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Mitochondrial Quality Control Pathways Sense Mitochondrial Protein Import

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Abstract

Mitochondrial quality control (MQC) mechanisms are required to maintain a functional proteome, which enables mitochondria to perform a myriad of important cellular functions from oxidative phosphorylation to numerous metabolic pathways. Mitochondrial protein homeostasis begins with the import of over 1000 nuclear-encoded mitochondrial proteins, and the synthesis of 13 mitochondrial DNA-encoded proteins. A network of chaperones and proteases helps to fold new proteins and degrade unnecessary, damaged or misfolded proteins. Meanwhile, more extensive damage can be removed by mitochondrial derived vesicles or mitochondrial autophagy (mitophagy). Here, we review the importance of mitochondrial protein import as a sentinel of mitochondrial function that activates multiple MQC mechanisms when impaired, with a focus on mechanisms in mammalian cells.

Impaired mitochondrial protein import induces multiple mitochondrial quality control pathways

Mitochondria mediate metabolic, biosynthetic, and signaling processes crucial for cellular function and survival [1]. The mitochondrial proteome underlying these functions consists of over 1000 different proteins, the vast majority of which are translated in the cytosol and must be imported into the organelle. Hence, **mitochondrial protein import** (see Glossary) is a fundamental mitochondrial process, as it underlies mitochondrial biogenesis and nearly all other mitochondrial functions [2]. Accordingly, impairments to mitochondrial protein import activate **mitochondrial quality control** (MQC) pathways to maintain overall mitochondrial function and thus cellular survival [3]. Here, we review recent research in mammalian cells showing how impaired mitochondrial protein import is directly monitored by multiple MQC proteins that enact protective responses (see Figure 1, Key Figure).

Mitochondrial protein import is a complex process mediated by dedicated protein import machinery with interrelated pathways importing proteins into different sub-compartments of the mitochondria, as described in Box 1. This import also involves mitochondrial bioenergetics to provide energy, chaperones to aid in protein folding, and proteases for further processing and quality control [2]. Critically, a broad range of mitochondrial stressors can perturb import, such as depolarization (loss of **mitochondrial membrane potential**, $\Delta\Psi_m$), disruptions to ATP production, oxidative stress [4] or clogging of protein import channels [5]. Disruptions to **mitochondrial protein homeostasis (proteostasis)** also impair protein import [6,7]. For example, misfolded proteins that accumulate in mitochondria can add excess load to mitochondrial chaperones, which are essential for both protein folding and mitochondrial protein import [7]. Meanwhile, accumulation of misfolded proteins can also cause dissociation of the protein import motor complex [6], signaling disrupted mitochondrial proteostasis through protein import. Altogether, the integrity of mitochondrial protein import reflects to a considerable degree the overall mitochondrial state, making it a key process to monitor for MQC.

Given the necessity of mitochondrial function for the cell at large, there are a wealth of MQC processes that help maintain a functional pool of mitochondria through improved mitochondrial functioning, or removal of damaged or dysfunctional mitochondrial components [3]. The selective elimination of entire mitochondria is mediated by mitochondrial **autophagy (mitophagy)** [8]. The PINK1/Parkin mitophagy pathway, one of the most well studied pathways, clearly demonstrates the link between impaired protein import and MQC. Normally, the kinase PINK1 is constitutively synthesized and partially imported into mitochondria before being degraded. However, when mitochondrial protein import is impaired (*e.g.*, with mitochondrial depolarization), PINK1 accumulates on the outer mitochondrial membrane and recruits the E3-ubiquitin ligase Parkin to activate mitophagy (Figure 2(a)). In this manner, PINK1 efficiently detects impaired mitochondrial import to activate mitophagy. Another well-established example linking impaired mitochondrial import to stress responses is the **mitochondrial unfolded protein response** (UPR^{mt}), best characterized in the model organism *Caenorhabditis elegans*. Here, the transcription factor ATFS-1 is normally imported into the mitochondria, but under protein import stress, instead translocates to the nucleus where it activates stress

response genes (Figure 2(b)) [9,10]. These two classic examples demonstrate a close link between protein import and MQC.

An accumulation of recent research has suggested that many other regulators of MQC mechanisms closely monitor protein import. Here, we review novel regulators of MQC processes in mammalian cells, focusing on mitophagy, **mitochondria-derived vesicles**, and the UPR^{mt}, collecting demonstrating that impaired mitochondrial protein import is a central signal for MQC.

Mitophagy and mitochondrial protein import

Mitophagy plays an important role in MQC. Low level mitophagy occurs constitutively for normal maintenance [11] while stress-activated mitophagy removes damaged mitochondria from the cell [8]. An overview of mitophagy and some of the key mediators are described in Box 2. Mitophagy can be activated by a diverse range of mitochondrial insults which converge on impaired mitochondrial protein import [8,12]. The most well studied trigger is depolarization [8], either directly induced by mitochondrial uncouplers, inhibition of the electron transport chain, mitochondrial reactive oxygen species (ROS) [13], or calcium oscillation [14]. Defective protein import in the absence of depolarization also activates mitophagy, including depletion of protein import machinery [6,15] and disruptions to mitochondrial protein homeostasis [6,16]. Building on the well-studied link between mitochondrial protein import and the PINK1-Parkin pathway of mitophagy, it is notable that several recent discoveries have uncovered additional modulators of mitophagy that also respond to impaired protein import.

