013).

Mitochondrial function and proteostasis are integrally linked with mitochondrial protein import. For example, mitochondrial uncoupling or dissipation of the inner membrane potential because of OXPHOS defects directly inhibits protein translocation into the mitochondria. Furthermore, proteostasis perturbations require mtHsp70 to facilitate protein folding, which may impact the chaperones' role in mitochondrial protein import. Because protein import capacity and proteostasis correlate with mitochondrial function, import efficiency can serve as a sensor for detecting perturbations in mitochondrial homeostasis. At least two mitochondrial stress responses are regulated by mitochondrial import capacity. Severe impairment of protein import leads to degradation of the entire compartment via mitochondrial autophagy (mitophagy) (Pickles et al. 2018). Impaired import also leads to activation of an adaptive transcriptional response known as the mitochondrial unfolded protein response (UPR^{mt}), which promotes recovery of mitochondrial function (Shpilka and Haynes 2018). In this review, we focus on the mechanisms that regulate UPR^{mt}

activation and its role in diverse physiological scenarios.

UPR^{mt} REGULATION

The UPR^{mt} was initially discovered in cultured mammalian cells in which depletion of mtDNA or overexpression of a mitochondrial-targeted protein harboring a deletion rendering the protein terminally misfolded (Δ OTC) elicited a transcriptional response that included mitochondrial molecular chaperones and quality-control proteases (Martinus et al. 1996; Zhao et al. 2002). Over the ensuing years, findings in *Caenorhabditis elegans* have identified components in multiple subcellular compartments providing insight into how cells detect mitochondrial dysfunction and communicate with the nucleus to adapt transcription accordingly. Importantly, homologous UPR^{mt} regulatory components have been identified in mammalian systems, demonstrating the evolutionary conservation of the adaptive mitochondrial stress response (Fiorese et al. 2016; Münch and Harper 2016; Quirós et al. 2017).

Stressors that Perturb Mitochondria and Activate the UPR^{mt}

A diverse number of stressors, which impair mitochondrial function, activate the UPR^{mt}. Stressors that perturb mitochondrial proteostasis components, such as molecular chaperones and quality-control proteases, are among the strongest inducers of the UPR^{mt}, possibly owing to the accumulation of misfolded proteins in the mitochondria, which exceeds the protein-handling capacity of chaperones and proteases, thereby hindering protein import (Yoneda et al. 2004). Consistent with this model, inhibiting import directly by depleting the import complex TIM (*timm-23*) strongly activates the UPR^{mt} (Nargund et al. 2012). Furthermore, inhibition of mitochondrial protein synthesis via RNA interference (RNAi) or mitochondrial ribosome inhibitors, such as doxycycline or chloramphenicol, activates the UPR^{mt} (Yoneda et al. 2004; Houtkooper et al. 2013).

In addition to perturbations in proteostasis, impairment of mitochondrial metabolic functions also elicits UPR^{mt} activation. For example, mtDNA depletion by treatment with ethidium bromide activates the UPR^{mt} (Yoneda et al. 2004; Houtkooper et al. 2013). Inhibition of OXPHOS by multiple compounds, including rotenone (complex I inhibitor), antimycin (complex III inhibitor), oligomycin (complex V inhibitor), or paraquat, also activates the UPR^{mt} (Yoneda et al. 2004; Runkel et al. 2013). Lastly, inhibition of fumarate hydratase, the enzyme responsible for converting fumarate to malate in the TCA cycle, activates the UPR^{mt}, indicating that the mitochondrial stress response is engaged by both mitochondrial proteostasis and metabolic perturbations (Wang et al. 2016b).

Transcriptional Program Mediated by UPR^{mt} Activation

Mitochondrial Network Recovery and Function

The mitochondrial recovery program elicited by UPR^{mt} activation includes induction of mitochondrial chaperones Hsp60 and mtHsp70, which promote protein folding and prevent aggregate formation. To eliminate the accumulation of damaged or misfolded proteins, the ATP-dependent m-AAA and i-AAA proteases, such as paraplegin and YMEL-1, are induced as are superoxide dismutases (SODs) to limit the accumulation of ROS from dysfunctional OXPHOS.

In addition to a transcriptional response induced for repair and recovery of damaged mitochondria, the UPR^{mt} activation also induces genes that promote mitochondrial biogenesis and function, including iron-sulfur cluster and ubiquinone synthesis required for OXPHOS complex biogenesis. UPR^{mt} activation also induces genes involved in mitochondrial fission, such as Dynamin-related protein (Drp1), to promote mitochondrial biogenesis as well as clearance of defective mitochondria.

Metabolic Adaptations

Most OXPHOS components are encoded by nuclear genes and assembled into complexes via

chaperones and assembly factors. UPR^{mt} activation limits the transcription of OXPHOS components possibly to reduce the load on the proteostasis machinery during stress (Nargund et al. 2015). Concomitantly, UPR^{mt} activation induces all glycolysis genes, suggesting that UPR^{mt} plays a role in balancing the metabolic landscape of the cell during mitochondrial stress likely by promoting an alternative means of ATP synthesis to facilitate mitochondrial recovery.

