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Mitochondrial protein import machinery conveys stress signals to the cytosol and beyond

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Abstract

Mitochondria hold diverse and pivotal roles in fundamental processes that govern cell survival, differentiation, and death, in addition to organismal growth, maintenance, and aging. The mitochondrial protein import system is a major contributor to mitochondrial biogenesis and lies at the crossroads between mitochondrial and cellular homeostasis. Recent findings highlight the mitochondrial protein import system as a signaling hub, receiving inputs from other cellular compartments and adjusting its function accordingly. Impairment of protein import, in a physiological, or disease context, elicits adaptive responses inside and outside mitochondria. In this review, we discuss recent developments, relevant to the mechanisms of mitochondrial protein import regulation, with a particular focus on quality control, proteostatic and metabolic cellular responses, triggered upon impairment of mitochondrial protein import.

KEYWORDS
metabolism, mitochondrial protein import, mitochondrial unfolded protein response, mitophagy, proteostasis

INTRODUCTION

Mitochondrial biogenesis is the process by which the mitochondrial network expands and takes over a larger volume of the cell. Transcriptionally, it is regulated by two genomes that express genes of mitochondrial proteins. These transcripts are translated by cytosolic or mitochondrial ribosomes and unfolded precursors of their protein products are targeted to the organelle through the function of specific protein translocases that reside on the outer and inner mitochondrial membranes. Yeast mitochondria are known to entail around 1000 different proteins, while its mammalian counterparts may contain up to 1500.[1,2] It is known that only 1% of them are encoded by the mitochondrial genome, while the majority are encoded by nuclear genes, translated in cytosolic ribosomes, and then targeted to the organelle. Targeting of mitochondrial precursors may happen post-translationally or co-translationally.[3]

Mitochondria have evolved an elaborate system capable of mediating the import and assembly of precursors to their respective sub-mitochondrial compartments, crossing one or even two membranes.[4–7] The main concepts of protein translocation in mitochondria have been pioneered by *in organello* studies on *Saccharomyces cerevisiae* (budding yeast) mitochondria, as they are readily isolated in adequate amounts and high purity. However, mitochondrial translocases display striking evolutionary conservation across eukaryotes.[8] Recent findings from system biology studies in whole cells (in vivo) have highlighted the mitochondrial protein import system as a critical

signaling hub receiving inputs from other cellular compartments and adjusting its function accordingly. In this review, we shortly describe the well-conserved mitochondrial protein import system of yeast, referring also to the mammalian counterparts. Moreover, we discuss the different levels of regulation that have been revealed for mitochondrial protein import. Finally, we focus on the quality control, proteostatic and metabolic cellular responses elicited by mitochondrial protein import impairment, in yeast and mammals.

Overview of the yeast mitochondrial protein import system

Mitochondrial precursors contain specific sequence signals that navigate them through the import translocases. These signals may be either located at the N-termini of the precursors (presequence) or dispersed internally in the mature part of the protein. The N-terminal presequences comprise 20–60 amino acids and form amphipathic α-helices with a net charge between +3 and +6.[9] These presequences are present both in yeast and mammalian mitochondrial precursors and target them in the mitochondrial matrix where they become cleaved and degraded by specific matrix proteases.[10,11] The internal signals are non-cleavable and target proteins in one of the other compartments.

The outer mitochondrial membrane separates the organelle from the rest of the cell. Therefore, all mitochondrial precursors need to cross this barrier to be properly targeted. The Translocase of the Outer Membrane (TOM) is the main import gate for most mitochondrial precursors. It comprises of the channel-forming subunit Tom40 (TOMM40 in mammals), three receptors that recognize and bind precursors from the cytosolic side of the complex (Tom20/TOMM20, Tom22/TOMM22, and Tom70/TOMM70A), and three small Tom proteins (Tom5/TOMM5, Tom6/TOMM6 and Tom7/TOMM7) that participate in the assembly and the stability of the TOM complex albeit not essential for its functions.[12]

Presequence-containing precursors follow the "presequence" import pathway to target the mitochondrial matrix. Presequences are initially recognized by the Tom20 receptor, and subsequently, there are handed over to the central receptor Tom22 to mediate translocation through the TOM channel. Upon exit from TOM, they are handed over to the Translocase of the Inner Membrane 23 (TIM23) complex of the inner membrane to mediate their targeting to the matrix in an ATP-hydrolysis- and membrane potential ($\Delta\psi$)-dependent manner. The channel is named after its channel forming subunit Tim23/TIMM23, but it also contains Tim17 (TIMM17A and TIMM17B in mammals), Tim50/TIMM50, Tim21/TIMM21, and Mgr2/ROMO1 subunits. Tim17 is required for the pore-forming capacity of Tim23 while Tim50 is involved in the recognition of presequences hanging from the TOM complex.[13] This transition is optimized by the formation of a TOM-TIM23 supercomplex which facilitates the transfer of matrix-targeted preproteins across the IMS.[14] Complete translocation into the matrix requires another complex that interacts with the TIM23 translocon, the PAM motor, which «pulls» the presequence-containing precursors into the matrix. The ATP-driven molecular chaperone mitochondrial Heat shock protein 70 (mtHsp70/HSPA9) is a prominent subunit of the presequence-associated protein motor (PAM). Inner membrane potential ($\Delta\psi$) and ATP-hydrolysis by the membrane bound mtHsp70 drives the inward movement of the precursor, while the soluble form of mtHSP70 is engaged in the folding of the preproteins to their mature conformation. TIM23 translocase may also sort preprotein into the inner membrane. In this case, Mgr2, guides precursors with a bipartite signal (stop transfer hydrophobic signal in addition to the amphipathic presequence) to lateral release into the inner membrane, a function that does not require the PAM motor or ATP hydrolysis, but still depends of $\Delta\psi$ of the inner membrane.[15] The polytopic membrane proteins of the inner membrane which belong to the metabolite carrier family of proteins (ADP/ATP carrier, phosphate carrier, etc.) use a different translocase to be inserted into their target membrane, the translocase of the inner membrane TIM22 complex. It comprises of Tim22/TIMM22, Tim54 and Tim18. Tim22 is the only essential subunit of the yeast TIM22 complex and the only one conserved in mammals.[16] In vitro reconstitution of the TIM22 complex revealed it is a voltage-activated and signal-gated channel, which does not rely on ATP hydrolysis.[16] The clients of the TIM22 complex are highly hydrophobic, as they contain several transmembrane segments, and they are inserted in the inner membrane only when a threshold of membrane potential is achieved. Upon translation, they are bound by cytosolic chaperones of the Hsp70 and Hsp90 classes which deliver them to the Tom70/TOMM70 receptor of the TOM complex.[17] Upon their export from the TOM complex, they are loaded onto the chaperone complexes of the intermembrane space to travel through this aqueous compartment toward their next target the TIM22 complex. These chaperones are heterohexameric complexes of the small Tim protein family and consist of three Tim9/TIMM9 and three Tim10/TIMM10 subunits.[18,19] Another chaperone complex of similar structure, Tim8/TIMM8A-Tim13/TIMM13 mediates the import of some precursors.[20] The small Tim12 protein complexes with the Tim9-Tim10 chaperone and assists in docking of the loaded complex onto the TIM22 translocon, so that the precursor remains protected from aggregation until they reach its final destination.