NIPSNAP1/2 maintain recruitment of autophagy receptors

NIPSNAP1/2 are multifunctional proteins implicated in regulating metabolism [17] that work together with PINK1/Parkin to recruit autophagy receptors to damaged mitochondria [18]. Normally an N-terminal mitochondrial targeting signal (MTS) drives NIPSNAP1/2 import into the mitochondrial matrix. However, in depolarized mitochondria, impaired import across the inner mitochondrial membrane (IMM) allows an internal MTS to stabilize NIPSNAP1/2 in the outer mitochondrial membrane (OMM). When localized to the OMM NIPSNAP1/2 interact with cytosolic autophagy receptors at a late stage of mitophagy to accelerate mitochondrial turnover.

NLRX1 recruits LC3 and promotes LC3 conjugation to PE

NLRX1, is a mitochondrial-localized member of the NOD-like receptor family implicated in both regulating mitochondrial metabolism and immunity [19]. Usually located in the matrix, when mitochondrial protein import impaired, NLRX1 relocalization promotes mitophagy [20]. Retention of NLRX1 in the cytosol promotes the conjugation of LC3 to PE and is necessary for efficient clearance of damaged mitochondria [20]. Intriguingly, NLRX1 responds to protein import stress affecting different pathways, including the TOMM complex, the presequence pathway, and the MIA40/ALR pathway. While exactly how diverse impairments of protein import causes cytosolic retention of NLRX1 is unclear, these findings suggest a generalized mechanism responsive to mitochondrial protein import mediated through NLRX1 relocation. As

inhibiting the MIA40/ALR pathway actually impairs PINK1 activation [21], NLRX1 extends the repertoire of protein import situations to which mitophagy can respond.

ATAD3B as a novel mitophagy receptor in oxidative stress

ATAD3B, a mitochondrial-DNA interacting protein also implicated in regulation of mitochondrial translation [22], mediates PINK1-independent mitophagy under oxidative stress [23]. Characteristic of mitophagy receptors, ATAD3B contains an L3C-interacting region (LIR) in its C-terminus, which is normally sequestered in the intermembrane space (IMS) [24]. Oxidative stress causes some ATAD3B to colocalize with the OMM, exposing the C-terminal LIR to the cytosol to recruit L3CB [23]. The exact mechanism for C-terminal relocalization is unclear, however the researchers proposed altered protein interactions with closely related ATAD3A may be responsible. Interestingly, ATAD3A does not relocate under oxidative stress. The difference may be due to a unique hydrophobic C-terminal extension on ATAD3B previously hypothesized to prevent import of the C-terminus into the matrix [24], which could render ATAD3B more sensitive to protein import defects allowing OMM relocalization.

TUFM recruits ATG12-ATG5

TUFM is normally localized in the matrix, where it functions as a mitochondrial translation factor. However, mitochondrial stressors such as infection or depolarization cause TUFM to accumulate on the OMM [25]. Exactly how TUFM ends up on the OMM is unknown; however, an alternative import pathway that inserts TUFM into the OMM is proposed. This pathway could be facilitated by impaired presequence import under mitochondrial stress, however this notion will require more research to elucidate. When located to the OMM, TUFM interacts with cytosolic factors and promotes mitophagy via recruitment of autophagy machinery ATG12-ATG5 to the mitochondria.

Intriguingly, TUFM plays a dual role in mitophagy regulation [26]. Under mitophagy-activating conditions like depolarization, a subset of OMM TUFM is phosphorylated by PINK1 on Ser-222 and dissociates into the cytosol where it inhibits ATG12-ATG5 recruitment. The dual role of TUFM may be a safety delay mechanism. As PINK1 accumulates on depolarized mitochondria, it phosphorylates both TUFM (anti-mitophagy) and Parkin-generated ubiquitin chains (pro-mitophagy). PS222-TUFM results in an initial inhibition of mitophagy, which may prevent aberrant activation of mitophagy due to fluctuations in PINK1 activity. But if mitochondrial damage persists, PS222-TUFM is more quickly degraded than phospho-ubiquitin, such that persistent PINK1 activity can then activate mitophagy. Presumably this mechanism depends on impaired protein import as it requires both PINK1 and TUFM stabilization on the OMM.

