How the mitoprotein-induced stress response safeguards the cytosol: A unified view

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Abstract

Mitochondrial and cytosolic proteostasis are of central relevance for cellular stress resistance and organismal health. Recently, a number of individual cellular programs were described which counter the fatal consequences of mitochondrial dysfunction. These programs remove arrested import intermediates from mitochondrial protein translocases, stabilize protein homeostasis within mitochondria and, in particular, increase the levels and activity of chaperones and the proteasome system in the cytosol. Here, we describe the different responses to mitochondrial perturbation, and propose to unify the seemingly distinct mitochondrial-cytosolic quality control mechanisms into a single network, the mitoprotein-induced stress response. This holistic view places mitochondrial biogenesis at a central position of the cellular proteostasis network, emphasizing the importance of mitochondrial protein import processes for development, reproduction and ageing.

Main text

The emerging role of mitochondria in the regulation of cellular and organismal protein homeostasis

Organization of the subcellular environment into distinct, membrane-bound organelles is a key feature of eukaryotic cells. While this allows cells to operate efficiently through the creation of functionally specialized environments, the spatial and temporal separation of protein synthesis, folding and degradation also presents a significant challenge to the cells' ability to maintain protein homeostasis (proteostasis).

In order to counteract proteostasis imbalances within compartments, cells have evolved dedicated, organelle-specific protein quality control programs, such as the heat shock response (HSR)(**see glossary**) of the cytosol and the unfolded protein responses of the endoplasmic reticulum (UPR^{ER}) and mitochondria (UPR^{mt}) [1-3]. These responses have been extensively studied, and are crucial for the functionality of cells, tissues and organisms. However, the classical view that organellar stress responses act in isolation has been challenged by observations in yeast, worms, flies and mammalian tissue culture cells. Rather, proteotoxic insults at the organellar level can have far-reaching consequences for protein quality control networks across the cell, or even for other tissues [4-6]. This has become particularly clear in the case of mitochondria, where the activity and composition of cytosolic proteostasis networks is tightly coordinated with fluctuations in mitochondrial activity and function, through several seemingly distinct protective responses. In this review, we present the different pathways that couple changes in mitochondrial function with cytosolic protein homeostasis, and discuss how these seemingly disparate mechanisms might be integrated into one coordinated mitoprotein-induced stress response that impacts development, ageing and disease.

Mitochondrial protein import is the nexus between mitochondrial and cytosolic proteostasis

Mitochondria are responsible for the bulk of cellular ATP production, and are classically referred to as the 'powerhouses' of the cell. Interestingly, a growing number of studies have connected mitochondrial function with susceptibility to, and protection against, cytosolic protein aggregation [7-13]. Impaired cell function as a consequence of mitochondrial dysfunction was initially attributed to changes in the levels of ATP or reactive oxygen species (ROS); however, another factor might be of even more direct relevance: the integrity of the mitochondrial protein import process [14].

Only a few hydrophobic core subunits of the respiratory chain (and a ribosomal protein in yeast) are encoded in the mitochondrial genome and produced within mitochondria. The other approximately 1000 mitochondrial proteins are synthesized on cytosolic ribosomes, subsequently targeted to the mitochondrial surface and then imported by dedicated translocases (**Figure 1**) [15]. Most mitochondrial proteins are synthesized as precursors with an N-terminal mitochondrial targeting sequence (MTS, also called presequence) which are cleaved upon arrival in the mitochondrial matrix. Mitochondrial functionality relies on an efficient protein import process and *vice versa*. Translocation across the mitochondrial membranes is dependent on the inner membrane potential ($\Delta\psi$) and the ATP level generated by the electron transport chain, as well as mitochondrial chaperones. Hence, perturbations of metabolism and protein homeostasis inside mitochondria translate into import defects (**Figure 1**).

In addition, protein import is sensitive to precursor state and load: The excessive synthesis of precursors, or stalling of prematurely folded import intermediates within translocases, can cause import defects [16-19]. In particular, proteins which are N-terminally anchored to the inner membrane are difficult to import due to the presence of stop-transfer signals after their mitochondrial targeting sequence [20, 21].

