

The mitochondrial UPR – protecting organelle protein homeostasis

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Summary

Mitochondria are required for numerous essential metabolic processes including the regulation of apoptosis; therefore, proper maintenance of the mitochondrial proteome is crucial. The protein-folding environment in mitochondria is challenged by organelle architecture, the presence of reactive oxygen species and the difficulties associated with assembly of the electron transport chain, which consists of components encoded by both the mitochondrial and the nuclear genomes. Mitochondria have dedicated molecular chaperones and proteases that promote proper protein folding, complex assembly and quality control. Work in cultured mammalian cells and *Caenorhabditis elegans* has yielded clues to the mechanisms linking perturbations in the protein-folding environment in the mitochondrial matrix to the expression of nuclear genes encoding mitochondrial proteins. Here, we review the current knowledge of this mitochondrial unfolded protein response (UPR^{mt}), compare it with the better understood UPR of the endoplasmic reticulum and highlight its potential impact on development and disease.

Key words: Mitochondria, Molecular chaperones, Proteases, Protein homeostasis

Introduction

Maintenance of protein-folding homeostasis is essential for all organisms and requires molecular chaperones, which promote a functional protein-folding environment by preventing the aggregation of newly synthesized, newly imported or stress-denatured proteins, as well as by facilitating efficient folding and complex assembly of nascent polypeptides. (Bukau et al., 2006; Young et al., 2004). Studies of chaperone expression in response to unfolded protein accumulation have revealed a conserved signaling paradigm, where signaling is repressed by free chaperones that are not engaged by client proteins. For example, in *Escherichia coli*, the transcription factor σ 32 is repressed by the Hsp70 chaperone DnaK (Guisbert et al., 2004). Upon accumulation of unfolded proteins, DnaK preferentially interacts with clients and releases σ 32 to activate transcription of the heat-shock operon. The subsequent increase in chaperones re-establishes protein-folding homeostasis and the excess of free DnaK is able to interact with σ 32 to repress the response (Straus et al., 1990; Tilly et al., 1983).

In compartmentalized eukaryotic cells, several pathways have evolved independently to ensure the integrity of the protein-folding environments in the cytosol, the endoplasmic reticulum (ER) and the mitochondria. All three compartments encounter nascent, unfolded polypeptides and each has a repertoire of compartment-specific chaperones to promote efficient folding. Unfolded protein stress is sensed in a compartment-specific manner and signaled to the nucleus for induction of the expression of compartment specific chaperone genes.

The cytosolic heat-shock response, which maintains protein-folding homeostasis in the cytosol, is mediated predominantly by the heat-shock factor (HSF) family of transcription factors (Fig. 1). Similarly to the bacterial response described above, Hsp70 binds to the transactivating domain of HSF1, thus repressing its

transcriptional activity. Following either heat shock or any other condition that perturbs protein folding within the cytosol, Hsp70 preferentially interacts with the accumulating unfolded proteins, thus releasing HSF1 and allowing it to transcriptionally activate genes with promoters containing HSF1 binding sites, such as Hsp70, Hsp90 and proteasome subunits (Perisic et al., 1989; Shi et al., 1998).

Signaling within the UPR^{ER} is significantly complicated by the need to transduce the signal across the ER membrane to the nucleus (Fig. 1). The UPR^{ER} is a tripartite signaling pathway that is regulated by three ER-localized transmembrane proteins. Inositol-requiring 1 (IRE1), protein-kinase-like endoplasmic reticulum kinase (PERK) and activating transcription factor 6 (ATF6) all monitor the protein-folding status in the ER lumen through direct interactions with the ER chaperone-binding immunoglobulin protein (BiP) (Ma and Hendershot, 2001; Ron and Walter, 2007). In the most conserved branch of the UPR^{ER}, IRE1 is activated by its dissociation from BiP; this results in IRE1 oligomerization and activation of its cytosolic domain, which contains a sequence-specific RNase activity that splices the X-box binding protein 1 (*XBPI*) mRNA. The spliced *XBPI* is then translated into a basic leucine zipper (bZip) transcription factor that traffics to the nucleus, where it upregulates genes encoding ER chaperones and the ER-associated degradation machinery (Calfon et al., 2002; Yoshida et al., 2001).

Here, we review current results from both mammalian cell culture and *Caenorhabditis elegans* on a conceptually similar pathway referred to as the mitochondrial unfolded protein response (UPR^{mt}). The UPR^{mt} is a stress response that activates transcription of nuclear-encoded mitochondrial chaperone genes to promote protein homeostasis within the organelle. We focus on recently identified components required for signaling the response and on the underlying biological conditions where it is active.