The small Tim chaperone complexes have been proposed to mediate the transport of outer membrane β-barrel proteins to the dedicated translocase of the Sorting and Assembly Machinery (SAM) of the outer membrane.[21,22] SAM complex consists of a membrane-integrated subunit Sam50/SAMM50 which forms a β-barrel channel into the outer membrane and two peripheral membrane proteins that are exposed to the cytosol, Sam35 and Sam37.[23] The human counterparts of Sam35 and Sam37, Metaxin 2 and Metaxin 1 and 3, respectively, show low sequence similarities.[24,25] The insertion of β-barrel proteins can be facilitated by the formation of a TOM-SAM supercomplex mediated by Tom22-Sam37 interaction.[26] The insertion of α-helical proteins into the outer membrane is less understood. Signal-anchored and polytopic proteins utilize the MIM insertase for their targeting.[27,28] Insertion of the signal-anchored outer membrane protein which faces the IMS is mediated by an alternative pathway that uses the translocases of the presequence import pathway together with the MIM

complex for their proper insertion.[29] However, it has been proposed that some tail-anchored and polytopic proteins are inserted into the OM in a spontaneous, lipid-assisted manner which needs further investigation.[30,31]

The small Tims are a family of essential proteins of the intermembrane space, which bear twin CX3C motifs, and together with other cysteine-rich proteins, utilize the Mitochondrial Intermembrane space import and Assembly machinery (MIA) redox-based machinery to be properly targeted. This system consists of the oxidoreductase Mia40 which works together with the sulfhydryl oxidase essential for respiration and vegetative growth Erv1 (Augmenter of Liver Regeneration -ALR in mammals) to introduce intramolecular disulfide bonds in their substrate precursors.[30,32] After translocation through the TOM complex in a reduced form, these precursors are bound by Mia40 (CHCHD4 in human cells) which forms a transient mixed disulfide intermediate with them. Mia40 binds the incoming substrates by its C-terminal hydrophobic pocket and oxidizes them via its redox-active cysteine pair, which remains reduced at the end of this interaction.[33] Erv1 mediates the reoxidation of Mia40 rendering it ready for another import cycle.[34–36] Electrons coming from reduced substrates are transferred to cytochrome *c* of the respiratory chain or molecular oxygen. In this disulfide relay mechanism these oxidases mediate the formation of intramolecular disulfide bonds within these Cys-rich proteins which stabilize their structure and therefore traps them in the mitochondrial intermembrane space.

The mitochondrial protein import machineries of yeast and mammalian mitochondria have been previously described in excellent reviews.[4,5,8,12,30,32] In the following sections, we will provide examples of how this delicate system is regulated by intracellular or external cues and we will describe the stress signals initiated by mitochondrial protein import impairments.

Regulation of the mitochondrial import system

Mitochondria protein import is a central regulatory hub that fine-tunes both mitochondrial and non-mitochondrial cellular functions. Therefore, it is imperative that it is itself carefully regulated to adjust its functions to the changing cellular environment (i.e., nutrient availability, stress signals, hormonal cues, cell cycle stage etc.). During the last decade several levels of regulation have been revealed. In the next section, we will discuss the regulatory mechanisms assisting mitochondrial translocases to balance their function with extra-mitochondrial and mitochondrial cues.

Post-translational modifications of translocase components or preproteins

Post-translational modifications of mitochondrial translocation machinery subunits hold a prominent role in this process (Table 1). The TOM translocase, as the main entry gate of the system, is a major target of regulatory PTMs. More than 30 phosphorylation sites were identified in vitro in Tom subunits.[37] Genetic ablation of the cytosolic casein kinase 2, CK2, in temperature sensitive *ck2* mutant yeast cells, significantly impaired both the biogenesis of TOM complex and its function, highlighting the importance of phosphorylation in mitochondrial protein import regulation.[37] CK2 constitutively phosphorylates Tom22 positions Ser44 and Ser46 thereby promoting its recognition by Tom20 and efficient import of phosphorylated Tom22 into the TOM complex.[37] Moreover, CK2 phosphorylates Mim1 protein channel at positions Ser14/16 assisting assembly of Tom70 and Tom20 but not Tom22 receptor.[37] The latter is thought to follow a Mim1-independent import route.[27]

The metabolic state of the cell is signaled to the TOM channel through two different kinases, the mitochondria-bound casein kinase 1 (CK1) and the protein kinase A (PKA). Yeast cells respond to the presence of glucose in their environment by shifting to fermentation for production of energy and regeneration of the NAD+ coenzyme pool. During fermentation glucose is reduced to pyruvate and then to ethanol and carbon dioxide, while mitochondrial respiration rate is reduced. In the presence of glucose, a fraction of CK1 translocates from the cytosol to mitochondria where it is anchored onto the outer mitochondrial membrane facing the cytosol, in a palmitoylation dependent manner.[38] CK1-dependent phosphorylation of Tom22 at position Thr57 under fermentative conditions, promotes Tom22 assembly into the TOM complex and interaction with Tom20.[38] Glucose-induced PKA negatively affects biogenesis and activity of TOM components. Specifically, PKA, upon stimulation by glucose and cAMP level increase, impairs biogenesis of Tom40 precursor protein by phosphorylation on Ser54. This site is not accessible by PKA in the mature and assembled Tom40.[39] Moreover, PKA phosphorylates Tom70 at position Ser174, impairing import of mitochondrial metabolite carriers into the inner mitochondrial membrane[37] and Tom22 at Thr76 to attenuate its assebly.[38]

Phosphorylation seems to critically regulate the mitochondrial protein import also in human cells. Recently it was shown human TOMM70 receptor is phosphorylated at Ser91 by DYRK1A, a modification that activated its assembly into the TOM complex, thereby promoting the metabolite carrier import pathway.[40] Inhibition of DYRK1A leads to decreased respiration rate, aberrant mitochondrial network and cell growth retardation. Interestingly, DYRK1A initiates a likely transient protective transcriptional response which increased the overall levels of several import components like TOMM70, TOMM20, and TOMM22, while TOMM40 levels remained unaffected. Induction of this response upon DYRK1A inhibition partially rescued the efficiency of the carrier import pathway while simultaneous knock-down of *TOMM70* with DYRK1A inhibition led to severe impairment of the metabolite carrier pathway.[40]

Apart from phosphorylation other types of post translational modifications have been shown to regulate mitochondrial protein import pathways. Interestingly, overexpression of the ubiquitin-ligase Parkin in human cells expressing a biosensor for mitochondrial protein import rate showed increased protein import rate through the presequence pathway, independently of mitophagy induction or PGC1α-induced biogenesis.[41] Ubiquitination has also been proposed to regulate

TABLE 1 Post-translational modifications regulating mitochondrial protein import in different species

