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A Review

An overview of response pathways for protection of mitochondria from protein misfolding stress

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Mitochondria are canonically known as cellular powerhouse but apart from ATP production, these organelles are hubs of critical metabolic and cellular pathways like fatty acid metabolism, calcium signalling, heme biosynthesis, and apoptosis. Mitochondrial proteome contains almost 1500 proteins to facilitate these cellular processes; however, maintaining a healthy mitochondrial proteome is an extremely challenging task. As mitochondria are continuously exposed to various cellular stresses, misfolding and aggregation of proteins is one of the most prominent outcomes of these stresses, protein misfolding inside the mitochondria or on the mitochondrial surface poses a severe threat to mitochondrial health as well as to cellular health. To cope with such proteotoxic stress, cells have evolved multiple stress response pathways, which help in the maintenance of healthy mitochondrial proteome, resulting in prolonged cellular survival. Here in this review, we have summarized the origin of mitochondrial proteotoxicity and cellular response to tackle these toxic proteins.

Keywords: Mitochondria, Mitophagy, Protein misfolding, Proteostasis, Proteotoxicity, UPR^{MT}

Introduction

Mitochondria are one of the major cellular organelles found in eukaryotic cells, however, the endosymbiotic theory explains that the emergence of mitochondria into eukaryotic cells occurred around ~2 billion years ago when an α-proteobacterium was absorbed by precursor eukaryotic cells. Structurally mitochondria are double membrane bound organelles and during the course of evolution, it has undergone many changes to finally acquire the modern-day structure. Because of its double membrane-bound architecture, they consist of two soluble sub-compartments; Matrix and Inter-Membrane Space (IMS). Both sub-compartments have their own microenvironment and different oxido-reductive atmospheres[1,2].

The vital functions of mitochondria are ATP synthesis by oxidative phosphorylation, heme synthesis, β-oxidation of fatty acids, stem cell regulation, calcium homeostasis, regulation of innate immunity, and programmed cell death[3–5]. All these events depend upon the signaling cascade of an elaborated network of proteins or functional proteostasis network of mitochondria. However, mitochondria encounter several challenges in maintaining the protein homeostasis or proteostasis. The first and foremost challenge for mitochondria to maintain its protein homeostasis is absolute dependence on nucleus for its constituent proteins as despite having its own genome, only 1% of its total proteins are synthesized inside each mitochondrion. The rest 99% of proteins are encoded by the nuclear genome and synthesized in the cytosol[6], and are subsequently translocated to mitochondria majorly in an unfolded conformation. The pores of the protein conducting-channels of the TOM (Translocase of Outer Mitochondrial Membrane) and TIM (Translocase of Inner Mitochondrial Membrane) translocases are narrow and cannot accommodate globular folded proteins. The predominantly unfolded precursor proteins containing some secondary structures are only able to pass through these translocases[7]. To prevent aggregation and misfolding of the non-native precursor states, the mitochondrial pre-proteins are assisted by chaperones to mask the hydrophobic regions of these proteins to prevent their aggregation during translocation[8]. Once translocated to the mitochondrial matrix, which has a basic (pH ~7.8) environment in comparison to the cytosol (pH ~7.4), the proteins experience a different environment. Because of this sudden change in the

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pH, folding and functionality of proteins may get impacted. Furthermore, ROS (Reactive Oxygen Species) and heat generation during OXPHOS (Oxidative Phosphorylation) may induce oxidative damage, denaturation, and aggregation of proteins[9]. Importantly, mitochondrial and nuclear genomes work under immense synchronization and any failure or miscommunication can cause changes in the emergence of damaged proteins and faulty pathways[10]. Together these factors can adversely affect the health of mitochondrial by disturbing the protein homeostasis of mitochondria, which may result in the rise of protein aggregation and misfolding inside the organelle.

To tackle these challenges, mitochondria utilize several quality control pathways. Mitochondrial unfolded protein response (UPR^{MT}) is a pathway that is elicited in response to the protein misfolding stress inside the mitochondria. During UPR^{MT}, the nuclear genome is informed by the reporter proteins to induce the expression of mitochondrial chaperones and proteases to reinstate protein homeostasis and subsequently healthy pool of proteins are restored in the organelle[11]. Apart from UPR^{MT}, in the recent years several other stress response pathways have been reported, such as mitochondrial ribosome quality control pathway (mitoRQC), which deals with the ribosome stalling during translation where nascent polypeptide chains are stalled with ribosome and tRNA due to some translational defect or mutation[12]. Ribosome stalling can give rise to aberrant or truncated proteins, which can accumulate and further aggregate to give rise to proteotoxic stress. Here, Vms1 (VCP/Cdc48-associated Mitochondrial Stress-responsive) acts as tRNA hydrolase to release the stalled peptide from the ribosome-nascent chain-tRNA complex and further assists in their degradation[13,14]. Mitochondrial Translocation Associated Degradation (mitoTAD) is a surveillance pathway, where Ubx2 (UBX domain-containing protein 2) continuously monitors the uninterrupted protein import through the TOM complex. During the translocation, if any precursor protein is found trapped in the TOM complex, Ubx2 recruits type II AAA-type ATPase protein Cdc48/VCP to remove the trapped precursor protein from clogged TOM complex and facilitates their degradation through ubiquitin-proteasomal degradation pathway[15]. Mitochondrial compromised protein import response (mitoCPR) is another pathway associated with mitochondrial protein import defects. MitoCPR deals with precursor proteins accumulated on the outer surface of mitochondria or translocases, when mitoTAD is not sufficient to unclog the TOM complex, mitoCPR gets activated. MitoCPR activation leads to Pdr3 (drug-responsive transcription factor) mediated upregulation of Cis1, which gets recruited on the outer mitochondrial membrane and acts as a bridge between TOM70 and Msp1 (mitochondrial sorting of proteins1) or Atad1 (ATPase family AAA domain-containing 1). Msp1/ATAD1 has an extractase activity that allows the removal of trapped precursor protein from the TOM complex followed by their degradation by ubiquitin proteasomal degradation machinery[16]. There are various other recently discovered pathways including UPR^{AM} (mitochondrial unfolded protein response activated by mistargeted protein), which deals with the mitochondrial import defect that leads to the aberrant accumulation of mitochondrial precursor proteins in the cytosol, and ultimately activates the efficient degradation of precursor protein by ubiquitin-proteasome system[17]. MAGIC (mitochondria as a guardian in the cytosol) is a pathway by which mitochondria rescue the cytosol to get rid of overburdening by misfolded proteins. Here, misfolded cytosolic proteins are transported inside the mitochondria for their efficient degradation. MISTERMINATE (mitochondrial-stress-induced translational termination impairment and protein carboxyl-terminal extension) explains the cellular proteostasis failure associated mitochondrial dysfunction[18]. Mitochondria-associated-degradation (MAD) pathway is another pathway that controls the overall balance of mitochondrial outer membrane protein turnover to make a synchronized balance between protein amount and their activity. Here Doa1 binds with ubiquitinated proteins on the outer mitochondrial membrane and further recruits VCP/Cdc48, which extracts the ubiquitinated proteins for their proteasomal degradation. During ROS-induced oxidative stress, Vms1 plays an accessory role in the MAD pathway and facilitates the removal of damaged proteins with the help of VCP/Cdc48[19]. In addition to all the pathways in mammalian and yeast cells, an alternative method to clear out the mitochondrial proteins by mitochondrial-derived vesicle (MDVs) formation[20].