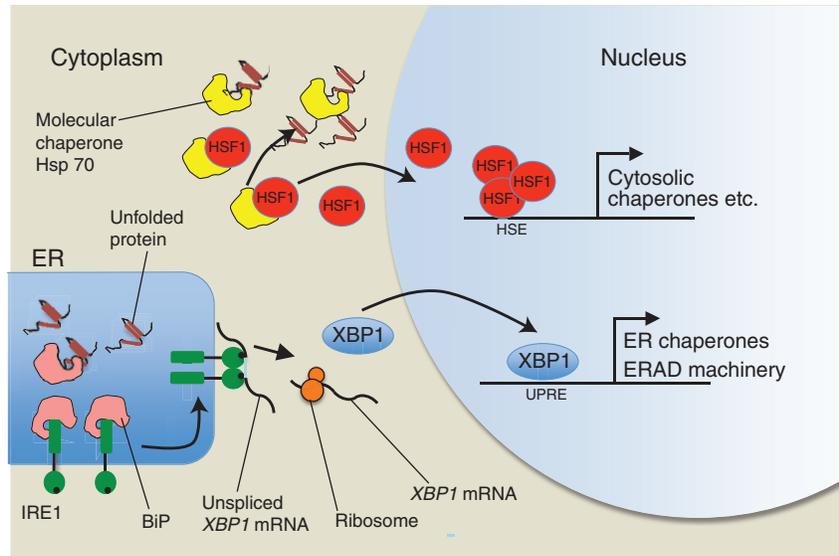


Fig. 1. The heat-shock response and ER unfolded protein response (UPR^{ER}). The heat-shock response and the endoplasmic reticulum UPR protect the cytosolic and ER protein-folding environments, respectively. The heat-shock response is regulated by the HSF family of transcription factors, of which HSF1 is depicted. In the absence of stress, the cytosolic chaperone Hsp70 binds to the transactivating domain of HSF1, thus repressing its transcriptional activity. Following a perturbation in cytosolic protein folding, Hsp70 preferentially interacts with the accumulating unfolded proteins, thus releasing HSF1. HSF1 trimerizes and transcriptionally activates genes with promoters containing HSF1 binding sites or heat-shock elements, such as those encoding Hsp70 and Hsp90. The UPR^{ER} is regulated by three transmembrane proteins; however, for simplicity, only the IRE1–XBP1 branch is illustrated. IRE1 is activated by its dissociation from the ER chaperone BiP, which results in its oligomerization and activation of its cytosolic domain. The cytosolic domain of IRE1 contains a sequence-specific RNase activity that splices and ligates the X-box binding protein 1 (*XBP1*) mRNA. Spliced *XBP1* is then translated into a basic leucine zipper (bZip) transcription factor that traffics to the nucleus, where it upregulates genes encoding ER chaperones and the ER-associated degradation (ERAD) machinery.

Unique challenges to mitochondrial protein-folding homeostasis

Mitochondria have dedicated machinery that functions to promote accurate protein folding and complex assembly within the organelle. Matrix-localized chaperones are required for protein import and facilitate protein folding, whereas proteases localized in the matrix and inner membrane degrade proteins that fail to fold or assemble correctly (Tatsuta and Langer, 2008). Mitochondria-localized chaperones include an Hsp70 family member, mtHsp70, as well as the orthologues to GroEL and GroES (Hsp60 and Hsp10), which both reside in the mitochondrial matrix and are encoded by nuclear genes (Neupert, 1997; Neupert and Herrmann, 2007). Studies in *C. elegans* and mammalian cell culture indicate that the protein-folding capacity within mitochondria can be adapted to deal with an increase in unfolded or misfolded proteins (Martinus et al., 1996; Yoneda et al., 2004; Zhao et al., 2002).

Several characteristics that are intrinsic to mitochondrial function, biogenesis and topology create a unique protein-folding environment prone to the accumulation of unfolded or unassembled proteins. Mitochondria are double-membrane-enclosed organelles containing proteins that are encoded by two different genomes. In mammals, the mitochondrial genome (mtDNA) encodes 13 proteins that make up ~10% of the approximately hundred components of the electron transport chain with the remaining encoded by nuclear genes. Nuclear-expressed proteins are translated in the cytosol and then directed towards and imported into each mitochondrion where they fold. Many proteins subsequently assemble into stoichiometric complexes with proteins that are encoded by the mitochondrial genome. It is not difficult to imagine how perturbations, such as an increase in mitochondrial biogenesis and protein import, mutations in client proteins, the presence of protein-altering reactive oxygen

species (ROS) or harsh environmental conditions, can affect these processes and negatively impact the mitochondrial protein-folding environment. The UPR^{mt} expands the folding capacity of the organelle during times of stress to maintain proteins in a folding or assembly-competent state, preventing deleterious protein aggregation.

UPR^{mt} signaling in mammalian cells

Pioneering experiments from the Hoogenraad laboratory showed that disturbing the stoichiometry of mitochondrial- and nuclear-encoded proteins by selectively lowered expression from the mitochondrial genome or overexpression of a nuclear-encoded aggregation-prone protein targeted to the mitochondrial matrix results in increased expression of HSP60 and the mitochondrial protease ClpP in cultured mammalian cells (Martinus et al., 1996; Zhao et al., 2002). Importantly, the response was shown to be organelle specific because ER-specific chaperones are not induced. Furthermore, this transcriptional program appeared to have a role in maintaining protein homeostasis, because the upregulation of HSP60 and ClpP reduces mitochondrial protein aggregation (Zhao et al., 2002). Therefore, these studies provided the first clue to the existence of a mitochondrial UPR.

The first component identified to be required for HSP60 upregulation was the transcription factor CHOP (C/EBP homology protein) because a CHOP binding site was found in the HSP60 promoter, and subsequently, its dimerization partner C/EBP β was identified (Fig. 2). Further support for a role of CHOP was illustrated by an increase in HSP60 transcription when CHOP is overexpressed and, conversely, expression of a dominant-negative CHOP repressed the response (Zhao et al., 2002). CHOP and C/EBP β are not only required for induction of the stress response,