Owing to their post-translational mode of import, mitochondrial precursors are transiently exposed to the cytosol, where they are stabilized by chaperones [22-26] and under the surveillance of the proteasomal degradation system [27-29]. The passage of precursors through the cytosol makes the import process vulnerable to proteotoxic insults outside of mitochondria. In fact, cytoplasmic aggregation of pathological protein species such as mutant huntingtin/polyQ proteins [30-32], α-synuclein [33, 34] or amyloid β [9, 35-37] all interfere with mitochondrial protein import. Moreover, it was suggested that aggregated proteins in the cytosol are imported into mitochondria for sequestration or subsequent degradation [38, 39], although the underlying mechanism and relevance of this pathway is under debate. Together, these observations place mitochondrial protein import at the center of cellular proteostasis networks, well beyond the mitochondrial compartment (**Figure 2**).

Consequences of impaired mitochondrial protein import

If mitochondrial protein import is defective, cells face two major challenges. On the one hand, the lack of protein supply leads to proteome imbalances *inside* mitochondria, comparable to consequences of defects in the expression of the mitochondrial genome [40]. On the other hand, import defects result in the accumulation of precursor proteins in the cytosol and challenge proteostasis *outside* mitochondria.

It has been estimated that under normal basal conditions, around 5% of nascent ER proteins might constitutively fail to reach the ER at steady state conditions [41]. A similar magnitude also seems likely for mitochondrial preproteins, especially because some mitochondrial precursor proteins can traverse the ER surface on their route to mitochondria [42]. Small amounts of orphaned proteins can be efficiently cleared from the cytosol by proteasomal degradation [27] or from membranes by more specific mechanisms that degrade or re-route mislocalized proteins [43, 44]. However, when mitochondrial protein import efficiency is globally reduced, the fraction of accumulating precursors

can increase substantially, thereby placing a burden on protein folding and degradation pathways. As pre-proteins are escorted to the mitochondrial translocases by chaperones of the HSP70, HSP90 and HSP40 families [22, 23], a higher load of precursors could sequester these chaperones, leading to reduced protein folding capacity in the cytosol. In addition, most mitochondrial proteins are unlikely to fold properly outside mitochondria and can associate with, and perhaps even induce, cytosolic aggregates [45, 46]. Thus, defects in the import of mitochondrial precursors induce a situation that is reminiscent of the widespread decline of proteostasis that is associated with protein conformational diseases or ageing [47, 48].

Cellular reactions to compromised mitochondrial protein import

Cells use a repertoire of means to prevent an overload of mitochondrial protein import and to counteract the consequences of import failure for both mitochondria and the cytosol. Initially described as individual phenomena, numerous studies have revealed that cells safeguard mitochondrial protein import and restore mitochondrial/cytosolic homeostasis by: (1) unclogging jammed translocases and removing accumulating precursor proteins from the mitochondrial surface [19, 20]; (2) adjusting the synthesis of mitochondrial proteins to match import capacity, and increasing the expression of mitochondrial biogenesis and quality control components to preserve mitochondrial integrity [18, 49]; and (3) engaging cytosolic protein folding and degradation machineries to relieve the burden of accumulating precursor proteins outside of mitochondria [18, 50] (**Figure 2**). These mechanisms have been described in different organisms using several experimental models, such as mutants of the mitochondrial import machinery [29, 50-53], overexpression of proteins whose translocation is challenging [17-20], and disruption of mitochondrial membrane potential [54-57].

Problem-solving at the outer mitochondrial membrane

To prevent clogging of the import channel by non-productive import intermediates, the translocase of the outer mitochondrial membrane (TOM) is continuously monitored by the mitochondrial protein translocation-associated degradation (mitoTAD) pathway. In yeast, the key component of this pathway is Ubx2, which also functions in ER-associated degradation (ERAD). Ubx2 is part of the TOM complex; upon the appearance of arrested precursors in the translocase, Ubx2 recruits the AAA ATPase Cdc48/VCP/p97 to extract trapped precursors and direct them to the proteasome for degradation [19].