Finally, when the proteotoxicity-induced mitochondrial damage is beyond the scope of repair

by any of the above-mentioned pathways, the ultimate resort for cell survival is to segregate the damaged and healthy mitochondria followed by the clearance of the damaged mitochondria *via* the autophagy pathway, popularly known as mitophagy. Mitophagy can be initiated by three mechanisms, the first is PINK1/Parkin-dependent mitophagy. When mitochondrial membrane potential is compromised, PINK1 (phosphatase and tensin homolog (PTEN) induced putative kinase protein 1) stabilizes itself on the outer mitochondrial membrane, followed by the autophosphorylation of PINK1[21]. Phosphorylated PINK1 recruits E3-ubiquitin ligase Parkin to the outer mitochondrial membrane which in turn polyubiquitinates various outer mitochondrial membrane proteins that provide the binding site for mitophagy adaptor proteins (p62, Optineurin, NDP52, TAXBP1). On the other hand, these adaptor proteins interact with autophagophore receptor proteins LC3 (microtubule-associated protein light chain 3) or GABARAP (gamma-aminobutyric acid A receptors) followed by the formation of autophagophore around the damaged mitochondria. Lastly, the fusion of the autophagophore with lysosome leads to the degradation of damaged mitochondria. The second type of mitophagy is known as receptor-mediated mitophagy. On the outer mitochondrial membrane, various mitophagy receptor proteins are present; during receptor-mediated mitophagy, these mitophagy receptors, without any direct involvement of PINK1/Parkin, interact with the autophagophore receptor proteins (LC3/GABARAP) and facilitate the removal of damaged mitochondria by mitophagy[22]. Lastly, there is another mechanism of mitophagy, known as lipid-mediated mitophagy, where cardiolipin present in the inner mitochondrial membrane is transferred to the outer mitochondrial membrane where it directly interacts with autophagophore receptor proteins (LC3/GABARAP) to initiate the mitophagy events[23].

In this review, we are highlighting the major mitochondrial stress response pathways reported during mitochondrial protein misfolding stress or proteotoxic stress that have been described in the recent years.

Mito UPR or UPR^{MT}: Mitochondrial unfolded protein response

The mitochondrial unfolded protein response (UPR^{MT}) is initiated by multiple forms of mitochondrial dysfunction (perturbation of OXPHOS, chemical stresses, protein misfolding stress *etc.*), which eventually triggers and activates the transcriptional response by some of the known transcription factors[24–26].

The mitochondrial unfolded protein response (UPR^{MT}) was initially studied as the stress response that arises from the damage of mitochondrial DNA in mammalian cells[11,27]. Later, the *Caenorhabditis elegans* system was employed to study the UPR^{MT}, where the Activating Transcription Factor associated with Stress-1 (ATFS-1) was found as a key transcription regulator of UPR^{MT}[28]. Apart from its nuclear localization signal, ATFS-1 also possesses a mitochondrial localization signal (MTS). In physiological condition, ATFS-1translocates to mitochondria and is degraded by Lon protease present in the mitochondrial matrix. When mitochondria are stressed, mitochondrial import of ATFS-1 is inhibited which allows it to localize into the nucleus and governs the upregulation of mitochondrial chaperones and proteases. Dual targeting signal in ATFS-1 is a surveillance as well as a regulatory mechanism to assess mitochondrial health and UPR^{MT} activation[29]. However, it was also reported that, removal of MTS leads to accumulation of ATFS-1 in the nucleus and UPR^{MT} activation[28]. There has been various mitochondrial dysfunctions reported to activate UPR^{MT}, including mitochondrial translation and protein synthesis defects, OXPHOS impairment, mtDNA damage, reactive oxygen species and mitochondrial protein import dysfunction (Fig. 1 upper panel)[11,30,31]. These defects lead to disruption of the mito-nuclear protein balance, allowing the accumulation of these proteins inside the mitochondria.

As stated earlier, the UPR^{MT} was first reported in mammalian cells where mtDNA depletion or misfolded protein accumulation inside mitochondrial matrix causes the upregulation of mitochondrial chaperones and proteases. Similar to ATFS-1 in *C. elegans*, mammalian cells have three bZIP (basic leucine zipper) transcription factors, C/EBP homologous protein (CHOP), activation transcription factor 4 (ATF4) and activation transcription factor 5 (ATF5), known to participate in integrated stress response (ISR)[32,33]. CHOP and ATF-4 do not possess mitochondrial localization signals like ATFS-1 and ATF-5[33]. When mitochondrial stress arises in mammalian cells, UPR^{MT} activation is initiated by GCN2 (General Control Nonderepressible 2) and PERK (Protein kinase R-like endoplasmic reticulum