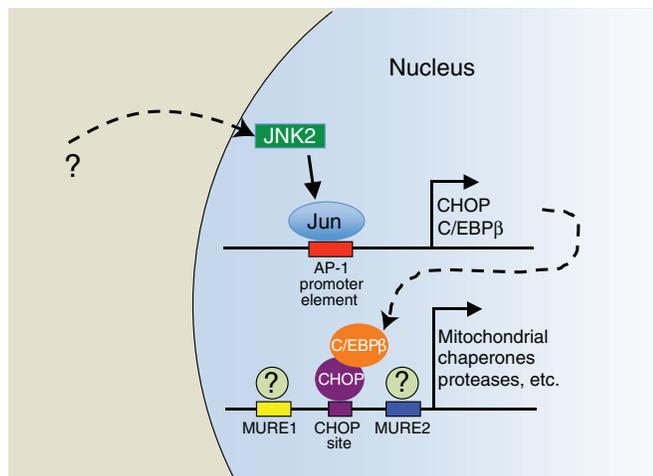


Fig. 2. Mitochondrial UPR signaling in mammalian cells. Transcriptional induction of mitochondrial chaperone and protease genes in mammalian cells requires several transcription factors, with CHOP and C/EBP β having important roles. CHOP binding sites along with two other conserved regions (MURE1 and MURE2) are found in a number of genes that are induced in response to mitochondrial stress (Aldridge et al., 2007). The identity of the transcription factors that interact with MURE1 and MURE2 are currently unknown. CHOP is itself transcriptionally induced in response to mitochondrial stress, which represents an early event in the pathway (Horibe and Hoogenraad, 2007). CHOP induction requires the kinase JNK2 and the transcription factor Jun, which binds to the AP-1 site within the promoters of the CHOP and C/EBP β genes. Signaling inputs that indicate mitochondrial stress and lead to activation of JNK2 signaling are currently unknown, but the upregulation of mitochondrial chaperone and proteases re-establishes protein-folding homeostasis within the organelle.

transcription of both is increased in response to mitochondrial stress and has been suggested to be an early event in the pathway (Horibe and Hoogenraad, 2007; Zhao et al., 2002). Analysis of the CHOP and C/EBP β promoters revealed the presence of an activator protein-1 (AP-1) site in both, suggesting the involvement of the Jun transcription factor, which is known to bind AP-1 sites. Horibe and Hoogenraad demonstrated the involvement of JNK2, a kinase upstream of Jun, because it becomes phosphorylated during mitochondrial stress and a JNK2 inhibitor attenuated the response.

CHOP, C/EBP β and Jun are unlikely to be the only transcription factors involved in the UPR^{mt} because promoters with CHOP binding sites that are activated in response to mitochondrial stress have two additional conserved sequences, the so-called mitochondrial UPR elements (MUREs) (Aldridge et al., 2007). This reveals the importance of stress-responsive transcription factors and kinases in the UPR^{mt} and suggests the potential for a crosstalk between UPR^{mt} signaling and other stress responses. However, such studies leave open the question of the identity of the sensing mechanisms that recognize the perturbation in the mitochondrial protein-folding environment.

UPR^{mt} signaling in *C. elegans*

A genetic approach was taken in *C. elegans* where we generated reporter animals in which the expression of GFP is under the control of a mitochondrial chaperone gene promoter. Under normal conditions, GFP expression from the *hsp-60* promoter is at a basal level, but increases significantly when mitochondrial stress is induced (Haynes et al., 2007; Yoneda et al., 2004). The UPR^{mt} in

C. elegans is activated by RNAi-mediated knockdown of factors required for mtDNA expression, mirroring the findings made in mammalian cells (Martinus et al., 1996). The worm UPR^{mt} is also activated by knockdown of mitochondrial chaperones and proteases (Yoneda et al., 2004).

A genome-wide RNAi approach was taken to identify components required to signal the UPR^{mt}. Cellular components localized in the mitochondrial matrix, inner membrane, cytosol and nucleus that compose a plausible signal transduction pathway were identified (Fig. 3). Consistent with a role for these components in protection of the mitochondrial protein-folding environment, knockdown or deletion of all components sensitizes organisms to mitochondrial stress, resulting in altered mitochondrial morphology, slowed development, shortened lifespan and reduced mitochondrial function (Benedetti et al., 2006; Haynes et al., 2007; Haynes et al., 2010).

ClpP and ClpX

Detection of unfolded proteins within the mitochondrial matrix is probably the initial signaling event that triggers the UPR^{mt}. The ATP-dependent protease ClpXP was shown to be required for induction of mitochondrial chaperone genes in response to stress (Haynes et al., 2007). We showed that it is localized within the matrix and potentially serves as the sensor by recognizing and degrading unfolded proteins that have accumulated beyond the mitochondrial chaperone protein-folding capacity (Haynes et al., 2007; Haynes et al., 2010). Eukaryotic ClpXP is involved in protein quality control because bacterial homologues perform a similar function to peptides in the degradation of substrates (Flynn et al., 2004; Yu and Houry, 2007). Furthermore, in the absence of ClpXP, proteolysis of unfolded mitochondrial proteins is impeded (Haynes et al., 2010). The requirement for a quality control protease in signaling the UPR^{mt} is intriguing, but a plausible mechanism by which proteolysis could contribute to signal transduction was not revealed until the discovery of the involvement of a mitochondrial peptide transporter.

HAF-1

HAF-1 is a mitochondrial inner-membrane-localized ABC transporter shown to have a positive role in mitochondrial chaperone induction in response to stress (Fig. 3). HAF-1 is homologous to the yeast protein Mdl1, which is also located in the mitochondrial inner membrane and to the TAP transporter located in the mammalian ER membrane, where both act as peptide transporters (Shepherd et al., 1993; Young et al., 2001). The orientation of HAF-1 in the inner mitochondrial membrane suggested that it fulfils a similar function in pumping peptides from the matrix to the inner membrane space. Using purified mitochondria, we showed that HAF-1 is required for mitochondrial peptide efflux (Haynes et al., 2010) similarly to Mdl1 (Young et al., 2001). The HAF-1-dependent recovery of peptides in mitochondrial supernatants is consistent with the observation that once they cross the inner membrane, small peptides (<20 amino acids) are able to diffuse freely through the mitochondrial outer membrane into the cytoplasm (Young et al., 2001).