In addition, the mitochondria-associated AAA ATPase Msp1 monitors the complete mitochondrial surface for aberrant protein species. Msp1 recognizes tail-anchored membrane proteins that are mistargeted to mitochondria, extracts them from the mitochondrial outer membrane and re-routes them to the ER [43]. In addition, upon blocked mitochondrial import, the adaptor protein Cis1 is expressed which recruits Msp1 to the TOM complex. There, Msp1 and Cis1 mediate the removal of the precursor proteins, a process known as the mitochondrial compromised protein import response (mitoCPR) [20].

Besides premature folding or weak translocation, precursor proteins can also arrest inside translocases due to stalling of the ribosome during translation. In the cytosol, arrested ribosome-nascent chain complexes are cleared by dedicated ribosome quality control (RQC) pathways, involving the addition of C-terminal amino acids to the stalled polypeptide (CAT tailing) to facilitate its degradation by the ubiquitin-proteasome system. However, when the ribosome-nascent chain complex associates with the mitochondrial import machinery, CAT-tailed polypeptides are no longer accessible to the cytosolic quality control machinery and tend to aggregate inside mitochondria [58]. The conserved quality control factor Vms1 recognizes ribosome-stalled proteins at the mitochondrial surface and prevents CAT tailing [59-61]. In addition to its role in this mitochondrial RQC pathway, Vms1 also recruits Cdc48 to mitochondria upon stress to assist with protein degradation [57].

Measures to restore proteostasis within mitochondria

Imbalances in the mitochondrial proteome are counteracted by a transcriptional program known as the mitochondrial unfolded protein response (UPR^{mt}). In a nutshell, protein import overload is prevented by three major measures: (1) Increased expression of mitochondrial chaperones, assembly factors and proteases [62]; (2) increased expression of mitochondrial translocases (in metazoa, not in yeast) [63]; and (3) reduced expression of many mitochondrial proteins, particularly the highly abundant enzymes of the respiratory chain and TCA cycle, the coordinated downregulation of which, presumably relieves the workload of the import machinery [49]. Similar to the role of the UPR^{ER} in homeostatic regulation of ER size, the responsiveness of the expression of mitochondrial enzymes to import overloading constitutes an elegant feedback mechanism to monitor and adjust the influx of proteins into mitochondria (**Box 1**).

The analysis of the UPR^{mt} was pioneered by studies in *C. elegans* and has been extensively reviewed elsewhere [1]. The master regulator of the UPR^{mt} in *C. elegans* is the transcription factor ATFS-1, which is a dually localized protein, present in mitochondria and the nucleus. A weak MTS efficiently targets ATFS-1 to mitochondria in well energized cells. However, when mitochondrial functions are compromised, ATFS-1 is no longer imported into mitochondria but instead accumulates in the nucleus, where it induces the UPR^{mt} [55, 63]. In human cells, the transcription factors ATF4 and ATF5 were proposed to fulfill a similar role [64, 65]. Yeast does not contain ATFS-1 homologs, but the HAP complex, which regulates the expression of most respiratory components, appears to play a comparable role. HAP-regulated genes are repressed upon protein import overload by inactivation of this transcription factor complex [18]. However, the underlying molecular mechanisms still remain to be discovered.

Measures to restore proteostasis in the cytosol

The accumulation of mitochondrial precursors in the cytosol is buffered by an upregulation of many cytosolic chaperones, including members of the HSP70, HSP90, HSP40, TRiC/CCT, and small heat shock protein families [18, 53, 66]. In addition, the abundance and activity of the proteasome is increased in a reaction known as the unfolded protein response activated by mistargeting of proteins (UPRam) [18, 50] or mitochondrial precursor over-accumulation stress (mPOS) [54]. The elevated proteasomal capacity helps to remove precursors from the cytosol and assists in the clearance of the outer membrane in conjunction with the mitoTAD and mitoCPR pathways. Although the protective responses described above were discovered as independent phenomena, recent evidence suggests that in fact, these pathways are amalgamated into a collective protective program, the mitoprotein-induced stress response [18](**Figure 3, Key Figure**).