Mitochondria-to-Nuclear Communication

Genetic screens in *C. elegans* have revealed multiple components required for UPR^{mt} activation, including the bZIP transcription factor associated with Stress-1 (ATFS-1) (Fig. 1). In addition to its bZIP DNA-binding domain, ATFS-1 also harbors both an MTS and a nuclear localization sequence (NLS) (Nargund et al. 2012). The net positive charge of the MTS facilitates its import into functional mitochondria, in which the MTS is cleaved and the remainder of the protein is degraded by the matrix-localized protease LONP1. However, during mitochondrial stress, a decline in mitochondrial function reduces import efficiency of ATFS-1 causing a fraction of the transcription factor to remain in the cytosol. Because ATFS-1 harbors an NLS, it then traffics to the nucleus to initiate a transcriptional program (Nargund et al. 2012). Thus, mitochondrial import efficiency of ATFS-1 allows cells to sense and monitor perturbations in mitochondrial network health and homeostasis (Haynes et al. 2010). In line with this model, mutations that reduce the strength of ATFS-1's MTS limit its mitochondrial import efficiency and constitutively activate the UPR^{mt} (Rauthan et al. 2013).

ATFS-1 is activated by impaired mitochondrial protein import. But, how are the induced proteostasis components, such as chaperones and proteases, imported into mitochondria to facilitate recovery? One possibility is that ATFS-1 activation transcriptionally induces genes encoding the import machinery, thereby gradually increasing mitochondrial protein import as well as protein-handling capacity. Another possibility is that because mitochondrial chaper-

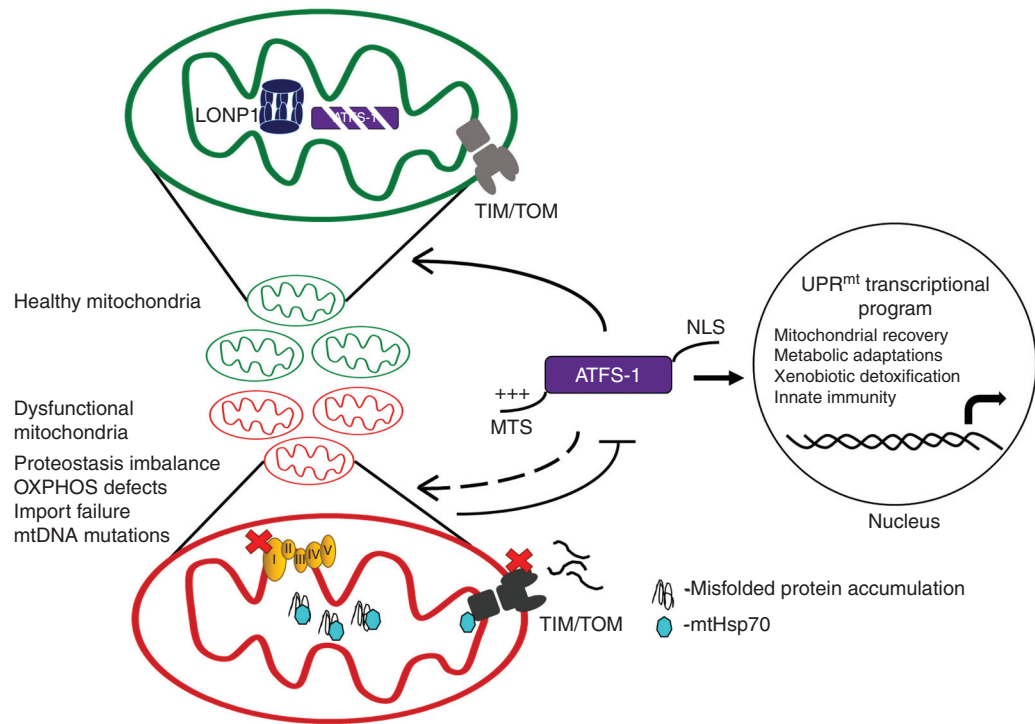


Figure 1. Regulation of the mitochondrial unfolded protein response (UPR^{mt}) by ATFS-1. UPR^{mt} activation is mediated by the transcription factor ATFS-1, which harbors a mitochondrial targeting sequence (MTS) as well as a nuclear localization sequence (NLS). When mitochondria are healthy, ATFS-1 is imported via the TIM/TOM (translocase of the inner and outer membrane) complex and subsequently degraded by the matrix-localized protease LONP1. Mitochondrial import of most precursor proteins and efficient folding within the matrix requires the matrix-localized chaperone mtHsp70 located at both the import channel as well as in the matrix. Mitochondrial dysfunction because of impaired proteostasis or oxidative phosphorylation (OXPHOS) reduces mitochondrial protein import efficiency of ATFS-1, allowing it to traffic to the nucleus to initiate a transcriptional program, which promotes the recovery of mitochondrial function.

ones, such as mtHsp70, play a dual role at the import complex and in the matrix for protein folding, reduced import capacity during stress may be concomitant with spatial redistribution of mtHsp70 and other chaperones from the sites of protein import to the sites of accumulated or misfolded proteins. Alternatively, ATFS-1 may simply have a relatively weak MTS, and the proteins induced by ATFS-1 may have strong MTSs, enabling them to be imported into dysfunctional mitochondria (Melber and Haynes 2018).