Organism	Subunit	Condition	Modification	Modifying enzyme	Result on protein import	Reference
Yeast	Tom40	Glucose-rich medium	Phosphorylation Ser54	PKA	Impaired assembly	[39]
	Tom22	Basal	Phosphorylation Ser44/46	CK2	Proper assembly and interaction with Tom20	[37]
	Tom22	Fermentable medium	Phosphorylation Thr57	Palmitoylated CK1	Proper assembly	[38]
	Tom22	Fermentable carbon source	Phosphorylation Thr76	PKA	Impaired assembly and interaction with Tom20	[38]
	Tom70	Fermentable medium	Phosphorylation Ser174	PKA	Impaired import of metabolite carriers	[37]
	Mim1	Basal	Phosphorylation Ser12/16	CK2	Proper import of Tom20 and Tom70	[37]
	IMS preproteins	Basal	Ubiquitination	Ubiquitin proteasome system	Proteasomal degradation	[42]
Mouse	Preproteins	Basal	Ubiquitination	MARCH5	Accumulation in cytosol-proteasomal degradation	[43,44]
	Preproteins	Basal	Deubiquitination	UPS30	Proper Import	[43,44]
Human	TOMM70	Basal	Phosphorylation Ser91	DYRK1A	Proper Assembly	[40]
	Membrane targeted precursors	Import failure	Ubiquitination	E3 ligase		[91]

the import capacity of specific precursors. In yeast, the ubiquitin-proteasome system determines the levels of intermembrane space preproteins targeted to mitochondria.[42] Specifically, it was shown that proteasomal inhibition enhances mitochondrial localization of Mia40 substrates. In mice, E3 ubiquitin ligase MARCH5 and a deubiquitinase UPS30 regulate the import of specific precursors at the TOM complex under basal conditions. Ubiquitinated precursors accumulated in the cytosol while deubiquitinated ones are properly translocated.[43] UPS30 was found to physically interact with a fraction of the TOM complex. Upon UPS30 genetic of pharmacologic impairment, MARCH5 drives degradation of UPS30 substrates by the proteasome.[43,44]

A growing body of evidence suggests that mitochondrial proteolysis is another node of regulation for several mitochondrial functions.[45] Mitochondrial protein import components are targets of proteolysis by mitochondrial proteases, or mitoproteases. For example, the ATP-dependent AAA+ zinc metalloprotease YMEL1, localized to the inner mitochondrial membrane limits mitochondrial protein import by mediating cleavage of TIMM17A subunit of TIM23 complex in several human cell lines.[46] YMEL1-dependent proteolysis of TIMM17A is induced by hypoxia, nutrient starvation and protein translation attenuation in the cytosol.[46,47] Interestingly, oxidative stress was shown to trigger YMEL1 degradation by the ATP-independent mitochondrial protease OMA1, thereby triggering cell death,[48] while OMA1 is degraded by the ATP-dependent YMEL1 upon $\Delta\psi$ diccipation.[49]

Mitochondria—ER contact sites

Mitochondria can form physical linkages with the ER mediated by tethering proteins. These molecular bridges that bring the two organelles in close proximity, are enriched in the mitochondria-associated membranes of ER (MAMs),[50] entail a high variety of ER and mitochondrial proteins and have been shown to regulate cellular homeostatic mechanisms, like Ca+2 homeostasis, lipid metabolism and mitochondrial quality control.[51,52] Although mitochondrial protein import has been extensively studied, targeting of mitochondrial precursors toward the organelle is less understood.[3] Increasing evidence suggest that the surface of endoplasmic reticulum (ER) is relevant to the targeting of certain precursors. In yeast, cooperation between an ER surface surveillance pathway, termed ER-SURF, with mitochondria has been shown to facilitate the import of Oxa1 precursor of the inner mitochondrial membrane and is proposed to enhance the import rate of mitochondrial receptors.[53,54] These findings are consistent with the fact that precursors of mitochondrial membrane proteins are preferentially translated on the ER surface.[55] The ER surface chaperone Djp1, which belongs to the J-protein/Hsp40 cochaperone family, is shown to genetically interact with Tom70 receptor to ensure proper localization of the precursor.[53]

In human cell lines, TOM complex participates in the ER-mitochondria contact sites by interaction with the ER protein B-cell

receptor-associated protein 31 (BAP31).[56] BAP31 is an integral membrane protein highly enriched on the ER surface, where it was known to constitute a complex with several apoptosis regulators, like Bcl-2, Bcl-XL, and procaspace-8L isoform.[57,58] Upon apoptosis, BAP31 is being cleaved, and its p20BAP31 product which remains anchored on the ER membrane further transmits the apoptotic signal. Moreover, BAP31 was shown to physically interact with mitochondrial fission protein Fis1 in mammalian cell lines, a complex that is known to transmit death signals from mitochondria to the ER, leading to procaspace 8 activation.[59] Recently, it was shown that BAP31 maintains mitochondrial homeostasis and respiration rates by physical interaction with TOMM40 subunit on mitochondria. NADH:ubiquinone oxidoreductase core subunit 4 (NDUFS4) and NADH:ubiquinone oxidoreductase subunit B11 (NDUFB11) are Complex I subunits and localize to the inner mitochondria membrane. Both subunits are binding-partners of BAP31 presumably as preproteins. ER stress (tunicamycin treatment) decreases the levels of BAP31 on MAMs, leads to BAP31-TOMM40 complex collapse and decrease mitochondrial import of NDUFB11 and NDUFS4 subunits of Complex I, lowering respiratory capacity of mitochondria.[56] Moreover, Bcl-2 can migrate from the ER to mitochondria through interaction with TOMM20 which is enabled by the existence of MAMs.[60] It has been suggested that this translocation could be bidirectional, however it needs further investigation.

Collectively these findings suggest that the close collaboration of mitochondrial protein import system with ER proteins promotes proper targeting of certain proteins under steady state conditions or upon specific stimuli. Further investigation is required to identify the aims and molecular players of such an interaction across species.

Lipid composition of mitochondrial membranes

Phospholipid composition of cellular membranes plays a critical role in the assembly, stability and function of membrane proteins and protein complexes. Mitochondrial protein import translocases are not an exception. Mitochondrial membranes have a rather unique phospholipid composition and differ significantly from each other. Phospholipids are the main lipid constituents of the membrane but they also house sterols and sphingolipids. Moreover, the protein content of each membrane differs remarkably as the outer mitochondrial membrane has a low protein content whereas the inner membrane is the most protein-dense membrane of the cell.[61] The inner mitochondrial membrane is the almost exclusive location of cardiolipin (CL) and its precursor phosphatidylglycerol (PG) which are synthesized by mitochondrial enzymes. Phosphatidylethanolamine (PE) is synthesized in yeast mitochondria, ER and Golgi apparatus and has been suggested to have redundant functions with CL. Simultaneous knock-down of mitochondrial cardiolipin synthase 1 (crd1) and phosphatidylserine decarboxylase 1 (psd1), the enzyme that catalyzes mitochondrial PE formation leads to synthetic lethality phenotype, suggesting that the "in house" PE synthesis cannot be complemented by the ER or Golgi pools.[62] On the contrary, mitochondrial PE synthesis contributes to cellular PE homeostasis.[63] Functional assays showed that both CL and PE are required for proper TOM and SAM complex assembly and thereby for the proper targeting of β-barrel and some α-helical precursors of the outer membrane.[64,65] In yeast, earlier studies showed the translocator and maintenance protein Tam41 is required for CL synthesis as well as mitochondrial protein import via TIM23 and TIM22 translocases.[66,67] Notably, early UPR^{mt} stress caused by a temperature sensitive mutation of MPP peptidase, triggered increase of protein import rate for all the main translocases accompanied by rapid CL remodeling.[68] Structural analysis by single particle cryo-electron microscopy of the yeast and human TOM channels corroborated these findings and showed that the pore is formed by two TOM40 β-barrel proteins tethered together by two TOM22 subunits in between them and one phospholipid with its head facing the IMS.[69,70] Although it was not clear which phospholipid participates in the formation of the pore, it is evident that its association further stabilizes the Tom40 β-barrel. Moreover, the bilayer-forming phospholipid phosphatidylcholine (PC) is required for the stability and function of both the SAM complex and the TIM23 translocase, while it is dispensable for TIM22 complex, in yeast.[71,72] Lipid metabolism is very responsive to several types of stress. Oxidative stress leads to abnormal remodeling of CL through the lyso-CL acyltransferase ALCAT1 in mammalian cell lines and mice.[73,74] Heat stress is able to decrease the unsaturation index and increase the chain length of mitochondrial phospholipids and free fatty acids in the small mammal *Meriones unguiculatus*.[75] The effects of hypoxia and Hypoxia induced transcription factors on lipid remodeling has recently started to emerge.[76,77] It is therefore expected that the mitochondrial import systems could mediate signals from remodeled membrane lipids adjusting their function accordingly. Further studies are needed to decipher the role of lipid remodeling under different stresses on mitochondrial protein import and assembly.