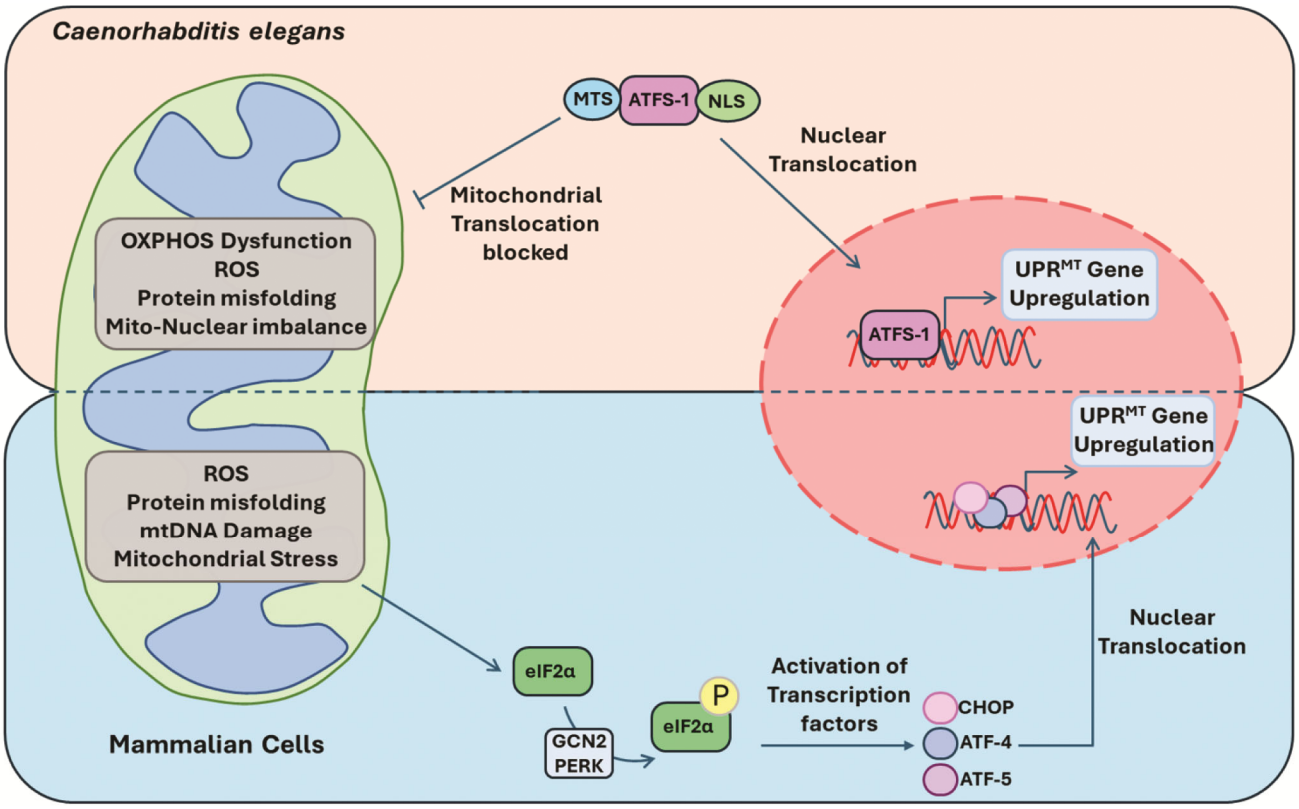

Fig. 1 — Mitochondrial unfolded protein response originating in response to mitochondrial dysfunctions in *C. elegans* and *H. sapiens*. Upper Panel: UPR^{MT} in *C. elegans* can initiated by various trigger factors, including OXPHOS dysfunction, ROS, protein misfolding and mito-nuclear imbalance. These triggers cause the ATFS-1 mitochondrial import block, leading to its localization in the nuclear and upregulating the gene expression of UPR^{MT} related markers. Lower Panel: In humans instead of ATFS-1 these are CHOP, ATF-4 and ATF5 which get activated by eIF2-α upon mitochondrial stress induction, and afterwards these transcription factors localized to the nucleus and upregulate mitochondrial chaperones and proteases to rescue mitochondria from stress

kinase) assisted phosphorylation of eukaryotic translation initiation factor 2 subunit 1 (eIF2α), which eventually activates the expression of CHOP, ATF4 and ATF5 (Fig. 1 lower panel). These transcription factors then induce the expression of mitochondrial chaperones and proteases to overcome the mitochondrial stress[34]. It is still debated how these transcription factors work individually or collectively; it was reported that they control each other's expression[26].

UPR^{MT} activation can also be linked to the mito-hormesis, which is the cytosolic and nuclear adaptive response that arises during the various types of mitochondrial toxicity. Both pathways (UPR^{MT} and mito-hormesis) act as cytoprotective by activating the cascade of signalling events to eliminate out the aftermath of mitochondrial toxicity[35]. At a broader perspective, this pathway may provide protection against cell death, eventually promoting healthy aging of an individual.

Mitophagy

Selective removal of mitochondria by autophagy termed as mitophagy, was discovered in mammalian cells, when increased mitochondrial segregation in lysosome was observed under an electron microscope (reviewed in[36]). Later Lemaster et. al. studied photodamage and starvation induced autophagy to show the encapsulation of mitochondria into autophagosomes, they named this phenomenon as "Mitophagy"[37]. One more group showed cells cultured in the presence of caspase inhibitors cause clearance of mitochondria prior to apoptosis, which links with the mitochondrial membrane permeabilization, suggesting damaged mitochondria are cleared by mitophagy[38]. Mitophagy is a quality control pathway to get rid of the damaged mitochondrial population from cells. It is also a synchronized mechanism to maintain a healthy pool of mitochondria to fulfil the cellular metabolic demands.

Mitochondria are dynamic organelles, that undergo constant fission and fusion, mitochondrial fission is

one of the earlier signature events of mitophagy initiation in yeast and mammalian cells. By governing fission, cells can segregate damaged and healthy parts of mitochondria. Apart from that, it also helps in the easy engulfment of mitochondria by autophagophore[39]. Mitophagy is a continuous process and is an essential part of mitochondrial metabolism that governs the equilibrium of healthy mitochondria based on cellular demand. It may change based on metabolic and energy requirements and may vary from different developmental stages of cells[40].

In yeast *Saccharomyces cerevisiae,* Atg32 (autophagy-related 32) is one of the key factors for mitophagy. Atg32 is a ~59kDa mitophagy receptor transmembrane protein spanning through the outer mitochondrial membrane. Atg32 is conserved throughout the budding yeasts[41]. Various studies showed that knockout of Atg32 leads to complete impairment of mitophagy; but it does not affect any other form of autophagy[41]. Atg32 can initiate mitophagy by two distinct ways; in the first mechanism, mitophagy induction causes phosphorylation of Atg32 at Ser114 and Ser119, facilitating the interaction of Atg32 with Atg11, followed by the Atg11 tethering with the PAS (Phagophore Assembly Site) for the selective sequestration of mitochondria[42]. Atg32 contains a conserved Atg8 family interacting motif (AIM), containing W/Y-X-X-L/I/V sequence where X can be any amino acids. The AIM of Atg32 helps in its interaction with Atg8, an autophagosome receptor protein (Fig. 2A)[43]. This interaction acts as a bridge between mitochondria and autophagosome. It helps in the engulfment of mitochondria into the autophagosome, which eventually is fused with the lysosomes for the ultimate degradation.