HAF-1 is required for development and normal lifespan in the presence of mitochondrial stress, supporting the functional importance of its role in mitochondrial protein homeostasis (Haynes et al., 2010). These observations suggest a model whereby ClpXP-mediated mitochondrial unfolded protein degradation generates peptides that are pumped out of the organelle and contribute

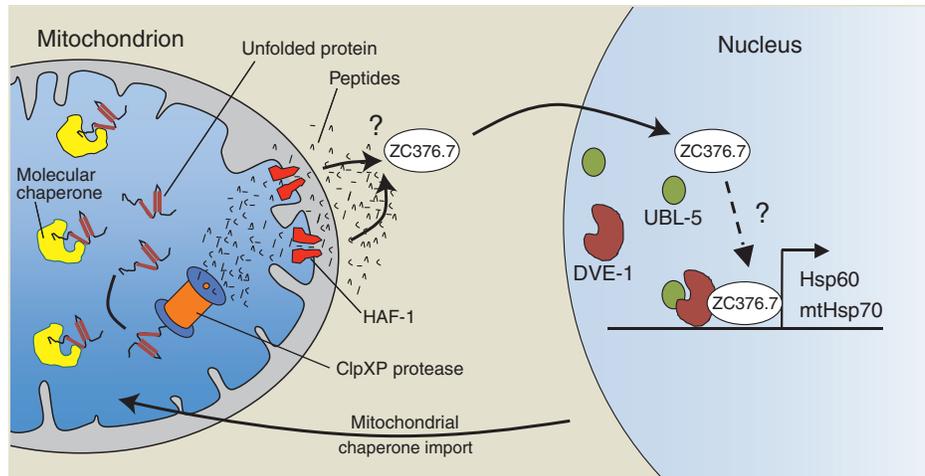


Fig. 3. A model for mitochondrial UPR signaling in *C. elegans*. It is assumed that signaling within the UPR^{mt} initiates when the unfolded protein load in the matrix exceeds the capacity of the mitochondrial chaperones. The AAA+ protease ClpXP degrades unfolded or unassembled proteins to peptides, which are pumped across the inner membrane by the ABC-transporter HAF-1 and then cross the more porous outer membrane to the cytosol. The presence of peptides in the cytosol, the process of peptide efflux or some linked activity of HAF-1 leads to the activation and nuclear translocation of the bZip transcription factor ZC376.7; however, the underlying mechanism(s) have yet to be identified. Additionally, the homeobox protein DVE-1 and UBL-5 form a complex and bind to the *hsp-60* promoter potentially remodeling chromatin structure to promote ZC376.7 binding and transcriptional activation. Transcriptional upregulation of mitochondrial chaperone genes leads to their subsequent import into mitochondria, thus relieving stress and re-establishing homeostasis.

somehow to downstream signaling in the UPR^{mt} and promote the return of the organelle to homeostasis.

The requirement for the quality control protease ClpXP suggests the UPR^{mt} in *C. elegans* relies on principles common to other UPRs, in which organelle-specific chaperone occupancy regulates signaling (Prahlad and Morimoto, 2009). However, in the case of the worm UPR^{mt}, our findings suggest that free chaperones do not directly repress the downstream transcription factor because they are in separate compartments (see below), but, more likely, mitochondrial chaperones bind to unfolded proteins, thus preventing their degradation by ClpXP into peptides that contribute to the stress response. It is interesting to note that this proposed sensing mechanism of the UPR^{mt} uses components found in bacteria because according to the endosymbiotic theory, both mitochondria and bacteria originate from a common precursor. However, the downstream signal transduction mechanisms require specific proteins that most likely arose as a consequence of the compartmentalized nature of the eukaryotic cell.

Downstream components of the UPR^{mt} pathway: ZC376.7, DVE-1 and UBL-5

Three nuclear components of the UPR^{mt} pathway have been identified that regulate downstream steps. ZC376.7 is a basic leucine zipper protein that resides in the cytoplasm of unstressed cells (Haynes et al., 2010). Upon induction of mitochondrial unfolded protein stress, ZC376.7 accumulates in the nucleus through a step that depends on ClpP and the mitochondrial peptide transporter HAF-1. How HAF-1-mediated peptide efflux affects ZC376.7 is presently unknown, but its regulation is required for worm development during stress (Haynes et al., 2010). Worms lack a conspicuous homolog of CHOP; given that both ZC376.7 and CHOP are small basic leucine zipper proteins, it is tempting to speculate on the parallels in their function in the UPR^{mt}.

DVE-1 is a conserved DNA binding protein with a homeobox domain. The homologous mammalian proteins SATB1 and SATB2

are global chromatin organizers implicated in transcriptional control (Galante et al., 2007). In response to perturbations in the mitochondrial protein-folding environment, DVE-1 redistributes within nuclei and binds to the *hsp-60* promoter (Haynes et al., 2007). Similarly, SATB1 and SATB2 have been shown to alter their nuclear localizations in response to stimuli (Cai et al., 2006; Dobreva et al., 2003); however, they have not been linked to mitochondrial unfolded protein stress in mammals.

UBL-5 is an ubiquitin-like protein that lacks a C-terminal diglycine motif (Benedetti et al., 2006). We previously showed that UBL-5 expression is increased in response to mitochondrial stress, upon which it binds to DVE-1 as determined by co-immunoprecipitation experiments in *C. elegans*. Interestingly, mammalian SATB2 and UBL5 also directly interact, as determined by co-immunoprecipitation experiments in cell culture (Benedetti et al., 2006; Haynes et al., 2007). In worms, both UBL-5 expression and complex formation with DVE-1 requires the protease ClpP, suggesting that they have a role in signaling downstream of this mitochondria-localized protease. However, it is unclear whether ZC376.7 and the DVE-1–UBL-5 complex interact with each other to increase UPR^{mt} signaling. Perhaps the complex between DVE-1 and UBL-5 facilitates chromatin rearrangements in facilitating access of ZC376.7 to the mitochondrial chaperone promoters?