Unconventional roles of Tom70

Apart from their role in mitochondrial protein import certain subunits of the mitochondrial import translocases have unconventional roles supporting several aspects of mitochondrial homeostasis. In yeast, Tom70 and Tom71 receptors facilitate the localization of lipid transport at contact site 1 (Ltc1) protein to the ER-mitochondria contact sites possibly to support transport and/or sense of sterols.[78] In mammalian cells TOMM70 depletion dampens Ca^{+2} flux from ER to mitochondria.[79] The reason behind this phenomenon was that TOMM70 recruits the ER protein IP3R3, responsible for Ca^{+2} transfer to mitochondria, in MAMs.[79] Recently, another unexpected function has been reported for yeast Tom70. It was found to participate in the transcriptional activation of mitochondrial components.[80] Interestingly, Tom70-dependent transcriptional program participated in the cellular response to mitochondrial import defect, triggered by temperature-sensitive *tim23* depletion.[80] The exact mechanism through which Tom70 participates in transcription regulation remains to be revealed. Finally, the conventional role of Tom70 has recently been revisited.[81] Specifically, it was proposed that its primary role is

to bind cytosolic chaperones rather than serve as TOM receptor for TIM22-destined substrates.[81] All these findings suggest that Tom70, independently of its role in protein import, is found at the cross-road of several pathways that interact and adjust the function of the mitochondrial import machinery.

Stress response pathways associated with impaired mitochondrial protein import

It is becoming increasingly evident that the mitochondrial protein import system, apart from its role in import and assembly of mitochondrial proteins, it functions as a sensor of cellular stress which adapts its activity accordingly. What happens when the mitochondrial protein import machinery fails? How does the cell cope with this stress? In the following section we will discuss stress-response pathways activated upon failure of the main mitochondria protein import translocases. Implications on cellular and organismal physiology will be discussed.

Proteostatic effects of impaired mitochondrial protein import

Since protein import is a major determinant of mitochondrial biogenesis, it is conceivable that its inhibition would confer proteotoxic effects both inside and outside mitochondria, as precursors that fail to be targeted will accumulate in the cytosol or on the mitochondrial translocases. Extensive investigation of this phenomenon in budding yeast has characterized several cytosolic and mitochondrial responses to impaired mitochondrial translocation[82–87] (Figure 1).

An RNAseq screening and quantitative proteomics of temperature sensitive *Mia40* mutants revealed decreased cytosolic ribosome proteins and proteins used in translation as well as a reduction in protein synthesis.[86] Under basal conditions, the ubiquitin-proteasome system (UPS) mediates the degradation of a fraction of Mia40 substrates which retro-translocate to the cytosol through the TOM40 pore.[42,88] *Mia40* mutants exhibit reduced protein translation and increased proteasomal assembly and activity, to counteract the increased cytosolic burden of mitochondria precursors. This protective response pathway is termed the unfolded protein response activated by mistargeting of proteins (UPRam).[86] Similar results were obtained in a yeast mutant of the ADP/ATP carrier (*AAC2*), a TIM22 substrate that is involved ATP/ADP exchange across the inner membrane. Although AAC2 is not a mitochondrial protein import component, its depletion affects inner membrane $\Delta\psi$ and ATP availability thereby compromising mitochondrial protein import. *aac2* mutants exhibit age-related mitochondrial depolarization, over-accumulation of unimported precursors in the cytosol, accumulation of the unprocessed Hsp60 chaperone in the matrix and progressive loss of the cells' proliferative capacity. This phenomenon leads to a distinct type of cell death called mitochondrial precursor over-accumulation stress (mPOS). In a genetic screen for suppressors of this type of cell death, several genes that regulate different aspects of cytosolic protein homeostasis, like target of rapamycin signaling (TOR), ribosomal function/translation, mRNA turnover/silencing, and ribosome-associated chaperoning, were identified.[85,89] Among them, Gis2, the yeast orthologue of human ZNF9/CNBP, which stimulates CAP-independent translation and the Nog2, inhibitor of nuclear export of 60S ribosomal subunit, are required for protection against mPOS-induced cell death.[85] Mammalian cells may experience mPOS upon overloading of mitochondrial carrier precursors.[90] Upregulation heat shock response and formation of large aggresomes in the cytosol, which are destined to proteasomal degradation, seem to protect the cells against mPOS-induced cell death.[90]

Overexpression of artificial precursors that clog the import pore is a more physiological way of modeling an overwhelmed protein import machinery. Interestingly, overexpression of clogger precursors can block the majority of import sites in yeast mitochondria and trigger an adaptive transcriptional response program. This program entails early activation of the heat shock response genes, via transcription factor Heat shock factor 1 (Hsf1), Rpn4-dependent activation of the Ubiquitin-proteasome system and silencing of the OXPHOS-related genes via inactivation of the multimeric transcription factor HAP.[82] This transcriptional response mediates proteostatic maintenance in the cytosol, and reduction of extra workload for the pores by reducing the number of precursors that need to cross them (Figure 1).

Cytosolic chaperones have a critical role in maintaining proteostasis also in mammalian cells. The ubiquilin protein family comprises cytosolic chaperones that bind on transmembrane domains of preproteins and prevent their aggregation while in the cytoplasm. Ubiquilins form a complex with the hydrophobic, aggregation-prone mitochondrial membrane protein precursors to allow for opportunities of successful import. However, upon import failure, ubiquilins recruit E3-ligases to ubiquitinate their client proteins directing their proteasomal degradation, thereby safeguarding cytosolic proteostasis[91] (Figure 1).

Interestingly, it was shown that import defects due to aging, pharmacologic depletion of inner membrane potential or Tom40, depletion may result in accumulation of unimported mitochondrial precursors in other cellular compartments, apart from the cytosol, like the ER and the nucleus, in yeast cells.[92,93] Certain mitochondrial precursors, like the mitochondrial carrier proteins of the inner membrane, utilize the GET pathway for their ER targeting, which normally facilitates ER targeting of tail-anchored proteins to the ER.[94] Other ER targeted mitochondrial precursors may not require the GET complex for their ER localization suggesting that alternative pathways exist. Mislocated mitochondrial precursors likely serve as targets of the ER-SURF pathway, for their proper mitochondrial targeting. Nuclear-targeted precursors are cleared through ubiquitination and proteasomal degradation, in a so called nuclear-associated mitoprotein degradation pathway (mitoNUC)[92] (Figure 1).