HSD17B10 inhibits fission to protect mitochondria from mitophagy

17- β hydroxysteroid dehydrogenase type 10 (HSD17B10) is a matrix protein involved in steroid metabolism and mitochondrial tRNA maturation [27]. Yet under conditions of impaired mitochondrial protein import, HSD17B10 accumulates on the OMM [28], where it promotes mitochondrial elongation via inhibition of the Drp1 fission protein. As elongated mitochondria are protected from mitophagy, OMM HSD17B10 represses mitophagy. Meanwhile, HSD17B10

on the OMM is also associated with improved $\Delta\Psi_m$ [28], suggesting its accumulation may act as an early protective response to mitochondrial stress to avoid mitophagy.

The examples above expand the repertoire of mitophagy mediators that respond to mitochondrial protein import beyond just PINK1. Intriguingly, a new paradigm is emerging whereby negative regulators of mitophagy are also important. For example, pS222-TUFM and HSD17B10, may provide early detection of impaired mitochondrial protein import that activates protective mechanisms to balance unchecked mitophagy, which can cause excessive loss of mitochondria, compromise cell survival, and cause disease [29]. Meanwhile, the fact that NLXR1 interacts with NIPSNAP1/2 [30] and TUFM [31] supports the notion mitophagy becomes activated as multiple signals accumulate.

Given the all-or-nothing nature of mitophagy, it does not form the only, and likely not the first, line of defense against cellular insults. Next, we will cover other mitochondrial stress responses that may act earlier or upon milder impairment of protein import, which does not necessitate removal of the entire organelle.

Mitochondria-derived vesicles and protein import

Mitochondria-derived vesicles (MDVs) are small vesicular structures released from mitochondria to transport select mitochondrial components [32]. MDVs sent to the lysosome mediate MQC by removing damaged mitochondrial components (see Box 3 for more details on the discovery and characterization of MDVs) [33]. MDVs are induced upon exposure to a variety of mitochondrial stresses that impact protein import, such as mild depolarization [34], oxidative stress [33,34], and protein import clogging [35]. Lysosome-destined MDVs are an MQC response to protein import stress, which occurs earlier than mitophagy, both kinetically and in response to milder mitochondrial damage [34]. We will discuss novel links between impaired protein import and MDV formation.

PINK1/Parkin-mediated MDVs

In addition to mitophagy, PINK1/Parkin also regulate the formation of PDH+/TOMM20-MDVs, which are sent to the lysosome for degradation [34]. Unlike mitophagy, which takes several hours to commence, MDVs formation is initiated within minutes following a stress [33,34] and can be triggered by milder stresses that do not activate mitophagy [34]. How do PINK1/Parkin alter their response to early, milder stressors? It is hypothesized that in response to local loss of protein import (conditions that are insufficient to meet the threshold for mitophagy) localized accumulation of PINK1 and subsequent Parkin recruitment triggers MDV formation. MDV-mediated removal of damaged mitochondrial components would help maintain mitochondrial function and $\Delta\Psi_m$, while mitophagy may come into play if sufficient or prolonged damage accumulates, which cannot be contained by MDVs.

Other subsets of MDVs

Another subset of lysosome targeted MDVs are TOMM20+ MDVs [33], which are also linked to impaired protein import. TOMM20+ MDVs produced under steady state conditions revealed a key cargo was assembled protein complexes, including the TOMM import complex [35].

Moreover, the formation of these MDVs could be stimulated by protein import stress. Blocking of the TOMM complex channel triggered formation of MDVs containing assembled TOMM complexes, suggesting MDVs help extract clogged protein import machinery to maintain mitochondrial protein import.

Proteomic profiling of MDV cargo has hinted at additional links to mitochondrial protein import. Cardiac cell-derived MDVs are enriched for proteins involved in mitochondrial protein import and processing, particularly under oxidative stress [36], while mouse brain-derived MDVs are enriched for small TIMM chaperones which help import mitochondrial carrier proteins [37]. Together, these findings suggest MDVs likely are important for maintaining mitochondrial protein import in the face of stress, though the mechanism(s) are still unknown and require further investigation.

SPOTs and SAM50

Reminiscent of MDVs, a recent report described “structures positive for outer mitochondrial membrane”, or SPOTs, which were initially observed in response to an intracellular parasite protein as part of a novel response to OMM import stress [38]. This OMM protein import stress causes release of OMM vesicles that extricate clogged protein import complexes from the mitochondria. The mechanism of SPOT formation hinges upon SAM50, which imports OMM proteins and is also a component of the mitochondrial intermembrane space bridging complex (MIB) that structurally connects the OMM and IMM. Disassembly of the MIB complex allows separation of the OMM and release of SPOTs containing the clogged SAM complex, posing another mechanism by which impaired protein import is recognized and used to activate MQC mechanisms.

While our discussion of MQC so far has focused on degradative processes, there are additional systems of MQC aim to restore mitochondrial function. Some of these processes, and how they relate to protein import, will be described next.