Sources of mitochondrial unfolded protein stress

In wild-type *C. elegans*, UPR^{mt} reporter gene activity is observed during the L3–L4 stage of larval development, which is approximately 30–40 hours after hatching and when the germline begins to proliferate. During the L3–L4 stage, a burst of mitochondrial biogenesis is known to take place (Tsang and Lemire, 2002). If protein folding is not perturbed by mutation or pharmacological agents, the UPR^{mt}, which is promoted by the physiological increase in unfolded protein load is relatively short-

lived and attenuates when mitochondrial biogenesis subsides (Yoneda et al., 2004). However, when the folding environment is perturbed, for example by RNAi-mediated knockdown of mitochondrial chaperones, a dramatic increase in UPR^{mt} signaling is observed at the L3–L4 stage of development and beyond (Yoneda et al., 2004). Although it is clear that the knockdown of molecular chaperones causes unfolded protein stress, this rather broad perturbation does not provide any information on the identity of the unfolded proteins triggering the response.

An interesting clue comes from earlier studies that examined conditions that activate the UPR^{mt} in mammalian cell culture (Martinus et al., 1996). Cells cultured in the presence of ethidium bromide, which inhibits mitochondrial genome replication and transcription, experience strong activation of their UPR^{mt} most likely as a result of the accumulation of orphaned subunits from nuclear-encoded proteins of the electron transport chain. This point was supported in follow-up experiments in *C. elegans*, where knockdown of individual components of hetero-oligomeric complexes was shown to activate the UPR^{mt}, presumably by promoting accumulation of orphaned subunits (Yoneda et al., 2004). Moreover, previous studies have shown that paraquat, an insecticide known to result in high levels of ROS, can also activate the UPR^{mt} (Cocheme and Murphy, 2008; Yoneda et al., 2004). Finally, direct evidence that accumulation of misfolded proteins activates the UPR^{mt} was shown in cell culture by targeting a mutant, aggregation-prone protein to the mitochondrial matrix (Zhao et al., 2002).

Unfolded protein stress in diseases associated with mitochondrial dysfunction

Numerous diseases are associated with mitochondrial dysfunction and failure of protein folding or assembly might contribute to their pathogenesis. These include spastic paraplegia, Parkinson's disease, Friedreich's ataxia and cancer, as well as disorders caused by mutations in the mitochondrial genome, such as Leigh Syndrome and Leber's hereditary optic neuropathy (Wallace, 2005). In addition to certain specific characteristics (some of which are discussed further below), these conditions have important features in common, such as increased ROS generation, mitochondrial DNA mutation accumulation and a reduction in metabolic outputs, including ATP production, all of which secondarily affect protein-folding homeostasis in the mitochondria.

Spastic paraplegia

Spastic paraplegia is a neurodegenerative disease often associated with mitochondrial dysfunction. Among the known 13 mutations that cause spastic paraplegia, two impair the mitochondrial chaperonin Hsp60 and the protease Spg7 (Casari et al., 1998; Hansen et al., 2002). Spg7 is an AAA+ protease localized within the inner mitochondrial membrane and is required for the processing and the quality control of electron transport chain components as well as for mitochondrial ribosome biogenesis (Nolden et al., 2005). The SPG13 mutation in the *HSP60* gene encodes a protein with compromised chaperone activity (Bross et al., 2008). Perhaps not surprisingly, knockdown of either HSP-60 or SPG-7 activates the UPR^{mt} in *C. elegans* (Yoneda et al., 2004). Another intriguing study has shown that in patients with *HSP60* mutations, ClpP expression is reduced as the disease progresses (Hansen et al., 2008). Assuming that the role of ClpP in the UPR^{mt} is conserved in humans, this feature would contribute to further deterioration in mitochondrial protein-folding capacity.

Friedreich's ataxia

Friedreich's ataxia (FA) is a neurodegenerative disease caused by a trinucleotide expansion within the first intron of the gene encoding frataxin, resulting in reduced expression (Rouault and Tong, 2005). Frataxin is a highly conserved mitochondrial-localized protein required for iron–sulfur (Fe–S) cluster biogenesis and intracellular iron homeostasis (Babcock et al., 1997). Reduced frataxin expression affects the assembly and function of proteins that require Fe–S clusters as a cofactor, including aconitase and several proteins within the electron transport chain (Rotig et al., 1997). Additionally, several cytosolic and nuclear proteins involved in DNA repair also require Fe–S clusters (Veatch et al., 2009). Interestingly, in a *C. elegans* model of FA, both the cytosolic heat-shock response and the UPR^{mt} are activated, indicating the accumulation of unfolded proteins within mitochondria and the cytosol (Ventura et al., 2009), which might be due to an accumulation of folding intermediates lacking Fe–S cofactors. Furthermore, in a mouse model of FA, ClpP expression was shown to increase over time (Guillon et al., 2009), similarly to what was shown earlier in cell culture following overexpression of a model misfolded protein (Zhao et al., 2002). These data provide support for a role of the UPR^{mt} in protecting the mitochondrial protein-folding environment when subcellular levels of frataxin and Fe–S clusters are reduced.

Cancer

Many tumors and cancer cell lines have increased levels of mitochondrial chaperones consistent with an activated UPR^{mt} and an accumulation of unfolded proteins (Ghosh et al., 2008; Kang et al., 2007). In most cases, the exact underlying stress is unclear, but alterations that affect mitochondrial biology found in cancer cells are numerous, ranging from the increase in protein-altering ROS to genomic instability. Commonly observed genomic alterations include increased point mutations in the mitochondrial (Chatterjee et al., 2006) and nuclear genomes, as well as aneuploidy, all of which can disrupt protein assembly by altering the stability and stoichiometry of protein complexes (Luo et al., 2009).