In addition to ATFS-1, several other proteins required for UPR^{mt} activation have been identified. The homeobox domain transcription factor DVE-1 forms a complex with the ubiqui-

tin-like protein UBL-5, and remodels chromatin by binding to UPR^{mt} gene promoters such as *hsp-60* (Benedetti et al. 2006; Haynes et al. 2007; Tian et al. 2016). Presumably, chromatin remodeling enables ATFS-1 to bind the UPR^{mt} element in the promoters of UPR^{mt} genes (Nargund et al. 2015).

The Role of Chromatin Remodeling in UPR^{mt} Activation

Epigenetic regulation by chromatin remodeling plays an important role in UPR^{mt} activation during mitochondrial dysfunction. A recent study has shown that mitochondrial stress caused by knockdown of prohibitin or a respi-



ratory chain complex IV subunit leads to nuclear localization of the chromatin modifier LIN-65 (Tian et al. 2016). LIN-65 function requires the H3K9 methyltransferase MET-2. Combined, these factors reorganize global chromatin structure and alter compaction to enable transcription of UPR^{mt} genes in response to stress (Tian et al. 2016).

The histone demethylases *jmjd-1.2* and *jmjd-3.1*, members of the H3K27 family containing jmC-domain, are also necessary to activate the UPR^{mt} when OXPHOS is impaired (Merkwirth et al. 2016). Furthermore, *jmjd-1.2* and *jmjd-3.1* overexpression is sufficient to induce the UPR^{mt} and increase longevity. Both demethylases remove repressive chromatin marks and, therefore, positively regulate gene expression by promoting access of the transcriptional machinery to UPR^{mt} gene promoters. The mechanisms that stimulate the activity of JMJD-1.2 and JMJD-3.1 during mitochondrial stress are unclear, but may relate to the regulation of these demethylases by metabolic cofactors, including α -ketoglutarate (a TCA-cycle intermediate) and iron (Tsukada et al. 2006). Production of such metabolic cofactors largely depends on healthy mitochondrial function and ATP generation (Teperino et al. 2010). During mitochondrial dysfunction, changes in metabolic pathways may alter the level of these metabolites, consequently affecting the activity of histone demethylases to promote UPR^{mt} activation.

UPR^{mt} Regulation and the Integrated Stress Response

Transcriptional regulation of the UPR^{mt} in mammalian cells is comparable to that in *C. elegans* and is mediated by multiple bZIP transcription factors including ATF5 (Fiorese et al. 2016). Much like ATFS-1, ATF5 harbors an MTS and is regulated by organelle partitioning. Importantly, ATF5 rescues UPR^{mt} activation in nematodes lacking ATFS-1 and cultured cells, ATF5 transcriptionally up-regulates mitochondrial chaperones and proteases during mitochondrial stress (Fiorese et al. 2016).

However, in mammalian systems, UPR^{mt} activation requires at least two additional bZIP

proteins, ATF4 and CHOP, as rotenone exposure transcriptionally up-regulates both ATF4 and CHOP (Horibe and Hoogenraad 2007; Silva et al. 2009; Quirós et al. 2017). Furthermore, mtDNA depletion causes induction of ATF4 and ATF5. All three transcription factors are linked to the integrated stress response (ISR), which is a conserved adaptive response activated by a wide variety of stressors. The ISR was first characterized in yeast as the “general control pathway,” which is activated during amino acid deprivation. The yeast pathway was later found to be a highly conserved stress-response mechanism in mammalian systems (Harding et al. 2000). ISR initiation is mediated by kinases that respond to specific stressors and phosphorylate serine 51 of the translation initiation factor subunit eIF2 α (Pathak et al. 1988). The mammalian ISR kinases are RNA-dependent protein kinase (PKR)-like ER kinase (PERK), heme-regulated eIF2 α kinase (HRI), double-stranded PKR, and general control nonderepressible 2 (GCN2). PERK, HRI, and PKR are specifically activated in response to perturbations in ER homeostasis, heme availability, and the presence of cytosolic double-stranded RNA, respectively (Meurs et al. 1990; Chen and London 1995; Harding et al. 1999). GCN2 is activated by amino acid or glucose deprivation, ROS, ribosome stalling, mitochondrial dysfunction, and UV light (Dever et al. 1992; Deng et al. 2002; Baker et al. 2012). Ultimately, eIF2 α phosphorylation causes attenuation of global protein synthesis while promoting the selective translation of messenger RNAs (mRNAs) harboring upstream open reading frames (uORFs) in the 5' untranslated region (5'UTR). The bZIP transcription factors CHOP, ATF4, and ATF5 are among the mRNAs selectively translated on eIF2 α phosphorylation.

In sum, ISR activation in mammals during stress causes eIF2 α phosphorylation, which promotes activation of the transcription factors ATF4, CHOP, and ATF5. However, the nuances of the interrelationship between the transcription factors during mitochondrial stress or which kinases are specifically activated by which mitochondrial perturbations is not yet fully understood.