The mitochondrial unfolded protein response and integrated stress response and protein import

Mitochondrial stress responses can extend beyond mitochondria to elicit cooperation of other parts of the cell. For instance, the mitochondrial unfolded protein response (UPR^{mt}) communicates mitochondrial stress to regulate nuclear gene expression [3]. The UPR^{mt} is classically described to respond to mitochondrial proteostatic stress by upregulation of mitochondrial chaperones and proteases such as HSP60, HSP10, ClpP and LONP1 [39,40] which help restore mitochondrial proteostasis. More recent research in mammalian cells has suggested that the **integrated stress response (ISR)**, a cellular stress response pathway, is also closely involved in responding to mitochondrial stress, possibly in conjunction with UPR^{mt} [41]. These two pathways are briefly reviewed in Box 4. Importantly, these pathways are closely linked to impaired mitochondrial protein import. Investigation of the UPR^{mt} in the model organism *C. elegans* revealed it primarily responds to a wider range of mitochondrial stresses which disrupt mitochondrial protein import [10], reflecting how the activation of the UPR^{mt} is mechanistically linked to impaired mitochondrial protein import [9]. This model is supported by

research in mammalian cells, where the UPR^{mt} and ISR can be activated by protein import stressors such as depolarization and ETC inhibition [42,43], oxidative stress [40,44], and disruptions to proteostasis including misfolded proteins [40,44], depletion of mitochondrial chaperones or proteases [44,45] and repression of mitochondrial genome expression [46,47].

Building on the paradigm of ATFS-1 as a sensor of mitochondrial protein import to trigger the UPR^{mt} in *C. elegans*, there is accumulating evidence linking impaired import as a UPR^{mt}/ISR trigger in mammalian cells.

Unimported mitochondrial preproteins are a signal to activate the mtUPR

Unimported mitochondrial precursor proteins can activate multiple MQC responses in yeast [48,49], and recent work has corroborated this in mammalian cells, showing unimported mitochondrial precursors can activate the UPR^{mt} [50]. Occupation of cytosolic chaperone HSP70 by mitochondrial precursors causes HSP70 to release the transcription factor HSF1, in a process also requiring leaked mitochondrial ROS. HSF1 translocates to the nucleus for activation of the UPR^{mt}. In this manner, failure of mitochondrial protein import is again implicated in the detection and signalling of mitochondrial stress responses.

DELE1 linking mitochondrial stress to the ISR

A striking advance in understanding how mitochondrial stress activates the ISR was the discovery of DELE1. DELE1 was identified as an ISR activator specific to mitochondrial stress through genome-wide screens [51,52]. DELE1 is normally imported into the mitochondria, but under mitochondrial stress, relocates to the cytosol where it binds and activates the ISR kinase HRI, initiating the ISR. Initial work on DELE1 established a mechanism by which OMA1, an IMS-facing stress-responsive protease, cleaves DELE1 and releases the C-terminus into the cytosol to interact with HRI.

Further research has established how activation of DELE1 is attuned to the efficiency of mitochondrial protein import [53]. DELE1 is imported into the matrix by the presequence pathway; if import is slowed, retention of partially imported DELE1 in the IMS facilitates cleavage by OMA1. This is aided by DELE1's unique MTS which seems to slow import across the IMM to allow for OMA1 cleavage. When DELE1's presequence was replaced with a more efficient MTS, it prevented DELE1 release under mild protein import stress as DELE1 was quickly imported into the matrix, bypassing OMA1 cleavage. This two-step requirement for both protein import impairment and OMA1 activation (which can occur due to many perturbations that affect protein import) may act as a second safeguard to ensure that activation of the ISR only occurs due to *bona fide* mitochondrial stress.

DELE1 also responds to impaired protein import upstream of the IMM independently of OMA1. If import across the OMM is impaired, such as through TOMM KD, full length DELE1 is retained in the cytosol and activates HRI [53]. In low iron conditions, DELE1 import is arrested across the OMM, allowing its C-terminus to remain in the cytosol to activate HRI, highlighting another way in which multiple stressors converge on the regulation of DELE1's import [54].

Contrary to a usual view of the nucleus as the control centre of the cell, it is apparent that signalling also travels from other parts of the cell to the nucleus, known as "retrograde signalling". In the case of mitochondrial dysfunction, which poses an existential threat to the

cell, this mito-nuclear communication is vital to responding to various stresses. Finally, it is important to acknowledge that exploration of the UPR^{mt} and ISR is ongoing. On that note, current definitions of the UPR^{mt} may not fully encompass what is likely a more extensive stress response beyond just chaperones and proteases, which may or may not always be part of the primary response. For instance, the UPR^{mt} and ISR are linked to remodelling of mitochondrial metabolism [44,55] amongst other adaptations. New terminology or definitions may emerge; the recently coined “mitochondrial ISR” refers to a mitochondrial stress response in muscle tissue, including the classic UPR^{mt} response, metabolic adaptations and cytokine production [56]. Our understanding will surely continue to evolve in coming years.