Protein-destabilizing mutations in three of the four components of the succinate dehydrogenase complex cause head and neck tumors, as well as renal cell carcinomas (Baysal et al., 2000; Gogvadze et al., 2008). Additionally, numerous missense mutations that directly affect the folding and stability of mitochondrial protein complexes are found in cancer cells and are thought to enhance tumor growth (Petros et al., 2005) and metastasis (Ishikawa et al., 2008). Several mechanisms have been proposed to explain how mitochondrial dysfunction confers an advantage to tumor cells, including increased ROS signaling, suppression of apoptosis and reduced aerobic respiration, leading to increased glycolysis – the so-called Warburg effect (Gogvadze et al., 2008). Regardless of whether mitochondrial dysfunction itself is pathogenic or not, it appears that it is prevalent in cancers, suggesting that cancer cells might depend on cellular pathways that protect mitochondrial function, such as the UPR^{mt}.

Conclusions and Perspectives

The UPR^{mt} is a stress-response pathway that adapts the protein-folding capacity of the mitochondrial matrix to the load of unfolded proteins in normal physiology and disease states. The protein-folding load of the mitochondrial matrix is monitored, and accumulation of unfolded proteins is signaled to the nucleus with subsequent upregulation of the respective chaperone-encoding genes. The UPR^{mt} is organized such that changes in chaperone

expression are intimately associated with the degradation of a percentage of accumulating unfolded proteins, thereby ensuring protein-folding homeostasis at the level of both protein folding and removal.

Important questions regarding regulation of the UPR^{mt}, as well as the precise signaling mechanisms within the pathway remain to be resolved. Of particular interest is the mechanism by which ClpP-dependent proteolysis and HAF-1 activity or peptide efflux impact nuclear activities of the downstream transcription factors. For example, the existence of a cytosolic signaling molecule that might interact with effluxed peptides to propagate the signal has been hypothesized, although none has been reported to date (Haynes et al., 2010; Young et al., 2001; Zhao et al., 2002).

A more comprehensive determination of UPR^{mt} transcriptional outputs dependent on each required transcription factor will potentially shed light on the global cellular response to mitochondrial stress and dysfunction (Aldridge et al., 2007). Further details of the conditions that activate the response and how unfolded proteins are recognized will provide a better understanding of upstream events in the UPR^{mt}, as well as the underlying causes of mitochondrial dysfunction. Finally, all of the signaling components identified in *C. elegans* have mammalian orthologues and they should be examined in the cell culture system generated by Hoogenraad and colleagues to determine the extent of conservation in UPR^{mt} pathways of different organisms.

What can studies of the UPR^{mt} and its signaling components in higher eukaryotes reveal about mitochondrial biology and disease states involving mitochondrial dysfunction? If ClpXP acts as the stress sensor, ClpXP substrates are likely to represent some of the primary protein-folding defects within that particular organelle or specific cell type. One prediction is that different forms of stress will perturb the folding of different substrates, all of which are able to activate the signaling pathway. Identification of ClpXP substrates might thus provide important insights into the underlying causes of mitochondrial alterations or rearrangements found in a variety of biological scenarios.

The UPR^{mt} is only one of the stress responses cells use to protect mitochondrial function. It will be interesting to learn how UPR^{mt} signaling integrates with the retrograde response to respond to lower mitochondrial metabolic output (Liu and Butow, 2006). Furthermore, recent work has shown that terminally defective mitochondria can be degraded through the autophagy pathway that requires the proteins PINK1 and Parkin (Geisler et al., 2010; Narendra et al., 2010). It is possible that the UPR^{mt} is the initial response to protect mitochondrial function and re-establish homeostasis, but organelles that are irreparably damaged are targeted by mitophagy. Furthermore, as damage accumulates and the majority of organelles become irreparable, programmed cell death pathways might be used. A possible mechanism was proposed whereby accumulating unfolded proteins within mitochondria contribute to mitochondrial permeability transition, an early event in apoptosis (He and Lemasters, 2002). However, the relationship between the UPR^{mt}, mitophagy and apoptosis remains to be examined.

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References

Aldridge, J. E., Horibe, T. and Hoogenraad, N. J. (2007). Discovery of genes activated by the mitochondrial unfolded protein response (mtUPR) and cognate promoter elements. *PLoS ONE* 2, e874.

