Review

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Stress-responsive regulation of mitochondria through the ER unfolded protein response

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The endoplasmic reticulum (ER) and mitochondria form physical interactions involved in the regulation of biologic functions including mitochondrial bioenergetics and apoptotic signaling. To coordinate these functions during stress, cells must coregulate ER and mitochondria through stress-responsive signaling pathways such as the ER unfolded protein response (UPR). Although the UPR is traditionally viewed as a signaling pathway responsible for regulating ER proteostasis, it is becoming increasingly clear that the protein kinase RNA (PKR)-like endoplasmic reticulum kinase (PERK) signaling pathway within the UPR can also regulate mitochondria proteostasis and function in response to pathologic insults that induce ER stress. Here, we discuss the contributions of PERK in coordinating ER–mitochondrial activities and describe the mechanisms by which PERK adapts mitochondrial proteostasis and function in response to ER stress.

ER stress impacts mitochondrial function through interorganellar signaling

The traditional view of ER and mitochondria as discreet intracellular organelles has been profoundly altered in recent years. Unlike the well-defined organelles described in cell biology textbooks, the ER and mitochondria are highly dynamic and undergo continuous structural and spatial reorganization in response to specific cellular signals. An interesting aspect of these organelles is that they form physical ER–mitochondrial contacts (reviewed in [1–3]). These contacts facilitate the transfer of metabolites, including lipids and Ca^{2+}, between the ER and mitochondria that are involved in the regulation of biologic functions including lipid homeostasis, mitochondrial metabolism, and the regulation of apoptotic signaling (Box 1). Thus, ER–mitochondrial contacts serve as a platform for interorganellar communication, essential for the coordination of cellular function.

A consequence of the physical and functional interaction between ER and mitochondria is that mitochondria function is sensitive to pathologic insults that induce ER stress (defined by the increased accumulation of misfolded proteins within the ER lumen). ER stress can be transmitted to mitochondria by alterations in the transfer of metabolites such as Ca^{2+} or by stress-responsive signaling pathways, directly influencing mitochondrial functions. Depending on the extent of cellular stress, the stress signaling from the ER to mitochondria can result in prosurvival or proapoptotic adaptations in mitochondrial function.

During the early adaptive phase of ER stress, ER–mitochondrial contacts increase, promoting Ca^{2+} transfer between these organelles [4]. This increase in Ca^{2+} flux into mitochondria stimulates mitochondrial metabolism through the activity of Ca^{2+}-regulated dehydrogenases involved in the tricarboxylic acid (TCA) cycle. The increased activity of these dehydrogenases promotes mitochondrial respiratory chain activity, resulting in a transient increase in mitochondrial ATP synthesis during the initial phase of ER stress. This surge in bioenergetic capacity increases the available energetic resources to mount an adaptive response and alleviate ER stress. Alternatively, chronic exposure to ER stress negatively impacts cellular metabolism by reducing mitochondrial respiration and decreasing cellular ATP levels [4,5]. This has been shown to lead to depletion of Ca^{2+} stores in the ER and increased Ca^{2+} within mitochondria ([6,7] and discussed below). Ultimately, this signaling results in mitochondrial fragmentation and the opening of the mitochondrial permeability transition pore (MPTP), which initiates intrinsic apoptotic signaling and programmed cell death. Varying levels of ER stress in multiple cell types have also been reported to impact other mitochondrial functions including mitochondrial DNA (mtDNA) biogenesis [8], the transcription of respiratory chain subunits [5], and increases in mitochondrial-derived reactive oxygen species (ROS) [5,9,10], further reflecting the capacity for ER stress to influence mitochondrial function.

Many metabolic diseases including nonalcoholic fatty liver disease, type 2 diabetes (T2D), and obesity are associated with unresolved ER stress, suggesting that mitochondrial dysfunction in these diseases may be dysregulated through mechanisms involving ER stress-dependent alterations in ER–mitochondria communication [11,12]. For example, stress-dependent alterations

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Box 1. Metabolite transfer through ER–mitochondrial contacts

ER and mitochondria form tight physical junctions stabilized by tethering complexes anchored in the ER and mitochondrial outer membrane (reviewed in [1–3]). In higher eukaryotes, these tethers are mediated by interactions between ER-localized MFN2 with MFN2 and MFN1 in the mitochondrial outer membrane. These tight interactions facilitate the transfer of metabolites between the two organelles (Figure I).