Future directions

Our view of mitochondria, as static energy producers, has long transformed to encompass a dynamic role as a metabolic signalling hub [57]. Beyond signalling to other organelles, mitochondria can communicate with the organism as a whole. Mitochondrial stress in one cell or tissue can generate a “mitokine” signal that activates a stress response in other locations [58]. A classic example in *C. elegans* is that mitochondrial stress in neurons activates the UPR^{mt} in the gut. In mammalian tissues, mitochondrial stress in skeletal muscles induces compensation in adipose by the release of FGF-21 [59]. Understanding MQC pathways in a multicellular or organismal context will improve our understanding of mitochondrial biology and conditions involving MQC dysfunction such as neurodegenerative disorders [60] and cardiac disease [61].

One area of future research may be steroid hormones, signalling molecules that function as long-range messengers throughout the body [57] and also influence mitochondrial function such as $\Delta\Psi_m$ [62]. Intriguingly, as described above, the steroid metabolism protein HSD17B10 is also a mitochondrial stress response protein [28]. Another interesting candidate is the protein STARD1, which mediates mitochondrial import of cholesterol, the rate-limiting step of steroid production. STARD1 promotes mitochondrial cholesterol import when it is transiently associated with the OMM, prior to its import into the matrix, and slowing mitochondrial protein import increases cholesterol import [63]. Thus, impairments in mitochondrial protein import homeostasis may alter steroid signalling through synthesis or metabolism, with the potential to translate mitochondrial defects to organism wide signalling.

Finally, while we have focused here on pathways understood in mammalian cells, additional MQC pathways monitoring mitochondrial protein import are recognized in yeast [48,49,64–66]. Evidence for many of these pathways is still lacking in mammalian systems, but they suggest much more is to be discovered in mammalian cells in the context of protein import and MQC. More questions to be considered are listed in Outstanding Questions.

Concluding remarks

Impaired mitochondrial protein import sets off a series of events. Early on, small scale perturbations in mitochondrial protein import can generate MDVs to remove damaged mitochondrial components. Meanwhile, activation of the UPR^{mt}/ISR stress response offer the opportunity to address the problem before it leads to larger issues. If these measures are

insufficient to restore mitochondrial protein import, prolonged defects stabilize a host of mitophagy mediators to remove unsalvageable mitochondria. Critically, impaired import of mitochondrial proteins triggers the activation of mitophagy, MDV formation and UPR^{mt}, indicating it is a common feature underlying many MQC pathways. This shared mechanism may reflect how impaired mitochondrial protein import is a common symptom of mitochondrial ailments, as well as an efficient mechanism to couple the detection of mitochondrial stress to recovery efforts. As we have described here, relocalization of key proteins outside of mitochondria or to the OMM facilitates distinct functions. In addition to the examples of MQC proteins that interact directly with the mitochondrial protein import machinery, even general cytosolic accumulation of mitochondrial precursors activates stress responses. Together, these examples emphasize how the cell at large is closely attuned to mitochondrial protein import stress.

Highlights

- Mitochondrial protein import is a fundamental mitochondrial process that can be disrupted by a wide variety of stressors, making it an inclusive readout of overall mitochondrial function.
- Many mitochondrial quality control pathways that maintain overall mitochondrial function monitor the integrity of mitochondrial protein import.
- Recent work has revealed that novel modulators of mitophagy, mitochondrial derived vesicles, and the mitochondrial unfolded protein response sense impaired mitochondrial protein import.
- Failure of protein import is a mechanistic step that helps trigger mitochondrial quality control responses.

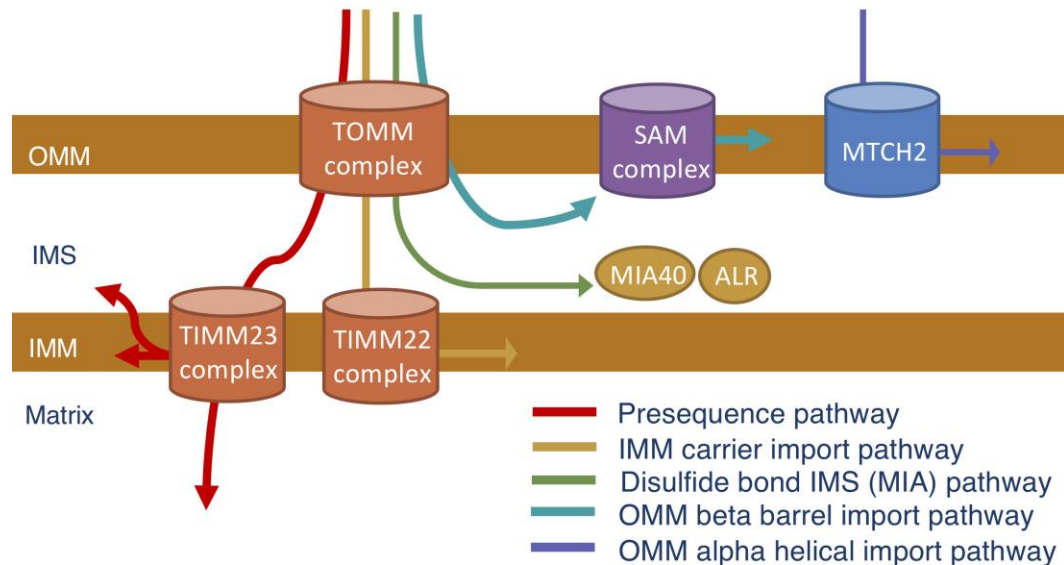
Box 1: Mitochondrial protein import pathways