- Babcock, M., de Silva, D., Oaks, R., Davis-Kaplan, S., Jiralerspong, S., Montermini, L., Pandolfo, M. and Kaplan, J. (1997). Regulation of mitochondrial iron accumulation by Yfh1p, a putative homolog of frataxin. *Science* 276, 1709-1712.
- Baysal, B. E., Ferrell, R. E., Willett-Brozick, J. E., Lawrence, E. C., Myssiorek, D., Bosch, A., van der Mey, A., Taschner, P. E., Rubinstein, W. S., Myers, E. N. et al. (2000). Mutations in SDHD, a mitochondrial complex II gene, in hereditary paraganglioma. *Science* 287, 848-851.
- Benedetti, C., Haynes, C. M., Yang, Y., Harding, H. P. and Ron, D. (2006). Ubiquitin-like protein 5 positively regulates chaperone gene expression in the mitochondrial unfolded protein response. *Genetics* 174, 229-239.
- Bross, P., Naundrup, S., Hansen, J., Nielsen, M. N., Christensen, J. H., Kruhoffer, M., Palmfeldt, J., Corydon, T. J., Gregersen, N., Ang, D. et al. (2008). The Hsp60 (p.V981) mutation associated with hereditary spastic paraplegia SPG13 compromises chaperonin function both in vitro and in vivo. *J. Biol. Chem.* 283, 15694-15700.
- Bukau, B., Weissman, J. and Horwich, A. (2006). Molecular chaperones and protein quality control. *Cell* 125, 443-451.
- Cai, S., Lee, C. C. and Kohwi-Shigematsu, T. (2006). SATB1 packages densely looped, transcriptionally active chromatin for coordinated expression of cytokine genes. *Nat. Genet.* 38, 1278-1288.
- Calfon, M., Zeng, H., Urano, F., Till, J. H., Hubbard, S. R., Harding, H. P., Clark, S. G. and Ron, D. (2002). IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 415, 92-96.
- Casari, G., De Fusco, M., Ciarmatori, S., Zeviani, M., Mora, M., Fernandez, P., De Michele, G., Filla, A., Coccoza, S., Marconi, R. et al. (1998). Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* 93, 973-983.
- Chatterjee, A., Mambo, E. and Sidransky, D. (2006). Mitochondrial DNA mutations in human cancer. *Oncogene* 25, 4663-4674.
- Cocheme, H. M. and Murphy, M. P. (2008). Complex I is the major site of mitochondrial superoxide production by paraquat. *J. Biol. Chem.* 283, 1786-1798.
- Dobrev, G., Dambacher, J. and Grosschedl, R. (2003). SUMO modification of a novel MAR-binding protein, SATB2, modulates immunoglobulin mu gene expression. *Genes Dev.* 17, 3048-3061.
- Flynn, J. M., Levchenko, I., Sauer, R. T. and Baker, T. A. (2004). Modulating substrate choice: the SspB adaptor delivers a regulator of the extracytoplasmic-stress response to the AAA+ protease ClpXP for degradation. *Genes Dev.* 18, 2292-2301.
- Galante, S., Purbey, P. K., Notani, D. and Kumar, P. P. (2007). The third dimension of gene regulation: organization of dynamic chromatin loopscape by SATB1. *Curr. Opin. Genet. Dev.* 17, 408-414.
- Geisler, S., Holmstrom, K. M., Skujat, D., Fiesel, F. C., Rothfuss, O. C., Kahle, P. J. and Springer, W. (2010). PINK1/Parkin-mediated mitophagy is dependent on VDAC1 and p62/SQSTM1. *Nat. Cell Biol.* 12, 119-131.
- Ghosh, J. C., Dohi, T., Kang, B. H. and Altieri, D. C. (2008). Hsp60 regulation of tumor cell apoptosis. *J. Biol. Chem.* 283, 5188-5194.
- Gogvadze, V., Orrenius, S. and Zhivotovsky, B. (2008). Mitochondria in cancer cells: what is so special about them? *Trends Cell. Biol.* 18, 165-173.
- Guillon, B., Bulteau, A. L., Wattenhofer-Donze, M., Schmucker, S., Friguet, B., Puccio, H., Drapier, J. C. and Bouton, C. (2009). Frataxin deficiency causes upregulation of mitochondrial Lon and ClpP proteases and severe loss of mitochondrial Fe-S proteins. *FEBS J.* 276, 1036-1047.
- Guisbert, E., Herman, C., Lu, C. Z. and Gross, C. A. (2004). A chaperone network controls the heat shock response in *E. coli*. *Genes Dev.* 18, 2812-2821.
- Hansen, J. J., Durr, A., Cournu-Rebeix, L., Georgopoulos, C., Ang, D., Nielsen, M. N., Davoine, C. S., Brice, A., Fontaine, B., Gregersen, N. et al. (2002). Hereditary spastic paraplegia SPG13 is associated with a mutation in the gene encoding the mitochondrial chaperonin Hsp60. *Am. J. Hum. Genet.* 70, 1328-1332.
- Hansen, J., Corydon, T. J., Palmfeldt, J., Durr, A., Fontaine, B., Nielsen, M. N., Christensen, J. H., Gregersen, N. and Bross, P. (2008). Decreased expression of the mitochondrial matrix proteases Lon and ClpP in cells from a patient with hereditary spastic paraplegia (SPG13). *Neuroscience* 153, 474-482.
- Haynes, C. M., Petrova, K., Benedetti, C., Yang, Y. and Ron, D. (2007). ClpP mediates activation of a mitochondrial unfolded protein response in *C. elegans*. *Dev. Cell* 13, 467-480.
- Haynes, C. M., Yang, Y., Blais, S. P., Neubert, T. A. and Ron, D. (2010). The matrix peptide exporter HAF-1 signals a mitochondrial UPR by activating the transcription factor ZC376.7 in *C. elegans*. *Mol. Cell* 37, 529-540.
- He, L. and Lemasters, J. J. (2002). Regulated and unregulated mitochondrial permeability transition pores: a new paradigm of pore structure and function? *FEBS Lett.* 512, 1-7.
- Horibe, T. and Hoogenraad, N. J. (2007). The chop gene contains an element for the positive regulation of the mitochondrial unfolded protein response. *PLoS ONE* 2, e835.
- Ishikawa, K., Takenaga, K., Akimoto, M., Koshikawa, N., Yamaguchi, A., Imanishi, H., Nakada, K., Honma, Y. and Hayashi, J. (2008). ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 320, 661-664.
- Kang, B. H., Plescia, J., Dohi, T., Rosa, J., Doxsey, S. J. and Altieri, D. C. (2007). Regulation of tumor cell mitochondrial homeostasis by an organelle-specific Hsp90 chaperone network. *Cell* 131, 257-270.
- Liu, Z. and Butow, R. A. (2006). Mitochondrial retrograde signaling. *Annu. Rev. Genet.* 40, 159-185.
- Luo, J., Solimini, N. L. and Elledge, S. J. (2009). Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell* 136, 823-837.
- Ma, Y. and Hersholt, L. M. (2001). The unfolding tale of the unfolded protein response. *Cell* 107, 827-830.