Transfer of Ca^{2+} between the ER and mitochondria is a major function for ER–mitochondrial contacts and is carried out through the IP_3R and VDAC transporters localized to the ER and mitochondria outer membranes, respectively (see [1–3]). These channels form a tight interaction stabilized by the cytosolic isoform of the mitochondrial HSP70 chaperone HSPA9/GRP75/mortalin. Ca^{2+} is imported into the mitochondrial matrix through the high-capacity, low-affinity mitochondrial Ca^{2+} uniporter (MCU). The close physical proximity between these various Ca^{2+} transporters at ER–mitochondrial contacts increases local Ca^{2+} concentration to levels sufficient to drive import through MCU into the mitochondrial matrix.

Flux of Ca^{2+} through the ER–mitochondrial contacts is highly regulated by accessory proteins both at the ER and mitochondria membranes [1–3,6]. ER-localized phosphofurin acidic cluster sorting protein 2 (PACS2) recruits the chaperone calnexin to the ER luminal face of MAMs to mediate their formation and stability. The ER Sigma-1 receptor stabilizes IP_3R and promotes protective ER to mitochondria Ca^{2+} exchange in response to ER Ca^{2+} depletion. Alternatively, MCU regulators including MICU1 and MCUR1 have also been identified to influence ER–mitochondria Ca^{2+} transfer and Ca^{2+}-regulated mitochondrial activities [1–3,6]. ER–mitochondrial Ca^{2+} transfer is also influenced by a truncated isoform of SERCA (S1T) localized to MAMs that can promote ER Ca^{2+} leakage and mitochondria Ca^{2+} overload associated with cellular death [1–3]. These regulators provide a significant level of control over ER–mitochondrial Ca^{2+} transfer, reflecting the importance of this process in cellular physiology.

Apart from Ca^{2+}, other metabolites including lipids are also transferred between the ER and mitochondria through ER–mitochondrial contacts [1–3]. Lipid biosynthesis enzymes involved in the synthesis of phospholipids, cholesterol metabolites, and sphingolipids localize to the ER and mitochondrial membranes. Lipid transfer between the ER and mitochondria is required for the biosynthesis of these critical metabolites, including cardiolipin (CL). CL has been shown to have a variety of essential functions in the mitochondria including maintaining membrane curvature at cristae tips and providing structural integrity to both electron transport chain and mitochondrial import complex components [93–96]. The synthesis of CL involves the transfer of ER-derived phosphatidic acid to the mitochondrial inner membrane followed by the action of a cascade of mitochondrial enzymes including cardiolipin synthase (CLS). Thus, maintaining ER–mitochondrial contacts is critical for the proper synthesis of essential lipids, such as CL, and for maintaining normal mitochondrial function and cellular physiology.