The transcription factor HSF1 is crucial for maintaining proteostasis in the cytosol and has emerged as a key component of the mitoprotein-induced stress response. HSF1 dictates protein folding and degradation capacity in the cytosol through the coordinated expression of molecular chaperones, co-chaperones and degradation factors. It has long been known that in yeast, the transition from fermentative to respiratory metabolism, which strongly induces the production of mitochondrial proteins, is accompanied by an HSF1-mediated upregulation of chaperones and other stress-responsive factors [67, 68]. Consistent with this, it was recently discovered that mitochondrial import stress, impaired respiration or perturbation of mitochondrial HSP70, leads to a rapid elevation in the levels of HSF1 target genes [18, 66, 69], thereby augmenting the function of the core cytosolic proteostasis network.

Under non-stress conditions, HSF1 activity is repressed by direct interactions with molecular chaperones. However, upon proteostasis imbalances in the cytosol, molecular chaperones are titrated away from HSF1 through preferential binding to misfolded protein species. This permits the activation of functional HSF1 heterotrimers, and results in the increased expression of genes that restore cytosolic proteostasis [70, 71]. It is highly likely that the accumulation of unstable

mitochondrial precursors in the cytosol triggers the activation of HSF1 through a similar mechanism. However, it remains unclear whether HSF1 activation results from a general overload of the cytosol by mitochondrial precursors, or whether specific (groups of) precursors trigger HSF1 activation. Since the signatures of mitoprotein-induced stress response and heat shock response are similar but not identical, it is possible that additional mechanisms tailor chaperone expression to the specific sources of misfolded proteins. For example, lipid signaling has been reported to activate HSF1 upon mitochondrial perturbation in nematodes and thus might represent an addition layer of regulation [66].

In yeast, HSF1 also promotes the expression of Rpn4, the master regulator of proteasomal subunits and components of the ubiquitin proteasome system (UPS). [72]. The transcriptional induction of Rpn4 by Hsf1 is responsible for the upregulation of the ubiquitin-proteasome system in response to mitoprotein-induced stress [18]. Rpn4 itself is also a substrate of proteasomal degradation with very efficient turnover. Therefore, occupancy of the proteasome by mitochondrial precursors might also directly lead to the stabilization and, hence, increased abundance of Rpn4, augmenting its transcriptional upregulation.

In addition to proteasomal subunits, Rpn4 also increases the expression of Ubx2 and Cdc48, the central mediators of mitoTAD, and the transcription factor Pdr3, which in turn drives the expression of the mitoCPR factor Cis1 [20]. Therefore, HSF1 acts as the primary initiator of an Hsf1-Rpn4-Pdr3 transcriptional cascade that directly connects the regulation of mitochondrial and cytosolic responses to proteotoxic stress. The Hsf1-Rpn4-Pdr3 transcriptional axis is an intriguing example of how cells can coordinate the activity of multiple stress-related regulators. This mechanism has clear similarities with how increased Rpn4 levels in response to impaired translocation of ER preproteins, complements the UPR^{ER} to maintain cell viability [73]. This suggests that cells have evolved a general ‘core’ response to protein misfolding in the cytosol that is converged upon by mistargeted proteins or proteins that are misfolded due to heat exposure or other stresses.

Mitoprotein-induced stress leads to attenuation of translation

The transcriptional response to mitoprotein-induced stress is accompanied by the attenuation of protein synthesis [18, 50, 51, 54, 74]. This decreases the load on both the cytosolic protein quality control and mitochondrial import machineries, and saves energy. In addition to the specific shutdown of the synthesis of mitochondrial OXPHOS components, translational attenuation further reduces the production of mitochondrial precursors. Moreover, gene expression from the mitochondrial genome is also repressed [18, 75]. This may help to balance protein synthesis in the matrix with the reduced influx of imported proteins.