UPR^{mt} and Translation Regulation within Mitochondria

mtDNA-encoded transfer RNAs (tRNAs) are essential for translation and RNA maturation within mitochondria. Following transcription, mitochondrial tRNAs are processed at the 5' and 3' ends by the RNase P and RNase Z complexes (Rossmann 2012). In humans, RNase P is comprised of the proteins MRPP1, MRPP2, and MRPP3. During mitochondrial stress, UPR^{mt} activation results in decreased MRPP3 caused by increased degradation by LON protease (Münch and Harper 2016). Therefore, UPR^{mt} activation reduces protein synthesis within the cytosol by phosphorylating eIF2 α and mitochondria by degradation of MRPP3 likely facilitating proteostasis and organelle recovery.

Cell-Nonautonomous UPR^{mt} Regulation

In addition to mitochondrial-to-nuclear communication within each cell, mitochondrial dysfunction can be communicated between tissues to induce the UPR^{mt}. Recent studies in *C. ele-*

gans and mammals indicate that intercellular communication involves secretion of signaling factors, called mitokines, from neurons with dysfunctional mitochondria. The mitokine(s) then act on peripheral cells, such as the intestinal cells, to activate the UPR^{mt} (Fig. 2) (Durieux et al. 2011; Kim et al. 2013b; Berendzen et al. 2016; Shao et al. 2016). For example, FLP-2, a neuropeptide secreted from interneurons, acts as a signaling factor to activate the UPR^{mt} in intestinal cells when mitochondrial stress is induced specifically in neurons (Shao et al. 2016). A separate study showed that during neuronal proteotoxic stress, secretion of the neurotransmitter serotonin is also required for communication with intestinal cells to activate the UPR^{mt} (Berendzen et al. 2016). In addition to FLP-2 and serotonin, Wnt signaling pathway is required for cell-nonautonomous UPR^{mt} activation. Mitochondrial stress induced in neurons leads to secretion of the Wnt ligand EGL-20, which acts as a mitokine to induce the UPR^{mt} in peripheral cells (Zhang et al. 2018).

In mammalian systems, the potential mitokine fibroblast growth factor 21 (FGF21), a met-

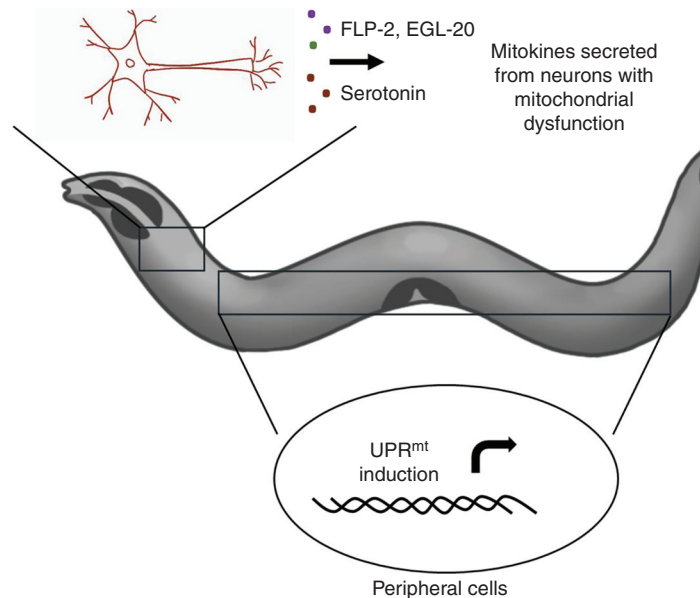


Figure 2. Cell-nonautonomous mitochondrial unfolded protein response (UPR^{mt}) signaling. Neuronal activation of the UPR^{mt} is communicated cell nonautonomously to the intestine by the secretion of mitokines (FLP-2, serotonin, EGL-20) to activate the UPR^{mt} in distal tissues such as the intestine.

abolic hormone expressed in multiple mammalian tissues, is induced by the ISR regulator ATF4 during mitochondrial stress caused by autophagy deficiency (Kim et al. 2013a,b). Growth differentiation factor 15 (GDF15) is also a potential mitokine, which is induced during mitochondrial stress in skeletal muscles (Chung et al. 2017). In mice, GDF15 induction causes changes in lipid metabolism and insulin sensitivity, thereby conferring tolerance to onset of obesity (Chung et al. 2017).

Physiologies and Pathologies Impacted by UPR^{mt} Signaling

UPR^{mt} Signaling and Organismal Longevity

Decline in mitochondrial function is a hallmark of aging in most organisms and associated with

accumulation of mtDNA mutations as well as reduced OXPHOS (Bratic and Larsson 2013). Surprisingly, considerable evidence indicates that modest mitochondrial perturbations cause increased longevity (Feng et al. 2001; Dillin et al. 2002; Liu et al. 2005; Copeland et al. 2009). In *C. elegans*, impairment of respiratory chain complex IV function (*cco-1* RNAi) both activates the UPR^{mt} and extends life span by ~50% (Fig. 3) (Durieux et al. 2011). Interestingly, the life-span modulation is dependent on the developmental stage during which mitochondrial function is impaired, that is, increased longevity occurs only when *cco-1* RNAi is administered before adulthood. This suggests that temporal regulation of mitochondrial function is associated with longevity, possibly owing to epigenetic level changes during different stages of development (Dillin et al. 2002; Durieux et al.