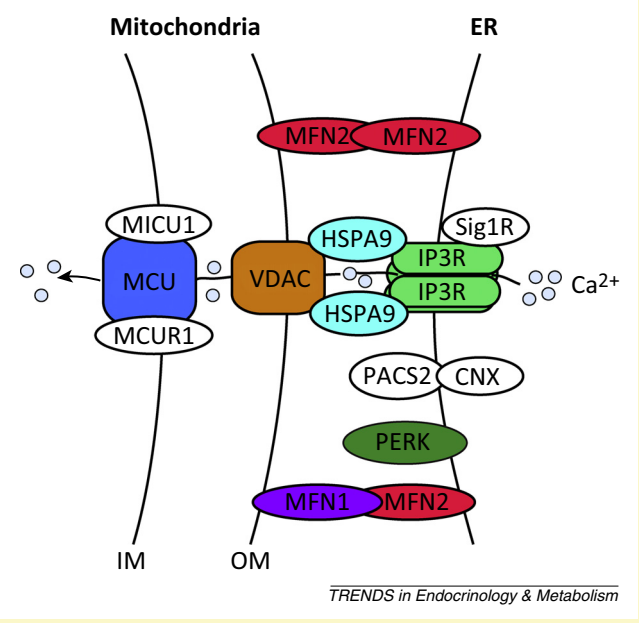

Figure I. Illustration of the components and interactions of proteins localized to ER–mitochondrial contacts. The colored proteins represent core components of ER–mitochondrial contacts required for organelle tethering (MFN2 and MFN1) or Ca^{2+} transfer between these organelles (IP_3R, VDAC, MCU, and HSPA9). The white proteins are regulatory factors that influence the Ca^{2+} signaling through ER–mitochondrial contacts. Abbreviations: ER, endoplasmic reticulum; MFN, Mitofusin; IP_3R, inositol trisphosphate receptor; VDAC, voltage-dependent anion-selective channel; MCU, mitochondrial Ca^{2+} uniporter.

in ER–mitochondrial Ca^{2+} transfer has been proposed to contribute to the pathophysiology of T2D where increased cytosolic calcium leads to aberrant insulin signaling in the pancreas and disrupted routine metabolic functions (e.g., gluconeogenesis) in the liver [13]. Increased activation of TOR signaling has also been linked to the development of metabolic disease [14]. Interestingly, in addition to promoting a diabetic phenotype, ablation of tuberous sclerosis complex (TSC), a suppressor of TOR activity, also induces chronic ER stress. Alleviation of this ER stress reestablishes insulin sensitivity even in the background of sustained TOR activation suggesting that chronic UPR activation has detrimental metabolic consequences [15].

ER stress and mitochondrial dysfunction are also intricately linked in the pathology of other diseases including α1-antitrypsin deficiency [16,17], cardiovascular disorders [18,19], and neurodegenerative diseases such as Alzheimer's disease [20,21], Parkinson's disease [22,23], and amyotrophic lateral sclerosis [24,25]. Despite the pathologic relationship between ER stress and mitochondrial dysfunction in these diseases, the specific contributions of altered ER–mitochondrial communication in disease pathogenesis are only beginning to come to light. For example, familial Alzheimer's disease is associated with mutations in presenilins 1 and 2 (PS1 and PS2), which are involved in the generation of the toxic Amyloid β (Aβ) peptide [20,21]. PS1 and PS2 are enriched in a subcompartment of the ER physically associated with mitochondria called mitochondrial-associated ER membranes (MAMs) and appears to be involved in coordinating ER–mitochondrial Ca^{2+} and lipid transfer, suggesting that these mutations could directly contribute to disease pathogenesis through alterations in ER–mitochondrial signaling [20,21,26–29]. These data suggest that dysregulation of ER–mitochondrial signaling could broadly contribute to the pathogenesis of human diseases with diverse etiologies. Therefore, the possibility of intervening in the transfer of chronic ER stress to mitochondria could be a promising avenue for therapeutic development for many of the debilitating diseases mentioned above.

The PERK signaling pathway of the UPR regulates mitochondrial function during ER stress

The predominant stress-responsive signaling pathway that regulates cellular physiology during ER stress is the UPR (reviewed in [30–32]). The UPR consists of three

integrated signaling pathways activated downstream of the transmembrane ER stress sensor proteins inositol requiring enzyme 1 (IRE1), activating transcription factor 6 (ATF6), and PERK. ER stress-dependent activation of these pathways protect essential ER activities and relieve ER stress through transient translational attenuation and the transcriptional upregulation of stress-responsive genes primarily involved in the regulation of ER protein homeostasis (or proteostasis). Although ATF6 and IRE1 primarily function to regulate ER proteostasis and function [30–32], it is becoming increasingly apparent that, in addition to its role in regulating ER proteostasis, the PERK arm of the UPR also has an important role in regulating mitochondrial proteostasis and function during conditions of ER stress.