- Martinius, R. D., Garth, G. P., Webster, T. L., Cartwright, P., Naylor, D. J., Hoj, P. B. and Hoogenraad, N. J. (1996). Selective induction of mitochondrial chaperones in response to loss of the mitochondrial genome. *Eur. J. Biochem.* **240**, 98-103.
- Narendra, D. P., Jin, S. M., Tanaka, A., Suen, D. F., Gautier, C. A., Shen, J., Cookson, M. R. and Youle, R. J. (2010). PINK1 is selectively stabilized on impaired mitochondria to activate Parkin. *PLoS Biol.* **8**, e1000298.
- Neupert, W. (1997). Protein import into mitochondria. *Annu. Rev. Biochem.* **66**, 863-917.
- Neupert, W. and Herrmann, J. M. (2007). Translocation of proteins into mitochondria. *Annu. Rev. Biochem.* **76**, 723-749.
- Nolden, M., Ehses, S., Koppen, M., Bernacchia, A., Rugarli, E. I. and Langer, T. (2005). The m-AAA protease defective in hereditary spastic paraplegia controls ribosome assembly in mitochondria. *Cell* **123**, 277-289.
- Perisic, O., Xiao, H. and Lis, J. T. (1989). Stable binding of Drosophila heat shock factor to head-to-head and tail-to-tail repeats of a conserved 5 bp recognition unit. *Cell* **59**, 797-806.
- Petros, J. A., Baumann, A. K., Ruiz-Pesini, E., Amin, M. B., Sun, C. Q., Hall, J., Lim, S., Issa, M. M., Flanders, W. D., Hossini, S. H. et al. (2005). mtDNA mutations increase tumorigenicity in prostate cancer. *Proc. Natl. Acad. Sci. USA* **102**, 719-724.
- Prahlad, V. and Morimoto, R. I. (2009). Integrating the stress response: lessons for neurodegenerative diseases from *C. elegans*. *Trends Cell Biol.* **19**, 52-61.
- Ron, D. and Walter, P. (2007). Signal integration in the endoplasmic reticulum unfolded protein response. *Nat. Rev. Mol. Cell Biol.* **8**, 519-529.
- Rotig, A., de Lonlay, P., Chretien, D., Foury, F., Koenig, M., Sidi, D., Munnich, A. and Rustin, P. (1997). Aconitase and mitochondrial iron-sulphur protein deficiency in Friedreich ataxia. *Nat. Genet.* **17**, 215-217.
- Rouault, T. A. and Tong, W. H. (2005). Iron-sulphur cluster biogenesis and mitochondrial iron homeostasis. *Nat. Rev. Mol. Cell Biol.* **6**, 345-351.
- Shepherd, J. C., Schumacher, T. N., Ashton-Rickardt, P. G., Imaeda, S., Ploegh, H. L., Janeway, C. A., Jr and Tonegawa, S. (1993). TAP1-dependent peptide translocation in vitro is ATP dependent and peptide selective. *Cell* **74**, 577-584.
- Shi, Y., Mosser, D. D. and Morimoto, R. I. (1998). Molecular chaperones as HSF1-specific transcriptional repressors. *Genes Dev.* **12**, 654-666.
- Straus, D., Walter, W. and Gross, C. A. (1990). DnaK, DnaJ, and GrpE heat shock proteins negatively regulate heat shock gene expression by controlling the synthesis and stability of sigma 32. *Genes Dev.* **4**, 2202-2209.
- Tatsuta, T. and Langer, T. (2008). Quality control of mitochondria: protection against neurodegeneration and ageing. *EMBO J.* **27**, 306-314.
- Tilly, K., McKittrick, N., Zylicz, M. and Georgopoulos, C. (1983). The dnaK protein modulates the heat-shock response of *Escherichia coli*. *Cell* **34**, 641-646.
- Tsang, W. Y. and Lemire, B. D. (2002). Mitochondrial genome content is regulated during nematode development. *Biochem. Biophys. Res. Commun.* **291**, 8-16.
- Veatch, J. R., McMurray, M. A., Nelson, Z. W. and Gottschling, D. E. (2009). Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell* **137**, 1247-1258.
- Ventura, N., Rea, S. L., Schiavi, A., Torgovnick, A., Testi, R. and Johnson, T. E. (2009). p53/CEP-1 increases or decreases lifespan, depending on level of mitochondrial bioenergetic stress. *Aging Cell* **8**, 380-393.
- Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* **39**, 359-407.
- Yoneda, T., Benedetti, C., Urano, F., Clark, S. G., Harding, H. P. and Ron, D. (2004). Compartment-specific perturbation of protein handling activates genes encoding mitochondrial chaperones. *J. Cell Sci.* **117**, 4055-4066.
- Yoshida, H., Matsui, T., Yamamoto, A., Okada, T. and Mori, K. (2001). XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* **107**, 881-891.
- Young, J. C., Agashe, V. R., Siegers, K. and Hartl, F. U. (2004). Pathways of chaperone-mediated protein folding in the cytosol. *Nat. Rev. Mol. Cell Biol.* **5**, 781-791.
- Young, L., Leonhard, K., Tatsuta, T., Trowsdale, J. and Langer, T. (2001). Role of the ABC transporter Mdl1 in peptide export from mitochondria. *Science* **291**, 2135-2138.
- Yu, A. Y. and Houry, W. A. (2007). ClpP: a distinctive family of cylindrical energy-dependent serine proteases. *FEBS Lett.* **581**, 3749-3757.
- Zhao, Q., Wang, J., Levichkin, I. V., Stasinopoulos, S., Ryan, M. T. and Hoogenraad, N. J. (2002). A mitochondrial specific stress response in mammalian cells. *EMBO J.* **21**, 4411-4419.

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