Reduced cytosolic translation has been proposed to occur through transcriptional downregulation and reversible cysteine oxidation of 80S ribosomal subunits. While still speculative, this suggests a model where mitochondrial stress can directly alter cytosolic translation through 'redox switches' in ribosomal subunits [51]. In addition, protein synthesis can be reduced through eIF2α phosphorylation as part of the integrated stress response and through inhibition of the target of rapamycin (mTOR) complex. Both eIF2α phosphorylation and reduced mTOR activity have been observed in response to mitochondrial stress [76, 77]; however, the precise contribution of these pathways to mitoprotein-induced slowdown of translation remains to be determined.

Conservation of the mitoprotein-induced stress response

Although well-described in yeast, the regulatory basis and composition of the mitoprotein-induced stress response in metazoans is less well understood. However, available evidence suggests that analogous mechanisms to those observed in fungi are present in animals. For example, the targeting of misfolding-prone substrates to mitochondria, genetic and chemical inhibition of respiration and perturbation of mitochondrial HSP70 have all been reported to increase the expression of HSF1 target genes in *C. elegans* and *Drosophila* [56, 66, 69, 78].

In addition to the immediate activation of acute transcriptional responses, mitochondrial status has also emerged as a critical determinant of HSF1 activity and susceptibility to protein aggregation later in adulthood. In *C. elegans*, the transition to reproductive maturity is accompanied by the programmed repression of the heat shock response [79]. This is mediated by changes in chromatin architecture at HSF-1 target promoters and leaves cells vulnerable to protein folding stress later in life. Mild perturbation of either respiration or mitochondrial import efficiently maintains the activity of the heat shock response in aged animals and protects against age-related protein aggregation, suggesting that exposure to mitochondrial stress can override age-related changes in chromatin organization and the heat shock response [56]. Although the precise mechanism by which mitochondrial impairment maintains the heat shock response is unknown, mitochondrial stress and full activation of the UPR^{mt} are also associated with changes in chromatin organization [78, 80, 81] **(Box 2)**. Together, these observations demonstrate the existence of a complex link between mitochondrial function, chromatin organization, HSF1 activity, cytsolic proteostasis and ageing.

While HSF1 activity is clearly linked to mitochondrial function in worms and flies, the regulation of the proteasome under mitoprotein-induced stress is far less clear in animals, particularly as orthologues of Rpn4 do not exist in metazoans. Potentially, the transcription factors NRF1 and NRF2 could fulfill a similar role as Rpn4. Like Rpn4, NRF1 and NRF2 control the abundance of proteasomal subunits in response to compromised proteasome activity. NRF2 has been shown to localize to the surface of mitochondria and is activated by mitochondrial ROS upon proteasome dysfunction [82, 83]. Furthermore, the *C. elegans* orthologue of NRF1, SKN-1A, promotes a UPRam-like cytoplasmic unfolded protein response to counteract various proteotoxic stresses [84].

Thus, the general regulatory principles appear to be conserved among eukaryotes. These make sure that upon mitoprotein-induced stress, proteostatic balance is maintained in both the cytosol and the mitochondria. This response employs regulators of general stress programs, in particular those of the heat shock response, as well as mechanisms that act at the level of specific steps of mitochondrial protein synthesis and import. Dependent on the severity and duration of

mitochondrial defects, the mitoprotein-induced stress response is also coupled with more global cellular homeostatic programs, which employ processes such as chromatin re-organization [56, 81] autophagy/mitophagy [85-87] and apoptosis [21] to promote transcriptional responses, signal to unaffected tissues, remove defective mitochondria or eliminate unviable cells (**Figure 4**).

Even though the general principles of these programs appear to be similar among eukaryotes, a considerable amount of heterogeneity exists with respect to the specific factors and regulatory elements that drive these programs in different organisms. One obvious example is that the expression of mitochondrial proteins is muted in nematodes by the stress response factor ATFS-1, whereas in yeast this is controlled by the general respiration control complex HAP. As such, understanding why these differences have emerged may provide important insight regarding the coordination of mitochondrial and cytosolic proteostasis across developmental states and/or cell types.