Figure I: Mitochondrial protein import pathways

Mitochondrial protein import is mediated by the translocase of the outer mitochondrial membrane (TOMM) complex and two related but distinct translocase of the inner mitochondrial membrane (TIMM) complexes (TIMM22 and TIMM23), which enable proteins to be sorted into one of four sub-organellar compartments: the outer mitochondrial membrane (OMM), the inner mitochondrial membrane (IMM), the intermembrane space (IMS), or the matrix (Figure I) [67]. We will only provide a brief overview of the mitochondrial protein import pathways here, as they are reviewed thoroughly elsewhere [2,67].

The presequence pathway recognizes N-terminal mitochondrial targeting signals (MTS), and sorts proteins primarily into the mitochondrial matrix, with some proteins being redirected to the IMM or IMS [2,67]. Following initial import through the TOMM complex, these proteins are transferred across the IMM by the TIMM23 complex. Importantly, translocation of proteins across the IMM into the matrix requires mitochondrial membrane potential ($\Delta\Psi_m$). ATP is also used to power the import chaperone Mortalin/HSPA9, which pulls proteins into the matrix. Consequently, protein import is interconnected with mitochondrial bioenergetics. Once in the matrix, a series of processing events can remove the MTS.

Additional pathways, which are not as well understood, mediate import to other compartments [67]. IMM metabolite carrier proteins are transferred from TOMM to the IMM-spanning TIM22 complex and released to the IMM. IMS proteins containing disulfide bonds are folded by MIA40/ALR after passing through TOMM. OMM proteins generally take two different paths. Beta barrel proteins are imported through the TOMM complex into the IMS and then inserted into the OMM via the sorting and assembly machinery (SAM) complex. Meanwhile, alpha helical proteins are directly inserted by the mitochondrial import (MIM) complex on the OMM, for which a mammalian counterpart, MTCH2, was only recently identified [68]. The SAM complex also appears to contribute to some alpha helical protein import [38,69].