We described mechanisms of cytosolic adaptation to mitochondrial import stress. But how do mitochondria cope with their clogged pores? A mitochondria degradation system for clogged import pores has been revealed in yeast.[84] This system termed mitochondrial protein translocation-associated degradation (mitoTAD) requires the

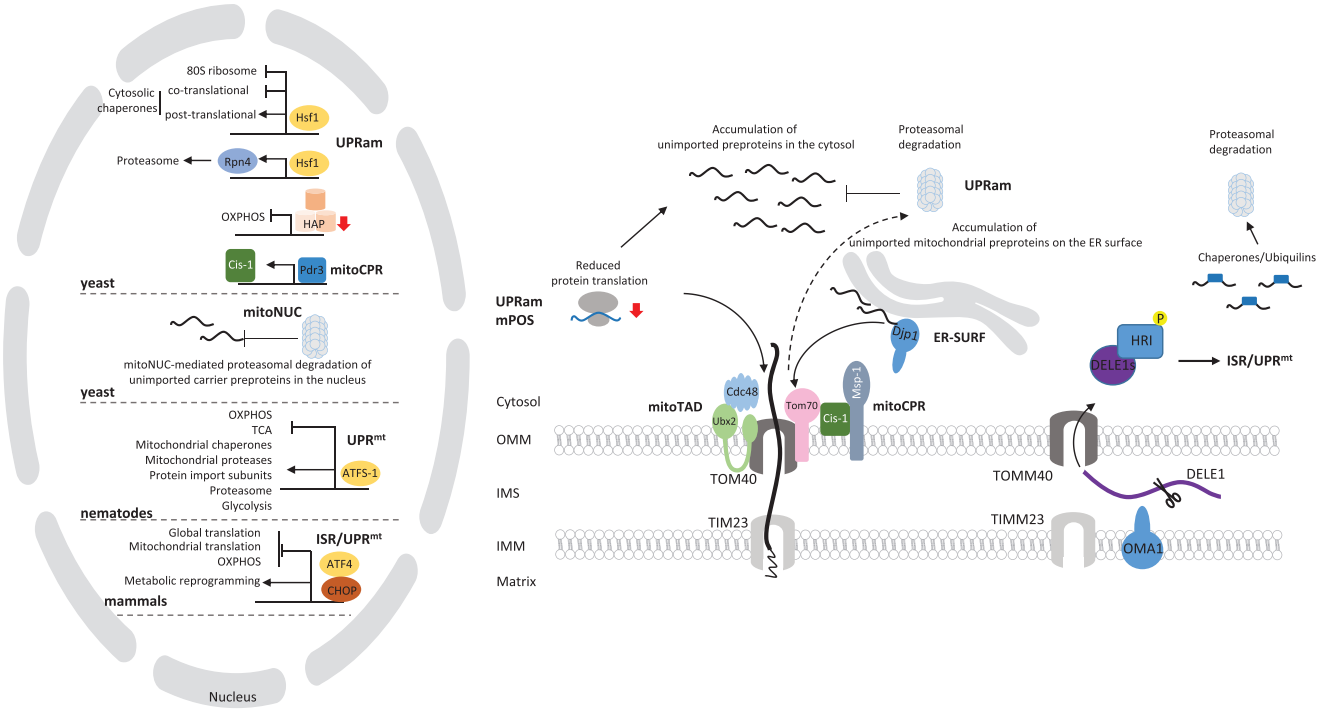

FIGURE 1 Proteostatic responses under the control of mitochondrial protein import system. Challenged mitochondrial protein import impairs the entry of newly synthesized preproteins into mitochondria and enhances their accumulation in the cytosol, the ER, and the nucleus of yeast cells. Unimported integral inner membrane and outer membrane proteins localize on the ER surface and their proper targeting is likely facilitated by the ER-SURF pathway, whereas, unimported matrix-localized enzymes are being degraded in the nucleus, by the mitoNUC pathway. Cytosolic accumulation of precursors of mostly soluble mitochondrial proteins triggers the unfolded protein response activated by mistargeting of proteins known as UPR^{am}, encompassing inhibition of protein synthesis and enhanced proteasomal activity. Clogging of the import machinery with stalled or overexpressed preproteins triggers a Ubx2-dependent Cdc48 recruitment (mitoTAD) and a Msp1-dependent Cis1 recruitment (mitoCPR) on mitochondria to mediate removal and proteasomal degradation of stalled preproteins. Transcriptional responses involved in adaptation to protein import stress entail the heat shock factor 1 (Hsf1) which activates expression of post-translational cytosolic chaperones and Rpn4-dependent expression of proteasomal subunits while repressing ribosomal factors and co-translational chaperones involved in protein translation. Reduction of the HAP complex leads to reduced expression of oxidative phosphorylation (OXPHOS) components. The transcription factor mediating multi-drug resistance, Pdr3, activates *CIS1* expression which executes mitoCPR response. In *C. elegans*, mitochondrial protein import stress leads to relocation of ATFS-1 transcription factor to the nucleus to activate the unfolded protein response (UPR^{mt}) activating mitochondrial and cytosolic proteostasis genes, protein import components and glycolytic enzymes while repressing mitochondrial respiration. In mammals, preproteins translated in free ribosomes are bound by cytosolic chaperones (ubiquilins) to mediate either their binding to the receptors of their respective translocase or their ubiquitination and degradation by the proteasome. Presequence import pathway impairment activates the mitochondrial protease OMA1 which cleaves DELE1, facilitating the translocation of a proteolytic fragment (DELEs) to the cytosol. Cytosolic DELE1 binds and activates the integrated stress response (ISR) heme-regulated inhibitor (HRI) kinase leading to its autophosphorylation and activation of the ATF4-CHOP-depedent transcriptional response.

ER-associated degradation (ERAD) component Ubx2.[95] Interestingly, at basal conditions, a fraction of Ubx2 co-purifies with several components of the TOM machinery but not with other outer membrane proteins of mitochondria.[84] Mitochondria localization of Ubx2 depends on Tom70 receptor and Mim1 channel. Upon import stress, Ubx2 recruits Cdc48 complex onto clogged pores and mediates the removal and proteasomal degradation of the stalled precursors[84] (Figure 1).

In both yeast and mammals, stalled ribosomes interact with a set of ribosome-associated quality control components (RQC) that mediate the dissociation of the faulty peptide from the ribosome and its subsequent proteasomal degradation.[96] Ubiquitination and proteasomal degradation of these peptides is facilitated by the C-terminal addition of peptides of various lengths consisting of alanine and threonine residues, known as CAT-tails. However, CAT-tails have been shown to induce aggregation of faulty polypeptides in case of impaired proteasomal degradation. Precursors that are imported co-translationally in mitochondria can also stall the ribosome due to nonstop mRNA defects.[96] Cdc48, a component of the mitoTAD pathway, interacts with the cytosolic protein Vms1 which is a component of the ribosome-associated quality control pathway on the outer surface of mitochondria (mitoRQC). Vms1 was shown to inhibit the addition of CAT-tails thereby preventing their aggregation after their release from the ribosome inside mitochondria and sequestration of mitochondrial chaperones and translation machinery.[97] Since the stalled peptides are eventually released from the import pores under normal conditions, these procedure does not severely attenuate the import capacity. However, under conditions of impaired ubiquitination and degradation, Vms1 rescues the faulty polypeptides from aggregation allowing

the mitochondrial quality control proteins to remove them (Figure 1). Within mitochondria, degradation of misfolded and unassembled small Tim proteins is mediated by Yme1l.[98]