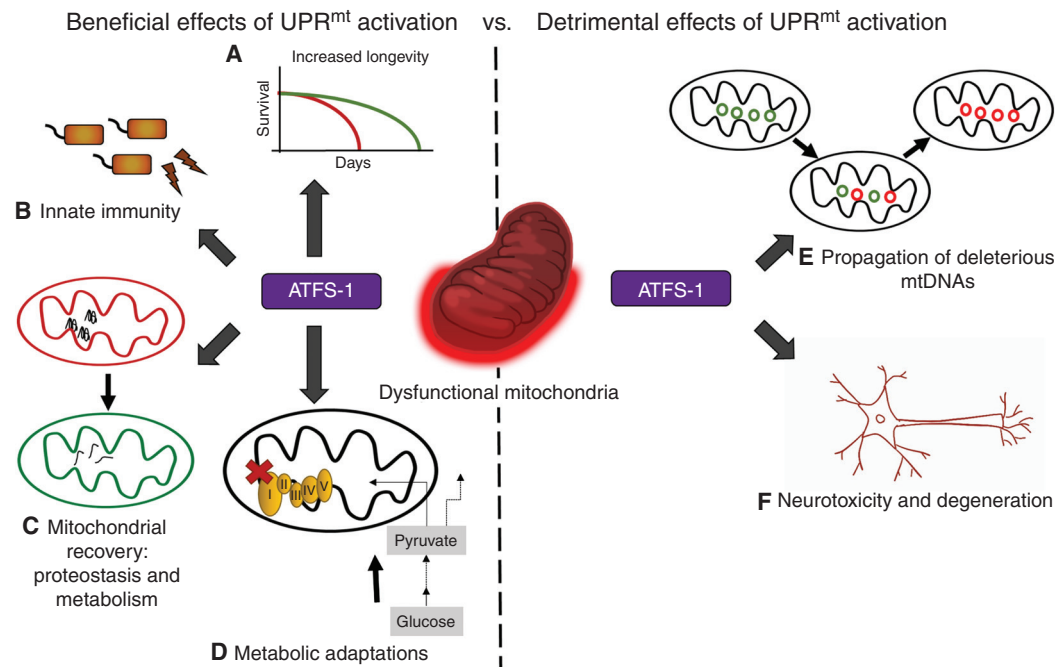


Figure 3. Functions and consequences of mitochondrial unfolded protein response (UPR^{mt}) activation. UPR^{mt} activation has beneficial, as well as detrimental effects, depending on the physiological scenario as well as duration and strength of activation. Beneficial outcomes include (A) increased longevity, (B) increased resistance against pathogen infection, (C) efficient recovery of the mitochondrial network, and (D) metabolic adaptations, including the induction of glycolysis. However, prolonged UPR^{mt} activation can result in (E) amplification of deleterious mitochondrial DNAs (mtDNAs), and (F) neurotoxicity, emphasizing the importance of proper UPR^{mt} regulation. ATFS-1, Activated transcription factor associated with Stress-1.



2011). Consistent with the role of mitochondria as central regulators of metabolic homeostasis, changes in metabolic inputs affecting mitochondrial function influence UPR^{mt} activation and, in turn, impact life span. For example, modulating levels of the metabolic cofactor NAD⁺ activates the UPR^{mt} and extends life span (Mouchiroud et al. 2013).

Parallel studies in multiple organisms have shown that modest mitochondrial perturbations, which cause UPR^{mt} activation, also extend life span. A study in flies showed that inducing mitochondrial stress in muscle cells by perturbing respiratory chain function activates the UPR^{mt}, specifically in muscles, and promotes longevity (Owusu-Ansah et al. 2013). In mice, impaired function of mitochondrial ribosomes by genetic or pharmacological means both activates the UPR^{mt} and extends life span (Houtkooper et al. 2013). Although considerable data indicate a role for the UPR^{mt} in extending life span, the cellular processes impacted by UPR^{mt} activation, which influence life span, remain to be identified.

UPR^{mt} Signaling in Propagation of Deleterious Mitochondrial Genome

Most cells harbor hundreds of copies of the mitochondrial genome distributed among the mitochondrial network, which are inherited via the maternal germline (Miller et al. 2003). As mtDNA harbors 13 protein-coding genes that are all essential for OXPHOS, mtDNA mutations can cause a wide range of pathologies, including neuropathies and myoclonic epilepsy (Tuppen et al. 2010). mtDNA acquires mutations at a higher rate than the nuclear genome, possibly because of the lack of efficient DNA repair pathways or the presence of a mutagenic environment. Given the high copy number of mtDNA, such mutations are generally well tolerated as they coexist with wild-type mtDNAs in a state known as heteroplasmy. However, as cells such as muscle and neurons age, the deleterious mtDNA (Δ mtDNA) often accumulates faster than the wild-type mtDNA, resulting in loss of OXPHOS. Although the mechanisms that underlie the preferential accumulation of

Δ mtDNAs remain largely unknown, recent work in two heteroplasmic *C. elegans* strains indicates a requirement for ATFS-1 and the UPR^{mt} in maintaining the Δ mtDNA (Gitschlag et al. 2016; Lin et al. 2016).