Concluding Remarks

Over the last five years, it has become increasingly evident that cellular stress resistance and organismal health are highly dependent on connections between mitochondrial and cytosolic proteostasis. While not all connections and causalities are understood (see **Outstanding Questions Box**), two major paradigms have emerged: First, the proteostasis and quality control programs from different subcellular compartments are distinct, but do not act in isolation from each other. Second, many seemingly disparate mechanisms are wired into a coordinated network that simultaneously restores mitochondrial function and safeguards cytosolic proteostasis. Therefore, we propose to amalgamate the existing independent mitochondrial-cytosolic quality control mechanisms into a single network, the mitoprotein-induced stress response. Taking a more holistic view of the mitochondrial-cytosolic protein quality control network will allow us to unravel the full complexity

of how mitochondrial function is coordinated with alterations in proteostasis and how this impacts development, reproduction and ageing.

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Box 1. The mitoprotein-induced stress response is conceptually distinct from the UPR^{ER}.

Protein misfolding in the lumen and the membrane of the ER is recognized by receptors located in the ER membrane. Upon stress, these elicit signaling pathways which induce or repress genes to buffer and counter problems within the ER. In contrast, the mitoprotein-induced stress response reacts to the presence of mitochondrial proteins that fail to be efficiently imported. Signaling can be triggered by specific stress-sensing factors, such as ATFS-1 of *C. elegans*, or by a more global accumulation of mitochondrial precursors, as proposed by the UPRam hypothesis for yeast (**Figure I**).

The distinction between transmembrane signaling from the ER and a "frustrated client" reporting model from mitochondria is not "black and white": Non-imported ER proteins are sensed in the cytosol [73, 88] and proteotoxic stress in the matrix of mitochondria can induce transcriptional changes in the nucleus [11, 89]. However, for most mitochondrial stress responses, signaling seems to occur mainly at the level of preprotein import from the cytosol and not via direct transduction across mitochondrial membranes.

Box 2. Mitochondrial stress regulates the expression of HSF1 target genes and the UPR^{mt} through chromatin reorganization

The rapid and effective activation of stress responsive transcriptional programs such as the HSR and UPR^{mt} is crucial for cells to successfully counteract proteostasis imbalances. In addition to the activity of dedicated transcription factors, it has recently been demonstrated that in *C. elegans*, changes in histone methylation and chromatin remodeling are crucial for effective induction of the UPR^{mt}, maximal induction of HSF1 target genes and full lifespan extension when respiration is compromised [56, 69, 80, 81].

In response to mitochondrial stress, chromatin architecture is reorganized through the chromatin remodeler LIN-65 in a process that is dependent on MET-2-mediated di-methylation of lysine 9 of histone H3 (H3K9me2). This results in a global chromatin conformation that generally represses transcription while favoring the induction of UPR^{mt} responsive genes [81]. Similarly, the HSF1-mediated induction of small heat shock protein genes upon electron transport chain dysfunction is also dependent on chromatin remodeling through the SWI/SNF-related factor, ISW-1 [69].

In addition to promoting immediate responses through HSF1 and the UPR^{mt}, mitochondrial stress-mediated changes in chromatin status can also promote long-term cell function. Upon electron transport chain perturbation, increased JMJD-1.2 and JMJD-3.1 activity results in reduced levels of di- and tri-methylation at lysine 27 of histone H3 (H3K27me2/3). This results in increased chromatin accessibility, prolonged activation of the UPR^{mt} and increased lifespan [80]. JMJD-3.1 activity has also been linked with the programmed repression of the HSR during early *C. elegans* adulthood. As worms reach reproductive maturity, signals from germ line stem cells result in decreased *jmjd-3.1* expression, increased levels of H3K27me3, and reduced chromatin accessibility at HSF1 target promoters. This leads to a dampening of the HSR and increased vulnerability to protein aggregation later in life [79]. While *jmjd-3.1* over-expression does not influence HSF1 activity early in life, it is sufficient to promote the UPR^{mt}, maintain HSF1 activity in aged cells and extend lifespan [79, 80]. Intriguingly, repression of the HSR and age-related cytosolic protein

aggregation can be suppressed by exposure to mitochondrial stress early in life [56]. While it is not clear to what extent these effects are mediated by altered histone modification and chromatin re-organization, these observations suggest that mitochondrial function is intimately coupled with the long and short-term activity of both HSF1 and the UPR^{mt} through changes in chromatin state.