Mammalian cells have an evolved mitophagy mechanism, it can be further sub-classified in three

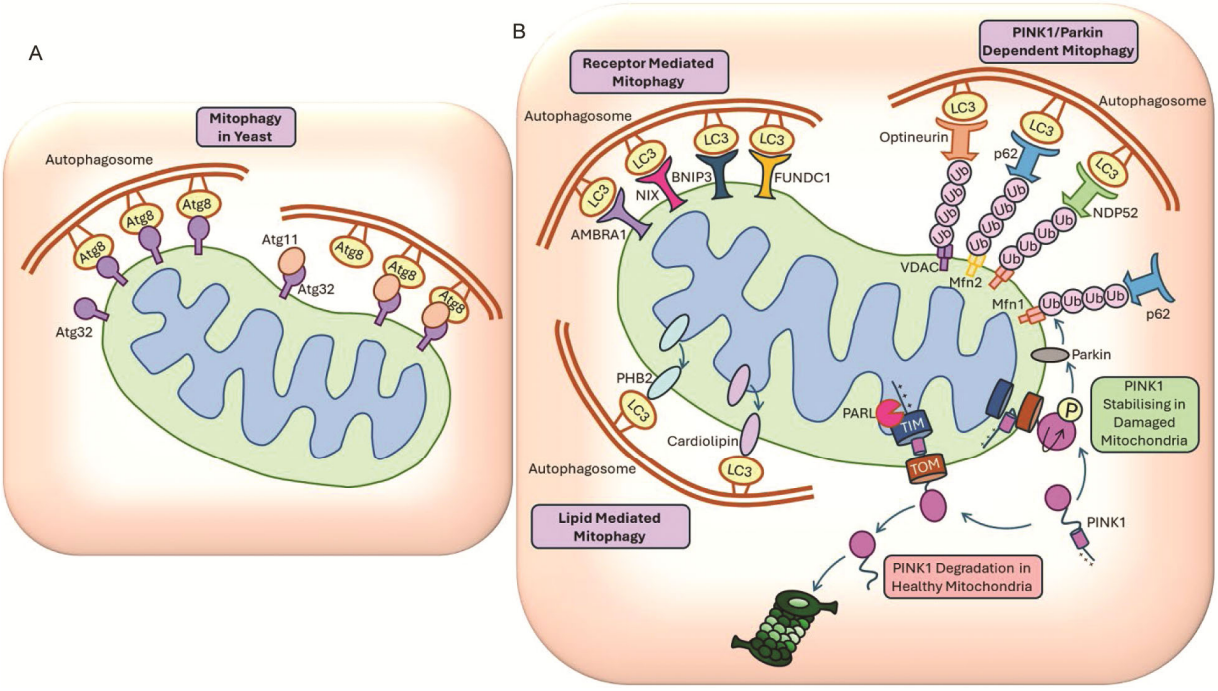

Fig. 2 — Schematic representation of mitophagy pathways takes place in yeast and humans. (A) In yeast, Atg32 is the key mitochondrial protein that Initiates mitophagy. Atg32 carries the LIR motif which helps in direct interaction with Atg8 present on autophagosome and initiates the engulfment of mitochondria. Another way Atg11 acts as the accessory protein which help in the bridging between Atg32 and Atg8 to initiate mitophagy; and (B) In humans, there are three pathways to initiate mitophagy 1. Receptor mediated mitophagy: The outer mitochondrial membrane contains several mitophagy receptors. AMBRA1, NIX, BNIP3, FUNDC1, *etc.*, which have conserved LIR motifs to directly interact with LC3 present on autophagosome. 2. PINK 1/Parkin dependent mitophagy: PINK I is an outer mitochondrial membrane protein in healthy mitochondria it gets degraded by the proteasome. While in damaged mitochondria it stabilizes on mitochondria and recruits Parkin (E3 ubiquitin ligase) which polyubiquitinates mitochondrial outer membrane proteins (Mfn1, Mfn2, VDAC, *etc.*), next the adaptor proteins present in cytosol recognizes the polyubiquitinated proteins and bind to them. Lastly, these adaptor proteins interact with autophagosome receptor LC3 *via* the LIR motif to initiate the encapsulation of mitochondria. 3. Lipid mediated mitophagy: Cardiolipin and PHB2 are inner mitochondrial membrane proteins however during mitochondrial stress they translocate to the outer mitochondrial membrane and directly interact with LC3 *via* the LIR domain to initiate the mitophagy

major categories, (1) PINK1/Parkin dependent mitophagy, (2) receptor-mediated mitophagy and (3) lipid-mediated mitophagy.

Firstly, PINK1/Parkin mediated mitophagy, is most widely studied mitophagy pathway. PINK1 (PTEN-induced putative kinase protein 1) is an outer mitochondrial membrane kinase protein. PINK1 acts as the surveillance protein which continuously checks for the health of mitochondria[44]. In healthy mitochondria, PINK1 spans through the outer mitochondrial membrane where the C-terminal of the protein faces the cytosol. On the other hand, the N-terminus of PINK1 spans through the TIM23 translocase into the mitochondrial matrix. The N-terminal MTS region present in the matrix gets cleaved by MPP (Mitochondrial processing peptidase), while the transmembrane domain of PINK1 is cleaved by PARL (PINK1/PGAM5-associated rhomboid-like protease)[45]. Thereafter, the cleaved PINK1 is translocated back to the cytosol, where it is cleared out by the proteasome system[46]. However, when the mitochondrial membrane is depolarised because of stress, the positively charged MTS domain of PINK1 fails to span through TIM23 translocase and resides in the IMS (Inter-membrane space) of mitochondria and bypasses the proteolytic cleavage. This event helps in the permanent establishment of PINK1 on the outer mitochondrial membrane allowing it to undergo autophosphorylation. Later it also phosphorylates the cytosolic E3 ubiquitin ligase Parkin at Ser65 position, which allows the conformational changes in Parkin structure by exposing the mitochondrial targeting region, facilitating the recruitment of Park in on the outer mitochondrial membrane[47]. As already stated, Parkin is an E3 ubiquitin ligase; it initiates the polyubiquitination of several of its substrate proteins like MARF (mitochondrial assembly regulatory factor), Mfn1, Mfn2 (mitofusins) and VDAC (voltage dependent anion channel) presenton outer mitochondrial membrane[48]. These polyubiquitinated proteins now act as degradation markers, which are recognized by the mitophagy adaptor proteins p62, Optineurin, NDP52 (nuclear dot protein 52 kDa), NBR1 (neighbour of BRCA1 gene 1) andTAX1BP1 (Tax1 (human T cell leukemia virus type I) binding protein 1) *via* their ubiquitin interacting domain. The other part of these mitophagy adaptors possesses a conserved LIR (LC3 interacting region) motif, W/Y/F-X-X-L/I/V where X can be any amino acid. LIR allows the interaction of tethered adaptor proteins with the autophagosome receptor protein LC3 and facilitate the autophagosome formation around the damaged mitochondria and facilitate the degradation *via* lysosomal fusion (Fig. 2B)[49].