Overexpression of non-defective mitochondrial precursors may also challenge the mitochondria protein import system. The majority of them are not stalled in the translocases. However, precursors with bipartite signal, that use a stop-transfer mechanism of import and are laterally released in the inner membrane via TIM23, can clog the translocases when overexpressed.[87] This type of stress, termed mitochondrial compromised protein import response (mitoCPR), initiates a Pdr3-dependent transcriptional response that entails the expression of multi-drug resistance genes, encoding ABC transporters, lipid metabolism-related protein and NADPH-dependent enzymes among others. The same response was observed in mutants with constitutive impairment of the import machinery.[87] *PDR3* expression was required for maintenance of mitochondrial functions upon import stress. Notably, the Pdr3 target *CIS1*, which is expressed and localized on mitochondria upon stress, mediates the clearance of unimported precursors, by interaction with the dislocase Msp1 known for its role in extraction of mistargeted ER and peroxisomal proteins from the mitochondrial outer membrane.[99] Interestingly, Hsf1-dependent expression of heat shock response genes and Rpn4-depedent transcription of proteasomal genes underline key processes of UPRam, while Rpn4 is involved in *PDR3* upregulation upon mitoCPR.[86,87] These findings suggest that these processes may be interconnected under the control of master regulators of transcription[82] (Figure 1).

Mitochondrial unfolded protein response (UPRmt)

In higher eukaryotes mitochondrial protein import perturbations initiate a similar response to those described in yeast, the mitochondrial unfolded protein response (UPR^{mt}). Initially, this pathway was described as response to mitochondrial proteotoxic stress sensed by the matrix protease ClpP, which cleaves the accumulated unfolded proteins and creates peptide signals to the nucleus.[100] UPR^{mt} was traditionally viewed as an adaptive induction of mitochondrial chaperones and proteases to mitochondrial proteotoxic burden.[101] Currently, it is known that UPR^{mt} is triggered by a variety of mitochondrial insults which likely converge on protein import impairment.[102,103] In *Caenorhabditis elegans* UPR^{mt} is activated mainly through the action of the basic leucine zipper (bZIP) transcription factor ATFS-1.[104] Recent findings on ATFS-1 shed light into the triggering event of UPR^{mt}. ATFS-1 contains a weak N-terminal mitochondrial targeting signal (MTS), followed by a nuclear localization sequence (NLS). A robust protein import system supports the import of ATFS-1, despite its weak MTS, into the mitochondrial matrix, where it gets degraded by the LON protease.[102,105,106] However, upon slight perturbation of the import machinery, ATFS-1 can no longer be imported and accumulates in the nucleus to activate the transcriptional response. This perturbation may be transient and/or timely, as the intense import of newly synthesized precursors in an expanding mitochondrial network.[105] UPR^{mt} regulates a range of genes from mitochondrial and cytosolic proteostasis components to glycolytic enzymes and innate immunity-related genes.[107,108] Interestingly, UPR^{mt} induces gene expression of mitochondrial import components, likely to support import of the required chaperones/proteases.[106,108]

Similarly, to the responses described in yeast, UPR^{mt} activates proteasome function and suppresses the expression of OXPHOS and TCA components, possibly to decrease the load of the challenged import machinery and to prevent an additional stress deriving from dysfunctional respiration.[108,109] Thereby, mitochondria have the time and tools to correct the damage before getting back to work. In nematodes, prolonged suppression of the main import translocases TOMM-40 and TIMM-23 throughout development and adulthood leads to reduction of the cellular mitochondrial load and extends lifespan of nematodes.[109,110] However, reduction of cellular mitochondrial load through mitochondrial protein import system suppression (MitoMISS) does not exhibit a robust proteotoxic response in the cytoplasm, while it induces the mitochondrial proteostatic response that characterizes UPR^{mt}.[110] Interestingly, mitochondrial Hsp60 induction is not required for the beneficial effects of MitoMISS on longevity.

In mammalian cells an Integrated Stress Response (ISR) pathway responses to various type of stresses, by tuning protein synthesis rates and gene expression. These responses are mediated by four main kinases that phosphorylate the eukaryotic translation initiation factor eIF2 in its alpha subunit. These are the general control nonderepressible 2 (GCN2), the protein kinase R (PKR), the protein kinase R-like endoplasmic reticulum kinase (PERK), and the heme-regulated inhibitor (HRI). The kinases respond to different stressors like amino acid deprivation, viral infection, ER-related proteotoxic stress, and mitochondrial stress, respectively. Depending on the intensity and the duration of the stress stimulus, ISR may convey adaptive, pro-survival signals that restore homeostasis or lead to apoptotic cell death.[111,112] The ISR branch of mitochondrial stress is often referred to as the mammalian UPR^{mt}. Mitochondrial stress in mammals is known to lead to upregulation of the CCAAT/enhancer binding protein (C/EBP) homology protein CHOP and the activating transcription factor 4 (ATF4).[113] Recently, it was shown that disruption of CHOP amplifies the ATF4-driven ISR and aggravates the disease phenotype type in a mice deficient in the mitochondrial aspartyl transfer RNA (tRNA) synthase DARS2 specifically in heart and skeletal muscle.[112]

Several types of mitochondrial stress may lead to CHOP and ATF4 activation. Mitochondrial membrane depolarization stress severely impacts mitochondrial import routes to the mitochondrial inner membrane and matrix. In human mitochondria, it was shown that treatment with the mitochondrial ionophore CCCP (carbonyl cyanide m-chlorophenyl hydrazone) that disrupts mitochondrial membrane potential, causes activation of OMA1 protease and subsequent processing of the mitochondrial protein DELE1 allowing for redistribution of one DELE1 proteolytic fragment to the cytosol. Upon redistribution, this short form of DELE1 (DELE1s) binds to HRI leading to its autophosphorylation and induction of CHOP- and ATF4-dependent ISR[114–116] (Figure 1). Targeting of DELE1 in human mitochondrial is mediated by the presequence import pathway similar to the route of ATFS-1 in *C. elegans* mitochondria.[116] Interestingly, depletion of

TOMM40 or TIMM23 translocases leads to accumulation of the unprocessed DELE1 in the cytoplasm which can still interact with HRI and activate CHOP. Thus, processing of DELE1 is not necessary for ISR activation.[116]

Contrary to the UPR^{mt} induced in nematodes and flies and the cytosolic response to mitochondrial stress in yeast, the heat-shock proteins (i.e., cytosolic HSP70 or mitochondrial HSPD1) are not a target of the DELE1-HRI pathway.[114,115,117] Specifically, it was shown that blockade of the OMA1-DELE1-HRI pathway upon mitochondrial stress blunts the expression of ATF4 and CHOP targets, whereas it leads to activation of heat shock protein expression.[114,115] The latter suggests that the transcriptional outcome upon mitochondrial protein import perturbations may vary depending on the trigger, and the molecular players involved. Amongst the most prominent ATF4 targets, upon mitochondrial import stress, are enzymes involved in one carbon metabolism, serine biosynthesis and transulfuration.[112,118]

A different type of stress derives from the accumulation of misfolded proteins within the organelle. Similar to nematodes and flies, induction of misfolded protein burden within mammalian mitochondria by overexpressing misfolded mitochondrial-matrix-localized ornithine transcarbamylase or pharmacological inhibition of mitochondrial HSP90/TRAP1 or LON protease induces a mitochondrial proteostatic response encompassing the expression of mitochondrial chaperonins HSPD1/HSP60 and HSPE1/HSP10 along with pre-RNA processing and mitochondrial translation components.[119] Moreover, this type of proteotoxic stress is independent of individual ISR kinases, inferring that the type of the stimulus for UPR^{mt} induction can differentiate the transcriptional outcome.