One such strain harbors ~60% Δ mtDNA and 40% wild-type mtDNA. The Δ mtDNA harbors a 3.1-kb deletion lacking four essential OXPHOS genes and perpetually activates the UPR^{mt} as a result of reduced OXPHOS. Impressively, deletion of the *atfs-1* gene results in nearly a complete loss of the Δ mtDNA, while having no effect on the wild-type genomes. Alternatively, hyperactivation of the UPR^{mt} causes a preferential accumulation of the Δ mtDNA, indicating that UPR^{mt} activation is both necessary and sufficient for the preferential accumulation of Δ mtDNAs (Fig. 3; Gitschlag et al. 2016; Lin et al. 2016). Although the outputs of UPR^{mt} activation that promote Δ mtDNA propagation remain unclear, evidence suggests that imbalanced mitochondrial biogenesis and mitophagy are involved in the process (Lin et al. 2016).

UPR^{mt} Signaling in Mitochondrial Diseases

Dysfunctional mitochondria are manifested in several diseases that are currently incurable (Suomalainen 2015). An example of this is mitochondrial myopathy, a progressive disease associated with defective respiratory chain function and muscular abnormalities often resulting from accumulating mtDNA mutations (Pfeffer and Chinnery 2013). A recent study showed the link between mTORC1 (mechanistic target of rapamycin complex 1) and UPR^{mt} regulation in a manner dependent on uORFs during mitochondrial myopathy. mTORC1 is a regulatory kinase that responds to nutrient availability, ATP production, and regulates protein synthesis through downstream effectors such as S6 kinase (Rohde et al. 2001; Magnuson et al. 2012). Using a mouse model of adult-onset mitochondrial myopathy caused by accumulating mtDNA deletions, the study showed that mitochondrial dysfunction stimulates mTORC1 (Khan et al. 2017). Interestingly, mTORC1 activation was required for induction of a transcriptional program, including cytokines such as FGF21 and

GDF15, consistent with the UPR^{mt}. Importantly, mTORC1 activation caused transcriptional up-regulation of ATF4 and ATF5, which is impaired by treatment with the known mTORC1 inhibitor rapamycin (Khan et al. 2017). However, the mechanistic details of mTORC1-mediated translational regulation of ATF4 and ATF5 remains elusive (Khan et al. 2017).

UPR^{mt} Signaling in Neurodegenerative Diseases

Alzheimer's disease, a progressive neurodegenerative condition associated with aging, is characterized by mitochondrial dysfunction along with proteotoxic aggregation of amyloid- β (A β) peptides (Hashimoto et al. 2003). Recent work has suggested that the UPR^{mt} is activated in neurons of Alzheimer's disease patients (Sorrentino 2017). Consistent with this data, expression of A β in *C. elegans* results in UPR^{mt} activation (Sorrentino 2017). Impressively, enhanced UPR^{mt} activation reduces the toxicity associated with A β accumulation and prolongs survival. In mouse models of Alzheimer's disease treatment with nicotinamide riboside (NR), which boosts NAD⁺ levels and activates the UPR^{mt}, alleviates proteotoxicity, as well as improves memory (Sorrentino 2017). In brief, these findings suggest that strategies to enhance UPR^{mt} activation may be useful in the treatment of Alzheimer's disease.

In contrast to the findings relating to Alzheimer's disease and the UPR^{mt}, prolonged or hyperactivation of the UPR^{mt} causes dopaminergic neuron death in a *C. elegans* model of Parkinson's disease in which the protein α -synuclein is overexpressed (Fig. 3) (Martinez et al. 2017). Combined, these results indicate that UPR^{mt} activation can have either beneficial or detrimental effects in the context of neurodegenerative conditions.

UPR^{mt} Signaling in Cancer

Cancer is a complex and multifactorial disease that is often associated with mitochondrial dysfunction because of the hypoxic environment within tumors, accumulation of mtDNA muta-

tions, and relatively high levels of ROS (Chatterjee et al. 2006; Liou and Storz 2010; Boland et al. 2013). Tumor cells rely more heavily on glycolysis rather than OXPHOS despite the presence of oxygen, a phenomenon known as the Warburg effect. Defects in mitochondrial function of cancer cells may contribute to altered flux in metabolic pathways giving rise to the Warburg effect. Accumulating evidence suggests that UPR^{mt} activation, in response to mitochondrial dysfunction, contributes to cancer cell growth and survival. For example, expression of Hsp60 is induced in multiple cancer types presumably to maintain mitochondrial function (Cappello et al. 2002; Tsai et al. 2008). Furthermore, activation of ISR kinases PERK, GCN2, and PKR has been implicated in tumor cell survival and neoplastic growth although the mechanistic consequences of mitochondrial dysfunction in tumor cells remain unclear (Kim et al. 2000, 2002; Ye et al. 2010; Nagelkerke et al. 2013). Specifically, in gastric cancers, evidence indicates that ROS activates ATF4 via GCN2 causing enhanced resistance to the chemotherapeutic drug cisplatin (Wang et al. 2016a). Numerous studies have shown a role for ATF5 in the growth of a variety of cancer cell types, suggesting UPR^{mt} inhibition may be a viable means to limit cancer progression (Chen et al. 2012; Wang et al. 2012; Nukuda et al. 2016; Angelastro 2017).