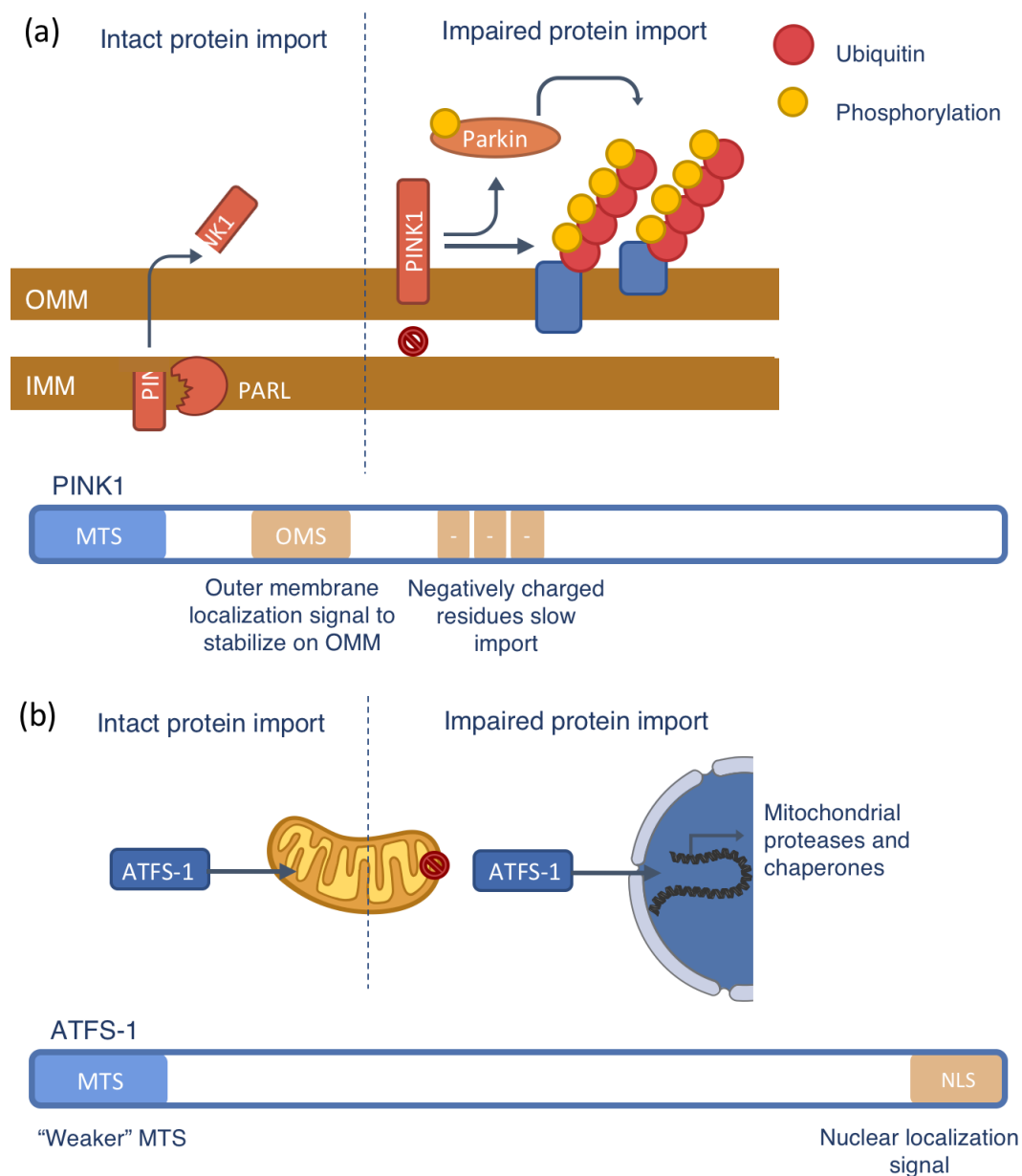


Figure 2: Canonical examples of detection of impaired mitochondrial protein import by MQC proteins

(a) PINK1/Parkin mediated mitophagy. Under basal conditions, an N-terminal MTS drives PINK1 import to the IMM by the presequence pathway, where it is cleaved by the IMM protease PARL [8]. The C-terminal fragment of PINK1 is released to the cytosol for degradation. However, when protein import is impaired, PINK1 fails to be imported to the IMM and is stabilized on the OMM. This process is mediated by an OMM localization signal domain and a cluster of negatively charged amino acids thought to antagonize import. Stabilized and activated PINK1 recruits and activates Parkin, an E3 ubiquitin ligase. Parkin ubiquitinates mitochondrial proteins, generating ubiquitin chains that are then phosphorylated by PINK1. Mitochondria coated in

phosphoubiquitin chains recruit degradative machinery to undergo mitophagy. (b) In the *C. elegans* UPR^{mt}, ATFS-1 contains both an MTS and a nuclear localization signal (NLS) [9,10]. Under normal conditions, the MTS directs ATFS-1 into the mitochondria where it is degraded. When protein import is impaired, ATFS-1 that fails to be imported accumulates in the nucleus where it activates transcription of stress response genes. ATFS-1 was found to have a “weaker” MTS compared to other mitochondrial proteins, making it more sensitive to loss of protein import. The Figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license. Abbreviations: ATFS-1, activating transcription factor associated with stress 1; IMM, inner mitochondrial membrane; MTS, mitochondrial targeting signal; NLS, nuclear localization signal; OMM, outer mitochondrial membrane; PARL, presenilin-associated rhomboid-like protein; PINK1, PTEN induced kinase 1.

Box 2: Mechanisms of mitochondrial elimination by mitophagy

Mitophagy, the autophagic elimination of mitochondria, makes use of the cellular autophagy machinery [70]. Briefly, select mitochondria are engulfed within a growing isolation membrane that encloses the cargo to form an autophagosome. The autophagosome then fuses with a lysosome containing hydrolytic enzymes to mediate degradation of the contents. Phosphorylated ubiquitin chains, such as those generated by the PINK1/Parkin pathway, act as signals to recruit autophagy receptor proteins, such as optineurin and NDP52 [71]. These receptors contain LC3-interacting regions (LIRs) that bind Atg8 family proteins (LC3s and GAPARAPs) to recruit the isolation membrane and other autophagy proteins to the mitochondria [72]. Atg8 family proteins are ubiquitously present on autophagosome membranes as they are conjugated to a modified membrane lipid, phosphatidylethanolamine (PE) in a process mediated by ATG12-ATG5 [70]. Atg8 family proteins are required for autophagosome-lysosome fusion [73] and act as further signal amplifiers for mitophagy [74].

It is important to note that in addition to PINK1/Parkin, several other mitophagy pathways exist [70]. For instance, mitophagy receptors such as FUNDC1 and BNIP are OMM proteins which contain LIRs. These receptors activate mitophagy under hypoxic conditions, generating additional pathways for damage-responsive mitophagy aside from PINK1/Parkin.

Box 3: Discovery and characterization of mitochondria-derived vesicles

Mitochondria-derived vesicles (MDVs) were first described in 2008 when researchers noticed that, despite suppression of mitochondrial fission, there were small vesicular structures being shed from the mitochondria [32]. MDVs are defined as being 70-100nm in size, formed in a manner distinct from canonical mitochondrial fission, and containing selective cargo. Different subsets of MDVs are targeted to distinct organelles (*e.g.*, peroxisomes [32], or lysosomes [33]), and functionally they are recognized to play roles in immune activation [75], pathogen defense [76], and MQC [34,35]. Notably, lysosome-targeted MDVs enriched in damaged mitochondrial components aid in steady state mitochondrial maintenance [35,36].