Mitophagy

Mitophagy is a mitochondria-specific type of autophagy that was initially described in mammalian cells by electron microscopy studies that suggested lysosomal sequestration of mitochondria upon starvation or glucagon-triggered catabolism.[120] Since then, it became clear that mitophagy mediates clearance of damaged or superfluous mitochondria under basal or stressed conditions across eukaryotes.[121–123] Several mechanisms of mitophagy have been described in different cell types and upon different stimuli. The most studied pathway is the PINK1/Parkin pathway. PINK1 is a Ser/Thr kinase that is constitutively being imported into the inner membrane of healthy mitochondria where it is being cleaved by PINK1/PGAM5-associated rhomboid-like protease (PARL) and subsequently retro-translocated to the cytoplasm to be fully degraded by the proteasome.[124–126] Upon $\Delta\psi$ dissipation, PINK1 can no longer be imported and accumulates on the outer mitochondrial membranes in a Tom7-dependent manner.[125] When on the mitochondrial surface, PINK1 is autophosphorylated at Ser228 and Ser402[127] and subsequently it phosphorylates Ser65 of the ubiquitin molecule on several basally ubiquitinated surface mitochondrial proteins. Ser65-ubiquitin has a high affinity for Parkin and recruits it from the cytosol onto mitochondria. Interaction of Parkin with the ubiquitinated substrates activates its E3-ligase function enabling it to ubiquitinate more targets on the mitochondrial surface, reinforcing the mitophagy signal in a positive feedback loop.[128] Ser65 ubiquitin chains on the damaged mitochondrion stimulate the initiation of mitophagy process. When Tom7 is absent, PINK1 is still imported in mitochondria even when $\Delta\psi$ is impaired, it is processed by the IMM-embedded metalloprotease OMA1 and then degraded by the proteasome.[125] Recently, it was shown that PINK1-dependent mitophagy can be activated by accumulation of misfolded protein into the matrix, which leads to sequestration of soluble PAM subunits and blocks the presequence import pathway, leading to PINK1 activation, in mammals[129] (Figure 2).

Apart from the PINK1/Parkin pathway, mitophagy may be initiated by mitophagy receptor proteins on the mitochondria outer membrane, such as Atg32 in yeast, and NIX/BNIP3, or FUNDC1 in mammals. The protein import machinery also plays a role in this process. Mim1/Mim2 complex and Tom70 in fission yeast *Schizosaccharomyces pombe* are crucial for starvation induced mitophagy, likely by mediating the import of the mitophagy receptor Atg43[130] (Figure 2). In mice, mitochondrial protein import stress has recently been reported to induce LC3 recruitment and lipidation in the vicinity of mitochondria.[131] LC3 is PE-conjugated on the surface of autophagosomes and is a critical player of autophagy/mitophagy as it contributes to cargo loading and autophagosome closure.[132,133] In this pathway of mitophagy, dissipation of inner membrane potential leads to relocation of the matrix-targeted Nod-like receptor (NLR) protein NLRX1 to the cytosol, where it interacts with a ribosome-binding transmembrane protein of the ER, RRBP1. The formation of the NLRX1/RRBP1 complex triggers LC3 lipidation and mitophagy induction.[131]

Mounting evidence suggests that mitochondria protein import machinery may direct mitochondrial fate by supervising the import of several mitophagy components. The impact of this process on cellular physiology is context specific. For example, removal of damaged mitochondria is essential for the maintenance of a healthy mitochondrial pool and neuronal viability.[134] However, the uncontrolled runaway mitophagy negatively impacts mitochondria content and can prove detrimental for cellular and organismal viabillity.[135] Future studies should investigate the potential of mitochondrial protein import to regulate mitophagy to the benefit of the cell, during aging and in disease background.

Metabolic adaptations to mitochondrial protein import stress

Besides the extensive investigation on proteostatic responses upon protein import stress, increasing evidence reports a concomitant metabolic adaptation to perturbed mitochondrial import. Mitochondria are major metabolic hubs for the cell, therefore it is anticipated that blocking mitochondrial protein import would greatly impact cellular metabolism. In *C. elegans*, transcriptomic analysis of UPR^{mt} triggered upon mitochondrial proteotoxic stress, points to a rewiring of cellular metabolism towards glycolysis and amino acid catabolism with concomitant reduction of tricarboxylic acid (TCA) cycle and