Figure legends

Figure 1. Mitochondrial protein import is challenged upon many conditions. Mitochondrial biogenesis requires the import of about 1,000 different proteins from the cytosol. About two thirds of these proteins are initially made as precursors with an N-terminal mitochondrial targeting sequence (MTS). These sequences are recognized by receptors on the mitochondrial surface (Tom70 and cytosol-exposed regions of the TOM complex) and direct precursor proteins through the protein-conducting channels of the TOM and TIM23 complexes. The membrane potential across the inner membrane ($\Delta\psi$) and ATP hydrolysis by HSP70 drive protein translocation. Proteins of the intermembrane space (IMS) and the outer membrane often lack N-terminal targeting sequences and use distinct import routes. Many IMS proteins contain cysteine residues and their import is associated with oxidative protein folding in the IMS, catalyzed by the oxidoreductase Mia40. There are different groups of outer membrane proteins, including pore-forming β-barrel proteins and tail-anchored proteins. In most cases, the import of Mia40 substrates and outer membrane proteins requires neither ATP nor a membrane potential across the inner membrane. The figure illustrates these key steps of mitochondrial protein biogenesis. The import process can be challenged by problems in the cytosol or by mitochondrial defects, some of which are indicated here in light boxes.

Figure 2. Import defects threaten cytosolic proteostasis. Under physiological conditions, precursor proteins are hardly detectable in the cytosol as they are rapidly imported or degraded. However, adverse conditions can lead to a slow-down of the import process and the accumulation of non-productive translocation intermediates. These can be removed by different mechanisms. Precursor proteins that are stalled in the TOM complex are degraded by the proteasome in a process referred to as mitoTAD. Ubx2 serves as a bridging factor in this process, which connects the TOM complex to Cdc48/VCP/p97, in order to extract the precursors from the TOM channel

and feed them to the proteasome. Missorted outer membrane proteins are recognized and extracted by Msp1, an AAA protein on the mitochondrial surface. Upon accumulation of translocation intermediates that are stalled in the TOM complex, Msp1 is recruited to Tom70 by the bridging factor Cis1. This process is called mitoCPR, and cooperates with mitoTAD-mediated TOM clearance. Ribosomes that are stalled on non-functional mRNAs, and thereby tethered to TOM complexes, are removed by a dedicated machinery, which employs the Cdc48 interactor Vms1, in a process called mitoRQC. If these measures on the mitochondrial surface fail, precursors accumulate in the cytosol, where they sequester chaperones and serve as substrates of the proteasome. If the level of cytosolic precursors exceeds the capacity of the chaperone and proteasome system, cytosolic proteostasis is challenged, leaving cells vulnerable to widespread protein aggregation.

Figure 3, Key Figure. Regulation of the mitoprotein-induced stress response. Mitoprotein-induced stress is countered by a concerted action of several transcription factors. In *C. elegans,* ATFS-1 serves as a major factor in the UPR^{mt}, which mutes the synthesis of mitochondrial proteins to relieve the burden on the mitochondrial import machinery. In yeast the HAP complex plays a comparable role, although the mechanistic details of this are still unclear. The accumulation of precursors in the cytosol leads to an induction of the heat shock response, triggered by HSF1. This attenuates protein synthesis and induces the expression of chaperones. In yeast, HSF1 also induces Rpn4, which serves as master transcription factor for the proteasome-ubiquitin system. NRF2 may play a comparable role in animals. Rpn4 also induces Pdr3, the transcription factor that induces components of the multidrug resistance response, and Cis1, which connects Msp1 to the TOM complex for mitoCPR-mediated TOM clearance. Thus, at least in yeast, the components that trigger the mitoprotein-induced stress response form a reaction cascade, which sequentially activates different programs to maintain proteostasis in both mitochondria and the cytosol.