Another mechanism of mitophagy that helps in the clearance of mitochondria is known as receptor-mediated mitophagy. As the name suggests, it requires mitochondrial receptors, which directly interact with autophagosome receptor protein LC3 *via* the LIR motif (Fig. 2B). As these receptors carry conserved LIR motif, it bypasses the requirement of PINK1 and Parkin[50]. There are several mitochondrial receptors reported to initiate mitophagy by their own, *e.g*. BNIP3 (BCL2 interacting protein 3), NIX (Nip3-like protein X), FUNDC1 (FUN14 domain-containing 1), AMBRA1 (activating molecules in Beclin1-regulated autophagy) and PHB2 (prohibit 2)[49]. The activation of receptor-mediated mitophagy occurs during certain stress and developmental stages. During hypoxic conditions, HIF1 (hypoxia inducing factor 1) activates the transcription of BNIP3 and NIX[51]. Phosphorylation of BNIP3 and NIX increases their interaction with LC3[52]. FUNDC1 is also reported to act in similar manner during hypoxic condition[53-55].

Lipid-mediated mitophagy is the third type of mitochondrial clearance mechanism. Cardiolipin is the key player in lipid-mediated mitophagy; it is a specific phospholipid present in the inner mitochondrial membrane. However, during mitochondrial damage and compromised membrane potential, cardiolipin moves to the outer mitochondrial membrane. Cardiolipin and ceramide have the potential to directly interact with autophagosome receptor LC3 to initiate the autophagosome formation around the damaged mitochondria in neurons and neuronal cells (Fig. 2B). Neuronal cell injury showed increased cardiolipin translocation from inner to outer mitochondrial membrane, suggesting the mechanism of mitophagy might vary from one cell type to another[56].

Mitochondria-associated degradation (MAD)

The Mitochondria- Associated Degradation (MAD) pathway is an important pathway of mitochondrial quality control, which ensures the disposal of imperfect or misfolded mitochondrial proteins *via* the ubiquitin- proteasome system (UPS). This pathway particularly targets the damaged mitochondrial

proteins rather than the entire organelle, therefore maintaining mitochondrial function and cellular homeostasis. Peripheral outer mitochondrial and membrane-embedded proteins are degraded by UPS co-factors by the MAD pathway. This pathway resembles the endoplasmic reticulum-associated degradation (ERAD) pathway[57]. In both instances, misfolded proteins are ubiquitinated, extracted from organelles or membranes by a protein complex containing Cdc48 (VCP/p97 in mammals) and are degraded by the proteasome machinery[19]. Cdc48/VCP binds to its various cofactors *e.g*., Npl4p (nuclear protein localization protein 4), Ufd1 (ubiquitin recognition factor in ER associated degradation 1), p47, Shp1 (Src homology region 2 domain-containing phosphatase 1), and Doa1, *etc. via* its N-terminal domain, which determines pathway selectivity and substrate processing[58]. Recent works show that Doa1 (Cdc48 cofactor) possesses a novel ubiquitin-binding domain that binds with ubiquitinated mitochondrial proteins and Cdc48 on the mitochondrial surface[59]. Doa1 in basal conditions as well as during oxidative stress condition, act as a regulator of MAD pathway[60]. *DOA1* deletion causes accumulation and mislocalisation of substrates on mitochondria[61]. Vms1 (VCP/Cdc48-associated mitochondrial stress responsive 1) has also been implicated in recruiting Cdc48p to mitochondria under oxidative stress[19]. Vms1, despite being a cytosolic protein harbours a non-canonical Mitochondrial Targeting Domain (MTD). N-terminal of Vms1 negatively regulates its MTD and restricts its localization to mitochondria under physiological conditions. During oxidative stress, the Vms1-N-terminal-MTD interaction is disrupted, allowing Vms1 to localize in mitochondria; this helps Cdc48 to bind with ubiquitinated mitochondrial proteins[61]. An additional role of Vms1 was also reported in mitoRQC pathway, where it protects mitochondria from toxicity of aberrantly formed truncated proteins synthesized on stalled ribosomes[62]. Vms1's mammalian homologue, ANKZF1 (ankyrin repeat and zinc-finger domain-containing 1) also interacts with VCP (Cdc48 homolog), but role of this interaction in the MAD pathway is not yet clear. MAD also plays role in mitochondrial fusion, as it regulates inhibition of mitochondrial fusion by degradation of Fzo1 (the first identified MAD substrate in yeast) which causes mitochondrial fission and mitophagy[63]. Fzo1 degradation is regulated by Doa1, Cdc48, Ufd1 and Npl4 complex, while in stressed condition it is regulated by Vms1, Cdc48 and Npl4 complex (Fig. 3 lower right panel)[19,61]. However, the role of Vms1 in Fzo1 degradation is still debatable[64]. Other identified MAD substrates are Msp1p, Tom70p and Mdm34p (Mitochondrial Distribution and Morphology Protein 34) in yeast and mitochondrial fusion proteins (Mfn1 and Mfn2) and anti-apoptotic protein like Mcl-1in mammalian cells[19,61,65]. So far, very less knowledge about MAD pathway is available in literature. Whether VCP (the mammalian homologue of Cdc48), ANKZF1 or Doa1 homolog function similarly, remains to be elucidated.