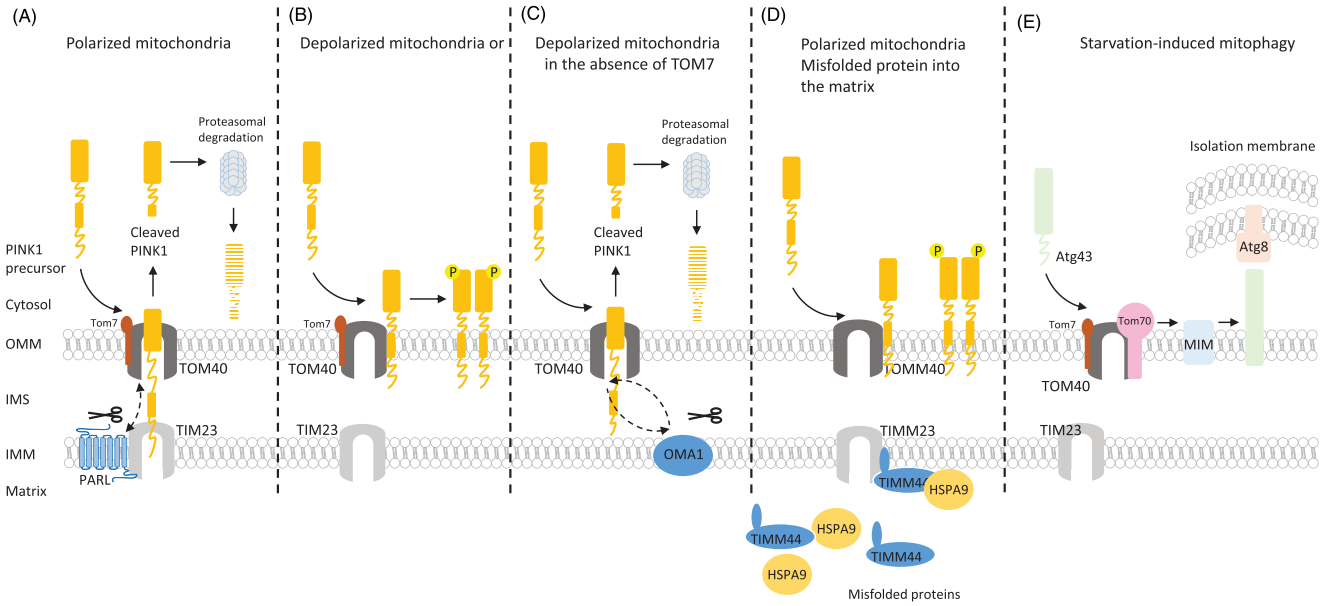

FIGURE 2 Regulation of PINK1-depedent mitophagy by the mitochondrial import system. (**A**) In healthy mammalian mitochondria, PINK1 precursors are imported into mitochondria through the TOM40/TIM23 complexes, and therein the presequences are cleaved by the presenilin-associated rhomboid-like protease (PARL). Cleaved PINK1 is ultimately targeted to the proteasome for degradation. (**B**) Upon dissipation of mitochondria membrane potential, PINK1 import into mitochondria is blocked, and it accumulates on the outer mitochondrial membrane (OMM) forming homodimers that facilitate its autophosphorylation in a Tom7-dependent manner. (**C**) In the absence of Tom7, PINK1 is still imported into mitochondria in a $\Delta\Psi$-independent manner and processed by the IMM-embedded metalloprotease OMA1. (**D**) Accumulation of misfolded proteins in the mitochondrial matrix sequesters TIMM44 and HSP9A subunits of the PAM motor complex thereby stabilizing and activating PINK1 on the OMM to induce mitophagy, in the presence of membrane potential. (**E**) Nitrogen-induced starvation in fission yeast promotes loading of Atg43 to the OMM through interaction with Tom70 receptor and MIM complex, where it exerts its role as a mitophagy receptor and couples the isolation membrane to mitochondria.

OXPHOS.[108] This response likely secures the energy resources of the cell and reduces function of dysfunctional mitochondria, until the mitochondrial stress is resolved (Figure 3). Similarly, MitoMISS triggers metabolic reprograming which entails increased glycolysis, glucose uptake, high activity of de novo serine biosynthesis and mobilization of fat stores. Interestingly, glycolysis and de novo serine biosynthesis are required for the beneficial effects of MitoMISS on organismal physiology.[110] These findings infer that the respective metabolic adaptation is a trait causally related to MitoMISS-related longevity. This notion complements our understanding on the significance of cytosolic and organellar proteostasis, for the UPR^{mt}-associated longevity.

De novo serine biosynthesis is increased in mammalian cell lines upon treatment with several mitochondrial poisons.[118] Serine is a non-essential amino acid that can be taken up by the cells or synthesized de novo from glycolytic intermediates.[136] Apart from its role in protein synthesis, serine is a one carbon donor in one carbon metabolism network of biosynthetic reactions supporting nucleotides synthesis, amino acid homeostasis, cellular redox environment, and epigenetics.[137] Activation of the OPA-DELE1-HRI pathway in human mitochondria elicits an ATF4-dependent response regulating one carbon metabolism, de novo serine biosynthesis and transulfuration.[114,118] Similarly, mitochondrial DNA depletion and respiratory defects activate de novo serine biosynthesis in an ATF4-dependent manner, which likely compensates for the impaired production of serine from formate within dysfunctional mitochondria.[138] The question that rises is whether or when this metabolic adaptation towards serine is beneficial for the cell or for the organism. In mammalian cells knock down of the DELE1-HRI pathway can be protective against oligomycin treatment or upon depletion of the mitochondrial ribosomal protein MRPL17, but it sensitizes cells upon LONP1 depletion.[115] In nematodes, de novo serine biosynthesis is required for MitoMISS associated longevity, while, folate and methionine intermediates display reduced levels across different canonical longevity mutants.[110,139] In flies, dATF4 activates the mitochondrial branch of one carbon metabolism conferring neuroprotection in pink1 and parkin mutants.[140] These findings suggest that UPR^{mt}/ISR-driven metabolic reprogramming may confer different outcomes upon distinct mitochondrial insults. It is anticipated that future studies will shed light on the metabolic fate of glucose and serine upon mitochondrial insults and reveal whether this is an adaptive or maladaptive response in different disease contexts.[136,139]

Future perspectives

During the last three decades, we have greatly advanced our understanding of the mitochondrial protein import machineries, in several

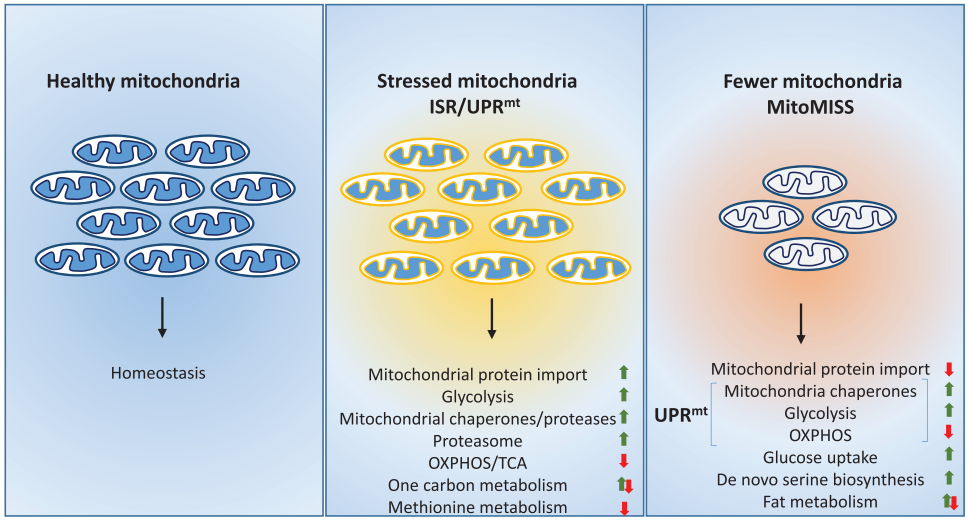

FIGURE 3 Working model on how UPRmt and MitoMISS impact cellular metabolism. Acute protein import stress and/or accumulation of unfolded proteins in mitochondria, in a fully developed mitochondrial network, leads to stressed mitochondria and activation of mitochondrial unfolded protein response/integrated stress response (UPR^{mt}/ISR) in nematodes and mammals. UPR^{mt} activates mitochondrial/cytosolic proteostatic responses and metabolic adaptations. Chronic reduction of protein import rate restricts biogenesis of the mitochondrial network leads to reduced mitochondrial load (MitoMISS). MitoMISS activates some features of UPR^{mt} response and initiates a metabolic shift which supports adaptation to reduced mitochondrial function. Induction of similar metabolic outputs may be adaptive or maladaptive in different contexts.

species. Nevertheless, there are still more mysteries to resolve. For example, our knowledge on the biogenesis of the outer membrane protein components, especially the tail-anchored proteins, is far from complete. Since outer membrane proteins participate in a variety of mitochondrial interactions with other compartments or the cytosol, regulation of their biogenesis and mapping of their interactome need urgent attention. Proteostatic stress response pathways triggered by protein import stress have been extensively studied in budding yeast, however their existence and regulation in higher eukaryotes and more importantly their therapeutic potential in disease-relevant backgrounds, is only starting to emerge. Finally, the mitochondrial import system is interdependent on cellular/lipid metabolism. State-of-the-art technologies and system biology approaches such as global metabolomics, tracer metabolomics and lipidomics are anticipated to shed light into this reciprocal relationship in the near future.

AUTHOR CONTRIBUTIONS

Eirini Lionaki, Ilias Gkikas, and Nektarios Tavernarakis conceived, wrote and edited the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of Interests.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

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