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Figure 4. Mitoprotein-induced stress elicits different programs depending on its severity and duration. Muting the expression of mitochondrial proteins by ATFS-1 is an elegant mechanism to adapt the amounts of produced precursors to the capacity of the mitochondrial import machinery. This ensures that HSF1 activation is only triggered once the level of precursors exceeds the import capacity. The heat shock response is triggered by the release of HSF1 from chaperones and tailored by chromatin re-organization at HSF1 target promoters. The modification of chromatin state may be particularly relevant for persistently occurring challenges as this may allow cells to respond more effectively to subsequent mitochondrial insults. If serious mitochondrial problems remain over longer periods of time, mitochondria are removed by autophagy/mitophagy and affected cells are eliminated by apoptosis. Both pathways can be triggered by incomplete translocation and, consequently, accumulation of effector proteins on the mitochondrial surface – PINK1 in the case of mitophagy [85], Nde1 in the case of apoptosis [21]. How these drastic reactions are connected to the mitoprotein-induced stress response still awaits to be unraveled.

Figure I for Box 1. Stress signaling from the ER and mitochondria

Glossary

ERAD: *Endoplasmic reticulum-associated protein degradation.* Mediates the removal of proteins from the ER lumen or membrane by proteasomal degradation.

HSR: *Heat shock response.* Signaling pathway that is induced by the accumulation of unfolded or misfolded proteins in the cytosol and/or nucleus. The HSR is triggered by exposure to high temperature but can be induced by any conditions that promote protein misfolding.

mitoRQC: *Mitochondrial ribosome quality control.* Mutated mRNAs can irreversibly arrest translating ribosomes. If these stalled translation intermediates are targeted to

mitochondria, a dedicated machinery recognizes and dissociates them to release the ribosome and degrade the non-productive nascent polypeptides.

mitoCPR: *Mitochondrial compromised protein import response*. Extraction system to remove arrested import intermediates from the TOM complex. Cis1 (together with Tom70) recruits the AAA extractor Msp1 to the TOM complex for back-translocation of precursors into the cytosol.

mitoTAD: *Mitochondrial protein translocation-associated degradation*. Degradation system to remove stalled translation intermediates from the TOM complex. For protein degradation of precursor proteins, the bridging factor Ubx2 recruits Cdc48 and the proteasome to the outer membrane receptor Tom70.

mPOS: *Mitochondrial precursor over-accumulation stress.* Describes the toxic accumulation of mitochondrial inner membrane proteins in the cytosol of yeast cells.

MTS: Mitochondrial targeting sequence or presequence at the N-terminus of mitochondrial precursor proteins. In most cases, presequences are removed after the import reaction by the mitochondrial processing peptidase giving rise to a mature mitochondrial protein.

UPRam: *Unfolded protein response activated by mistargeting of proteins*. Signaling pathway that is induced by mitochondrial precursor proteins which accumulate in the cytosol.

UPR^{ER}: *Unfolded protein response.* Signaling pathway that is induced by the accumulation of unfolded or misfolded proteins in the lumen or the membrane of the endoplasmic reticulum (ER).

UPR^{mt}: *Mitochondrial unfolded protein response.* Signaling pathway that is induced by the accumulation of unfolded or misfolded proteins in the mitochondrial matrix.

ROS: *Reactive oxygen species.* Highly reactive molecules including superoxide, hydrogen peroxide and hydroxyl radicals that are formed by electron transfer to oxygen. Are produced as byproducts by the mitochondrial respiratory chain.

TOM: *Translocase of the outer membrane of mitochondria.* The central pore-forming subunit Tom40 serves as general entry gate for mitochondrial precursor proteins. Receptors such as Tom70 and Tom20/22 recognize cytosolic precursors and direct them to Tom40.

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