MitoRQC: Mitochondrial ribosome quality control pathway

The cellular proteome requires constant monitoring of the quality of its components since many factors lead to the production of aberrant toxic proteins. One of the factors that disrupts protein homeostasis is aberrant translation, which can accumulate defective proteins and impede cellular function. RQC (Ribosome Quality Control) is one of the co-translational quality control pathways conserved from bacteria to eukaryotes which detects ribosome stalling or aberrant translation, eliminates aberrant proteins that have been erroneously produced, and is essential to protein homeostasis[66]. The greater portion of research on the RQC pathway has focused on cytosolic proteins. Still, proteins that must be delivered to membrane-bound organelles or secreted to the exterior may also undergo stalling during synthesis. When mitochondrial pre-proteins are subjected to similar problems, then this specific quality control is called the mitoRQC (Mitochondrial Ribosomal Quality Control)[13].

Translational stalling on damaged or truncated mRNAs is detrimental to all cells because it prevents ribosomes from producing active proteins and may promote the synthesis of cytotoxic truncated proteins. To deconstruct such stalled complexes, ribosome-associated quality control (RQC) pathways have evolved in all domains of life. Pelota/Dom34 (ribosome dissociation factor) and ABCE1 (ATP-binding cassette subfamily E member 1)/Rli1 recognize stalled 80S ribosomes and split them into small 40S and large 60S subunits in eukaryotes. The RQC pathway processes the molecules that come from the synthesis of 60S-peptidyl-tRNA complexes. Here, conserved NEMF (Nuclear export mediator factor)-family proteins, such as Rqc2p in yeast and

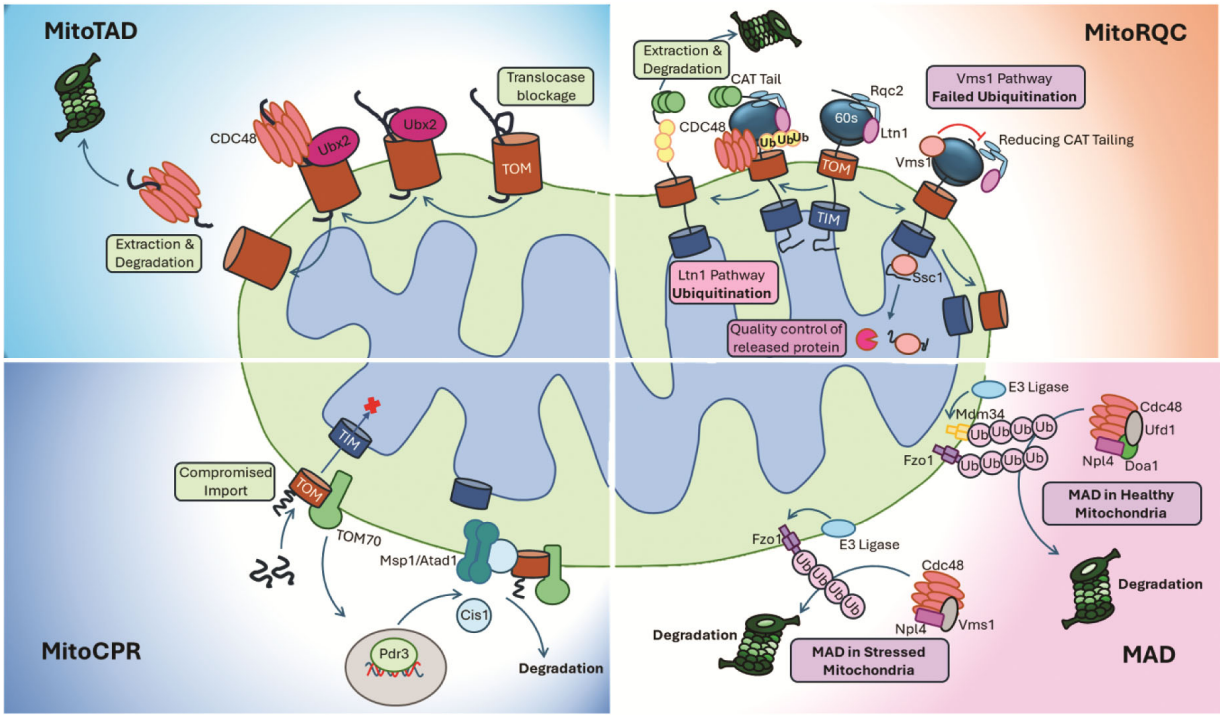

Fig. 3 —Mitochondrial Ribosome Quality Control pathway (MitoRQC): During translation defect and ribosome stalling, Rqc2 and Ltn1 facilitate the C-AT tailing and polyubiquitiation of nascent peptide for its degradation *via* the proteasome. Sometimes these proteins can cause aggregation inside mitochondria. so Vms1 helps in reduced C-AT tailing of nascent protein, and with the help of mitochondrial matrix chaperones and proteases degrades these problem causing nascent peptide chains. Mitochondria Associated Degradation (MAD) pathway: Doa1 with the help of Np14 and Udf1 recruits Cdc48 which extracts the polyubiquitinated outer mitochondrial membrane proteins and facilitates their degradation *via* the proteasome. In some cases, Vms1 assists Np14 during the recruitment of Cdc48 to extract the membrane proteins from mitochondria. Mitochondria Translocation Associated Degradation (MitoTAD): In healthy mitochondria when the mitochondrial precursor proteins are clogged in TOM complex during translocation, Ubx2 recruits Cdc48 which helps in the extraction of the clogged precursor proteins from translocases and facilitates their degradation *via* proteasome system. Mitochondria Compromised Protein Response (MitoCPR): Similar to mitoTAD, but when the situation cannot be controlled by mitoTAD, mitoCPR gets activated. Compromised protein import sends signal to nucleus where Pdr3 upregulates the expression of Cisl which translocates to outer mitochondrial membrane and recruits Atad1/Msp1 to initiate the extraction of clogged precursor proteins from TOM complex

NEMF in humans, aid in adding C-terminal alanine and threonine (CAT) tails to the developing polypeptide chains. Furthermore, Ltn1 (Listerin) ubiquitinates the nascent polypeptides extracted by p97/Cdc48 prior to proteasomal degradation[67].