While the formation and targeting of MDVs is not fully understood, research suggests that there are multiple distinct subsets of MDVs. The delineation of MDV types (and differentiation from intact mitochondria) is often accomplished by immunofluorescent imaging for marker proteins, taking advantage of selective cargo incorporation into different types of MDVs. Two subsets of MDVs characterized as positive or negative for either TOMM20 or the E2 and E3 subunits of matrix enzyme pyruvate dehydrogenase (TOMM20+/-PDH- or PDH+/TOMM20-) are targeted to lysosomes and are formed through distinct mechanisms [33–35].

Box 4: Mitochondrial unfolded protein response and integrated stress response

The mitochondrial unfolded protein response (UPR^{mt}) was first discovered in mammalian cell lines, where expression of a misfolded protein in the mitochondrial matrix or depletion of the mitochondrial DNA (mtDNA) triggered expression of nuclear-encoded mitochondrial chaperones and proteases [39,46]. Further characterization of the UPR^{mt} was accomplished in the model organism *Caenorhabditis elegans*, particularly the identification of ATFS-1, which activates the UPR^{mt} under mitochondrial protein import stress [9]. As only a small amount of ATFS-1 is needed to relocate to the nucleus to activate the mtUPR [9], this process likely responds to milder protein import stress than PINK1/Parkin.

While still only partially understood, the mammalian UPR^{mt} seems to be more complex. In mammals, four main transcription factors are implicated in activating stress response genes: ATF4 [42,45], ATF5 (which is proposed to be regulated similarly to ATFS-1) [40], CHOP [39] and, more recently, HSF1 [50,77]. Unlike ATFS-1, the expression of ATF4, ATF5 and CHOP are usually repressed, and their activation is closely tied to another stress response, the integrated stress response (ISR) [7,41].

The ISR is signalling pathway which converges on suppression of global protein translation while activating translation of certain stress response genes [78]. A full overview of this complex process is outside the scope of this review, however we will summarize its relevance to the UPR^{mt}. Four stress-activated kinases, PERK, PRK, GCN2 and HRI, can phosphorylate a component of the translational initiation complex, eIF2, to inhibit translational initiation. Overall translation is reduced, while the translation is turned on for certain transcripts which are usually repressed – namely ATF4, ATF5 and CHOP -- which then activate a program of stress response genes. Thus, the ISR allows the cell to focus resources specifically to handle the stress at hand and is proposed to be a prerequisite for the mammalian UPR^{mt} [41]. In this regard, all four ISR kinases are activated by various types of mitochondrial stress [79]. Further, translational attenuation is protective to mitochondria by reducing protein load, likely making reduced translation an important part of the protective response [80]. Exactly how the UPR^{mt} and ISR are related, which pathways respond under different mitochondrial stressors, and what exactly comprises the response is still being determined [41].

Outstanding questions

- To what extent does the import efficiency of individual mitochondrial proteins influence how they sense impacts on mitochondrial protein import? What sequences and domains within these proteins impact their relocalization?
- How do different degrees of protein import stress facilitate a graded response to the severity of mitochondrial dysfunction, and do they affect the type of MQC response initiated?
- Under what situations are different MQC responses activated? To what degree are these responses and different MQC mediators complementary or redundant?
- How do mitochondrial stress responses affect cells, tissues, organisms, and impact human health and disease?

Glossary

- **Autophagy:** Process of recycling cellular components through lysosomal degradation.
- **Integrated stress response:** Cellular stress response that leads to global translational repression and activation of stress response genes.
- **Mitochondria-derived vesicles:** Small vesicles with selective cargo released from mitochondria independently of mitochondrial fission that mediate processes including mitochondrial quality control, transport, antigen presentation, pathogen defense.
- **Mitochondrial membrane potential:** Electrochemical gradient across the inner mitochondrial membrane generated by pumping of protons into the intermembrane space.
- **Mitochondrial protein import:** The process by which mitochondrial proteins translated in the cytosol are translocated into the mitochondria and sorted into the appropriate subcompartment.
- **Mitochondrial proteostasis (protein homeostasis):** Complex balance maintained by coordination of mitochondrial and nuclear genome expression, protein import, folding, multiunit complex assembly and protein turnover, disruption to this balance results in proteostatic stress.
- **Mitochondrial quality control:** Processes that help maintain a functional pool of mitochondria within the cell.
- **Mitochondrial unfolded protein response:** Pathways typically understood to respond to disrupted mitochondrial proteostasis through increased expression of mitochondrial chaperones and proteases.
- **Mitophagy:** Autophagic degradation of mitochondria.

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Declaration of Interests

The authors declare no competing interests.

Author Contributions

LPL-G was involved in Conceptualization, Visualization, Writing – original draft, Writing - review & editing. TES was involved in Conceptualization, Funding acquisition, Project Administration, Supervision, Writing – original draft, Writing -review & editing.

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