UPR^{mt} Signaling during Pathogenic Infection

Bacterial pathogens secrete a wide array of virulence effectors that curb host functions to establish a persistent infection. Multiple pathogen-secreted virulence factors specifically target host mitochondrial function. For example, the opportunistic pathogen *Pseudomonas aeruginosa* perturbs mitochondria by secreting toxins, such as cyanide and siderophores, that impair OXPHOS (Kirienko et al. 2015; Managò et al. 2015). Recent studies in *C. elegans* show that pathogenic and nonpathogenic strains of *Pseudomonas* perturb mitochondrial function and activate the UPR^{mt} (Fig. 3) (Liu et al. 2014; Pellegrino et al. 2014). Surprisingly, these studies revealed that in addition to mitochondrial-protective genes, ATFS-1 mediates the induction

of antibacterial genes, including secreted lysozymes and antimicrobial peptides, as well as xenobiotic detoxification genes (Pellegrino et al. 2014). Importantly, worms lacking ATFS-1 were susceptible to infection, whereas hyperactivation of the UPR^{mt} limited infection and prolonged host survival. These findings indicate that the UPR^{mt} functions as an innate immune response and one mechanism by which eukaryotes can detect the presence of bacterial species that produce mitochondrial toxins, including OXPPOS and mitochondrial ribosome inhibitors (tetracyclines), is by monitoring mitochon-

drial function via the UPR^{mt} (Pellegrino et al. 2014). In turn, the UPR^{mt} activates a response to maintain mitochondrial function, and rid the host of the pathogen as well as the toxins.

Additional Mechanisms for Mitochondrial Quality Control

In addition to the UPR^{mt}, several other mechanisms are activated during mitochondrial dysfunction to promote recovery of the mitochondrial network as well as alleviate pleiotropic toxic effects throughout the cell (Fig. 4). Intriguingly,

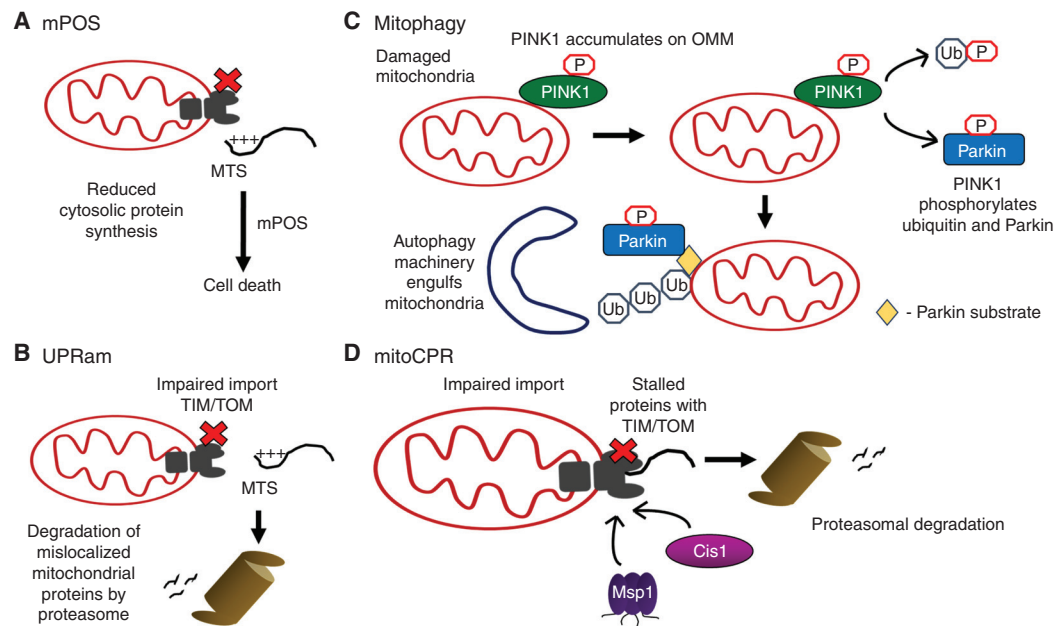


Figure 4. Mitochondrial stress-response pathways that potentially interact with the mitochondrial unfolded protein response (UPR^{mt}). (A) Impaired mitochondrial protein import caused by mitochondrial metabolic or proteostasis defects causes accumulation of mitochondrial precursor proteins in the cytosol and cell death; a pathway termed mPOS (mitochondrial precursor overaccumulation stress). (B) UPRam (unfolded protein response activated by mistargeted proteins) is activated during mitochondrial protein import failure, which causes accumulation of mitochondrial precursor proteins in the cytosol. To rid the cytosol of the toxic mislocalized proteins, proteasomal activity is increased. (C) Mitophagy is a process by which severely damaged mitochondria are degraded. The kinase PINK1 accumulates on the outer membrane of damaged mitochondria (OMM), where it phosphorylates ubiquitin and the E3 ubiquitin ligase Parkin, which leads to polyubiquitination of many OMM proteins. Subsequently, recognition by the autophagy machinery results in engulfment of the defective organelle, which is then delivered to lysosomes for degradation. (D) mitoCPR (mitochondrial compromised protein import response) is a stress-response activated by accumulation or stalling of mitochondrial precursor proteins within TOM (translocase of outer membrane) channels. The linker protein Cis1 is transcriptionally induced to recruit the ATP-dependent translocase Msp1 to the TOM channel to extract the stalled precursor proteins for proteasomal degradation. MTS, Mitochondrial targeting sequence; TIM, translocase of inner membrane.