Using the yeast model it has been shown that Vms1, a cytosolic protein along with other proteins like Ltn1- a RING domain E3 ubiquitin ligase, Rqc2 and Rqc1 make the RQC protein complex that protects against mitochondrial toxicity due to ribosome stalling. Ribosome stalling happens due to a lack of a stop codon in the mRNA, truncated mRNA generation, stable secondary structure in the mRNA, or an insufficient supply of charged tRNAs. Stalling causes activation of the machinery which further causes dissociation of the 80S ribosome and the subsequent retention of the peptidyl-tRNA by the 60S subunit. Then the RQC component Rqc2 and the E3 ubiquitin ligase Ltn1 recognize these 60S ribosome-nascent peptide complexes. To effectively recruit Ltn1 to the 60S ribosome and ubiquitylate the developing peptide, Rqc2 is necessary. Next, Rqc1, together with Cdc48 and Npl4/Ufd1, makes it easier for the ubiquitylated chain to be removed from the ribosome so that it can be destroyed by proteases[68].

Apart from recruiting Ltn1, Rqc2 modifies the stalled nascent chains by the C-terminal addition of variable length sequences consisting of alanine and threonine residues as non-canonical elongation known as CAT-Tailing as a fail-safe mechanism that enables the degradation of a far broader range of substrates by exposing lysine(s) sequestered in the ribosome exit tunnel. Lysines must be present close to the Ltn1p RING domain for RQC-dependent degradation of nascent polypeptides, and CAT tailing precisely

facilitates this since occasionally premature termination leaves the ubiquitylation site (lysine) inside the large ribosomal tunnel[69]. Accumulation of excessive CAT-tailed product can be detrimental to the cell as it can sequester the chaperone and other cellular machinery's activity, cytosolic Vms1 as an inhibitor of CAT-tailing by inhibiting Rqc2, which prevents mitochondrial dysfunction caused by imported CAT-tailed polypeptides (Fig. 3 upper right panel).

Diseases have been associated with defective RQC, especially those that impact the neurological system. While mutations in RQC-related variables have been reported in patients with early-onset neuromuscular problems and autism, malfunctioning RQC in mice results in progressive neurodegeneration[70,71].

Mitochondrial translocation-associated degradation (Mito TAD)

Mitochondria precursor proteins tend to fold prematurely or aggregate despite the various quality control mechanisms. So, when there is clogging of the TOM complexes by the improper precursor protein, elicits a strong stress response. The Mitochondrial translocation-associated degradation (Mito TAD) is a surveillance system that continuously keeps a check on the TOM complex[72] (Fig. 3 Upper left panel). Ubiquitin regulator X (Ubx2)/UBXUD8 containing a UBX domain that binds to the AAA protein cdc48/p29 (VCP) and Ubx2 also possess the ability to localize to the ER (Endoplasmic reticulum) and lipid droplets. Ubx2 that localizes to the ER acts as a docking site for cdc48 and promotes the ER-associated degradation (ERAD) of the misfolded protein on the TOM complex[73]. cdc48 being an AAA ATPase extractase acts on the clogged TOM complex by removing the accumulated protein by ATP hydrolysis followed by proteasomal degradation. Npl4 and Ufd1 act as co-factors with cdc48[73]. Recently a newly discovered E3 ubiquitin ligase MARCH 5 (membrane-associated RING-CH-type finger 5) binds to the TOM70, a subunit of the TOM complex leading to the proteasomal degradation of mitochondrial precursor protein[74]. A recent study showed, that a deubiquitylating enzyme USP30 is responsible for removing the ubiquitin from the accumulated precursor protein from the clogged TOM complex and their subsequent import into the mitochondria[75]. The mechanism of recognition of the accumulated precursor proteins on the TOM complex is not understood clearly yet and needs to be further explored.

Mitochondrial compromised protein import response

Protein synthesis and targeting of the newly synthesized proteins to their cognate organelle is an essential process for the appropriate functioning of a eukaryotic cell. To ensure the successful delivery of newly synthesized protein/membrane proteins to its cognate organelle requires a specific signal sequence with the machinery of the target compartment to recognize it. The universally conserved co-translational protein sorting pathway involved in majority of the sorting process is mediated by the Signal recognition particle (SRP), its membrane receptor, and the Sec61 protein translocation channel[76]. However, a subset of membrane proteins exists known as Tail Anchored (TA) proteins. The unique characteristic of TA proteins is the presence of a single transmembrane (TMD) at the C-terminus and most of the protein extending into the cytoplasm. TA proteins play numerous critical roles in cellular processes like intracellular trafficking, example, SNARE proteins, regulation of apoptosis *e.g*. Bcl_2 family proteins; various maturation and import proteins like TOM20; proteins involved in the organelle biogenesis for example Peroxisomal membrane protein 15 (Pex15); proteins involve in organelle ultrastructure likeFis1 (Fission-1) and proteins involved in metabolism *e.g*. CPT1 (Carnitine palmitoyltransferase1) and Cyb5 (cytochrome b5)[77]. Moreover, TA proteins possess a distinct topology that excludes them from being targeted by the canonical co-translational Signal recognition pathway. Instead, TA proteins are targeted by the non-canonical post-translational GET (Guided Entry of Tail anchored proteins) pathway in yeast and by TRC (TMD Recognition Complex) in mammals[78]. The majority of the TA proteins are targeted to two distinct organelle membranes, ER membrane and mitochondrial outer membrane TA proteins targeted to the ER and Mitochondrial OMM (Outer Mitochondrial Membrane) are partitioned mainly based on the several properties encoded at the extreme C-terminal of the proteins and the TMD. TA proteins targeted to ER have longer trans-membrane regions, higher hydrophobic residues. ER TA proteins are more likely to form an alpha helix. While the outer mitochondrial membrane TA proteins possess shorter TMDs, lesser hydrophobic residues, and lesser propensity to form alpha-helix[79].

However, all cellular pathways are prone to error and during several instances, proteins designated for

one particular organelle get mistargeted to mitochondria, which leads to problems in folding and maturation of the mistargeted protein mitochondria leading to proteotoxic stress[80]. TA proteins possessing these subtle differences are often mistargeted due to malfunction of the GET machinery[81].