ingly, all the mechanisms are stimulated by impaired mitochondrial protein import. Severely or irreversibly damaged mitochondria are eliminated via mitophagy, which is a form of selective autophagy. Damaged mitochondria are detected by the protein kinase PINK1, which, like ATFS-1, is regulated by mitochondrial import efficiency. PINK1 is imported into healthy organelles in which it is processed and then degraded (Narendra et al. 2010). During mitochondrial stress, if PINK1 fails to be imported across the mitochondrial inner membrane, it accumulates on the outer membrane with the kinase domain facing the cytosol. Here, PINK1 phosphorylates ubiquitin and the ubiquitin ligase, Parkin, which in turn leads to polyubiquitination of multiple mitochondrial outer membrane proteins (Kondapalli et al. 2012; Kane et al. 2014; Kazlauskaitė et al. 2014; Koyano et al. 2014; Ordureau et al. 2014). The accumulation of ubiquitinated mitochondrial proteins recruits adaptor proteins, which facilitate cargo recognition by the autophagy machinery (Heo et al. 2015). In the physiological context, mitophagy plays a role in dynamically modulating the levels of deleterious mitochondrial genome in addition to the UPR^{mt}. Mutations in genes encoding both PINK1 and Parkin cause early-onset Parkinson's disease, suggesting that mitophagy plays a prominent role in maintaining the mitochondrial network within dopaminergic neurons (Lucking et al. 2000; Valente et al. 2004).

Because nearly all mitochondrial-localized proteins are encoded on cytosolic ribosomes, impaired mitochondrial import results in the accumulation of mislocalized mitochondrial precursor proteins within the cytosol. If not reduced, the toxic mislocalized proteins cause cell death via a pathway dubbed mPOS (mitochondrial precursor overaccumulation stress) (Wang and Chen 2015). Although the mechanism by which mPOS causes cell death remains to be determined, at least two approaches are used to alleviate toxicity, including increased proteasomal activity to degrade the mislocalized proteins and reduced cytosolic translation to decrease the expression of mitochondrial precursors, which ultimately cannot be imported. These studies

emphasize the damaging effects that mitochondrial dysfunction can have on the cytosol, and highlight cellular activities or pathways that serve to restore cytosolic proteostasis, collectively known as UPR^{am} (unfolded protein response activated by mistargeting of proteins) (Wrobel et al. 2015).

Furthermore, a novel pathway that is activated specifically to resolve mitochondrial import stress known as mitoCPR (mitochondrial compromised protein import response) was discovered recently (Weidberg and Amon 2018). mitoCPR induces a transcriptional program that comprises more than 200 genes, including ABC transporters, and is regulated by a transcription factor PDR3 (pleiotropic drug resistance 3). Intriguingly, PDR3 induces the expression of *Cis1*, which recruits the ATP-dependent translocase *Msp1* (mitochondrial sorting protein) to the TOM channel to facilitate the extraction and degradation of stalled proteins. Thus, mitoCPR functions to alleviate import stress and restore homeostasis by facilitating clearance of stalled precursor proteins.

The existence of multiple quality-control pathways, in addition to the UPR^{mt}, illustrates the difficulty and importance of maintaining mitochondrial proteostasis. For the most part, each of these pathways has been studied in isolation. But, because all are regulated or impacted directly by impaired mitochondrial protein import, it will be exciting to understand how, or if, they interact both temporally and spatially.

PERSPECTIVES

Over the years, many studies from different organisms have shed light on the pathways that contribute to the maintenance of mitochondrial health and function. We have focused on the UPR^{mt}, which monitors activity of the mitochondrial network and responds to a wide array of stressors, including proteostasis and metabolic perturbations. Reduced mitochondrial protein import, arising as a consequence of either mitochondrial proteostasis or metabolic stress, leads to nuclear translocation of the transcription factor ATFS-1, which mediates UPR^{mt} activation, which in turn elicits an adaptive



transcriptional program. ATF5-1 up-regulates expression of chaperones-encoding genes and promotes mitochondrial biogenesis. UPR^{mt} activation also modulates protein import and restores mitochondrial proteostasis. This is mechanistically conserved in mammals as the transcription factor ATF5 is also regulated by impaired protein import and organelle partitioning. However, UPR^{mt} regulation in mammals requires translation regulation, which necessitates one of four eIF2 α kinases, as well as two additional transcription factors (ATF4 and CHOP), which are intimately associated with the ISR. The ISR has been best characterized as a response to ER stress, but how the pathway is specified to mitochondrial stress is yet to be discovered. It will also be of interest to further understand mechanisms that regulate ATF5 and ATF5-1 mitochondrial protein import efficiency, which may occur by direct protein modifications, such as phosphorylation, or by binding of metabolites and protein partners.

As discussed in this review, the diverse physiological consequences resulting from mitochondrial dysfunction repeatedly underscores the need for further research in understanding the UPR^{mt}. UPR^{mt} activation has been shown to have multiple beneficial effects such as promoting mitochondrial recovery, mounting an innate immune response against pathogenic infection, metabolic adaptations, and increasing longevity. However, prolonged UPR^{mt} activation can result in neurodegeneration and propagation of deleterious mitochondrial genomes, suggesting that precise regulation of UPR^{mt} activation is essential.

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