Recently, to combat the mitochondrial import stress a surveillance pathway was first discovered in budding yeast known as Mitochondrial Compromised Protein Import Response or MitoCPR. MitoCPR which is Mitochondrial Compromised Protein Response gets triggered upon protein import stress (stress due to inefficient import mitochondrial IMS) in mitochondria. The transcriptional factor PDR3 that is known to be associated with multi-drug resistance response (MDR) is activated in this pathway. Cis1 is the downstream transcriptional effector of PDR3[80]. Cis1, is the key regulator that recruits the AAA ATPase Msp1 in budding yeast[80]. Msp1 facilitates the removal and proteasomal degradation of un-imported precursors and mistargeted TA proteins located on the mitochondrial surface as well as translocase (Fig. 3 lower left panel)[82]. Msp1's mammalian homolog ATAD1, which is also an AAA ATPase known to function in a similar way. Deleting *MSP1* or a gene from the GET pathway individually do not result in severe growth defect in yeast, but the deletion of both leads to significant growth defects[82]. Msp1 functions by removing the mistargeted protein from the OMM in an ATP-dependent manner[83]. Some TA proteins upon extraction from the OMM retrace back to the ER, where they are targeted to their cognate organelle[84]. Mito-CPR is an overall protective mechanism of the mitochondria. It helps mainly with mitochondrial import stress. The functions and exact properties of the various proteins including Msp1 and ATAD1, are yet to be fully investigated.

Mitochondria derived vesicles (MDVs)

The mitochondria derived vesicles or MVDs were discovered in 2008 as a signaling and transport mediator between Mitochondria and Peroxisomes[85]. Mitochondrial-derived vesicles are a subtype of extracellular vesicles with a diameter ranging from 50-150 nm (Fig. 4). The vesicle formation capacity of the mitochondria is a mechanism that suggests it retains its bacterial ancestry[86]. MDV formation is evolutionarily conserved, as it has been observed is yeast, plants and mammals[87,88]. MDVs can be single-membrane or double-membrane bound, they can also act as cargo for specific mitochondrial nucleic acids, lipids, proteins, fragmented mitochondria, or other mitochondrial components[89] (Table 1). MDV formation under the Mitochondrial Quality Control (MQC) reported many sub-types of mitochondria-derived vesicles. MDV formation and heterogenicity

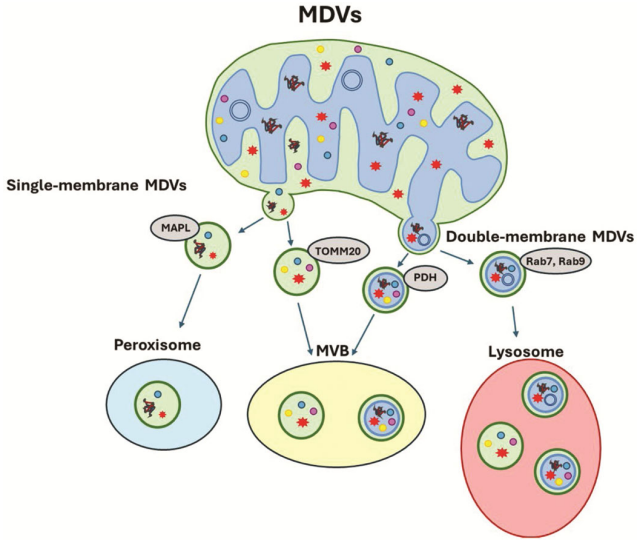

Fig. 4 — Mitochondria Derived Vesicles (MDVs) There are different subtype of MDVs. MVDs are classified based on their cargo and origin from the membrane. Mitochondria-anchored protein ligase (MAPL) and translocase of outer mitochondria membrane (TOMM20) are the markers of single membrane MDVs. The single membrane MDVs contain proteins from the outer membrane of mitochondria (OMM). MAPL+MDVs are destined for Peroxisome, and TOMM+MDVs fuse to multivesicular body (MVB) processes like exosomes. The double-membrane MDVs contain proteins from the outer membrane, inner membrane protein (IMM), and mitochondria matrix proteins. Pyruvate dehydrogenase is a marker for double-membrane MDVs, which are excreted by the MVB process. Rab7/Ras9 (Ras-related protein) can mediate MDVs fusion to late endosome/lysosome and mediated antigen presenting *via* MHCI

Table 1 — Types of MDVs

	Origin	Protein Marker
Single Membrane Bound	Buds intensively from the outer mitochondrial membrane consisting of outer mitochondrial proteins.	Translocase of outer mitochondrial membrane 20 (TOMM20) and Mitochondrial anchored protein ligase (MAPL)
Double Membrane Bound	Buds from outer and inner mitochondrial membranes consist of inner mitochondrial membranes and matrix proteins.	Pyruvate dehydrogenase subunits (PDH), Rab7/Rab9 and Mitochondrial stress protein 70 (mtHSP70)

are related to various processes like biogenesis of peroxisomes, fission of mitochondria, oxidative stress resistance, innate immunity signaling, and infections[90]. Different stresses like oxidative stress are known to cause mitochondrial damage; these damaged part can also be pinched off from mitochondria in the form of MDVs which later get fused with late endosome or lysosome for degradation[91]. Another mechanism of biogenesis of MDVs involves the recruitment of the Ras-related Protein 9 (Rab9) and SNX9 (sorting nexin 9) involving the clathrin protein[85], this links mitochondria to innate immunity by mediating mitochondrial antigen presentation followed by the proteasomal degradation of mitochondrial component and fusing to lysosomes, presenting mitochondrial antigen to Major Histocompatibility Complex I (MHCI) at the ER and subsequent transfer to the cell surface[92]. another study showed MDV biogenesis in the resting state stage based on the microtubule-associated motor protein, MIRO1, MIRO2 (Mitochondrial Rho GTPase), and DRP1 (Dynamin Related Protein1)[93]. Specific mitochondrial compartments direct the generation of vesicles[89].

The formation of MDVs helps to sustain the mitochondrial proteome (>1000 proteins) and maintain the functional integrity of the cell[94]. During increased hypoxia conditions, there is an elevation in the production of MDVs carrying the B-cell lymphoma (BCL-2) factors and subsequent release surge mitochondrial-mediated apoptosis and ease myocardial ischemia[9]. Further investigation of MDVs related to the molecular mechanism of their formation, selection of cargo, and its implication in physiological role are yet to be explored.

Conclusion

Mitochondria are undoubtedly the hub of the major metabolic processes and face major challenges due to imbalanced proteostasis. In this review, we have outlined the possible ways of mitochondrial proteotoxicity and the important mitochondrial stress response mechanisms that help in restoring the homeostasis. In this review we illustrated the stress response pathways individually, although during any environmental or other stress, these pathways may work in a highly collaborative manner to increase the possibility of cell survival.

In the past decade, researchers have decoded several mysteries regarding mitochondrial stress response mechanisms, but still there are many voids which needs to filled to get a complete understanding of each of these mechanisms.

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Conflict of interest

All authors declare no conflicts of interest.

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