PERK activation influences cellular physiology during ER stress through translational attenuation and transcriptional signaling

PERK signaling is initiated in response to ER stress by the dissociation of the ER heat shock protein 70 (HSP70) chaperone binding immunoglobulin protein (BiP) from the PERK sensing domain localized to the ER lumen (Figure 1) [30–32]. Dissociation of BiP leads to the activation of PERK through a mechanism involving PERK dimerization and autophosphorylation of the cytosolic PERK kinase domain. The active, phosphorylated PERK kinase selectively phosphorylates the serine 51 residue of the α subunit of eukaryotic initiation factor 2 (eIF2α) [33]. This phosphorylation inhibits the activity of the eIF2B GTP exchange factor involved in ribosomal translation initiation [34,35]. Thus, the initial impact of PERK on cellular physiology is a global attenuation of new protein synthesis that reduces the folding load of newly synthesized proteins entering the ER lumen, freeing ER proteostasis factors to alleviate the toxic accumulation of malfolded proteins within the ER lumen.

Interestingly, eIF2α phosphorylation also results in the activation of stress-responsive proteins. Transcripts of these proteins are selectively translated during conditions of eIF2α phosphorylation through a mechanism dependent on upstream open reading frames (uORFs) in their mRNA [34,35]. One of the predominant genes translated downstream of PERK-dependent eIF2α phosphorylation is the stress-responsive transcription factor ATF4 [9,36]. ATF4 induces the expression of a variety of stress-responsive genes involved in critical biologic processes including cellular redox maintenance, amino acid biosynthesis, and other stress-responsive transcription factors including the C/EBP homologous protein (CHOP/GADD153) [9,36]. CHOP in turn is involved in the induction of additional stress-responsive genes including ER oxidase 1 (ERO1) and the protein phosphatase 1 (PP1) regulatory subunit GADD34/PPP1R15A [37–39], which dephosphorylates eIF2α and restores translational integrity in a well-established negative feedback loop of PERK signaling [40,41]. Additionally, in response to chronic ER stress and subsequent PERK activation, high levels of CHOP can also promote activation of intrinsic apoptotic signaling pathways (discussed below). Thus, through this dual prosurvival/proapoptotic signaling mechanism, PERK serves as a critical regulator of cellular fate during conditions of ER stress. Importantly, whereas ER stress induces eIF2α phosphorylation through PERK, other eIF2α kinases such as PKR (interferon-induced, double-stranded RNA-activated protein kinase), GCN2 (general control nonderepressible 2), and HRI (heme-regulated inhibitor) induce eIF2α phosphorylation in response to other cellular insults including viral infection, nutrient deprivation, heme deficiency, and oxidative stress [35,42].

PERK coordinates ER–mitochondrial activities in the presence and absence of ER stress

PERK is intimately involved in defining ER–mitochondrial interorganellar signaling. PERK is enriched in MAMs (the subcompartment of the ER that interacts with mitochondria), localizing this ER stress sensor to ER–mitochondrial contact sites [43,44] (Box 1). PERK has been proposed to interact with the mitochondrial tethering protein Mitofusin-2 (MFN2), indicating that PERK may stabilize ER–mitochondrial contacts [45]. Consistent with this prediction, genetic depletion of PERK disturbs ER morphology and reduces the number of ER–mitochondrial contacts [43]. Furthermore, PERK depletion in MFN2$^{-/-}$ mouse embryonic fibroblasts (MEFs) reduces ROS, normalizes mitochondrial respiration, and improves mitochondrial morphology [45]. These results suggest a functional interaction between MFN2 and PERK involved in dictating ER–mitochondrial contacts, although the underlying physical relationship between these proteins requires further characterization. The stabilizing effects of PERK on ER–mitochondrial interactions appear to be dependent on the PERK cytosolic kinase domain as overexpression of PERK lacking this domain does not rescue defects in ER–mitochondrial contacts [43]. By contrast, overexpression of a kinase dead PERK restored ER–mitochondrial contacts to levels similar to those observed in wild type cells [43]. This indicates that stabilization of ER–mitochondrial contacts afforded by PERK is likely to be structural in nature and not dependent on canonical PERK signaling through eIF2α.

Apart from the structural role for PERK in stabilizing ER–mitochondrial contacts, cells deficient in PERK signaling have defects in mitochondrial functions. PERK-deficient cells show defects in regulating electron transport chain activity reflected by increased basal and maximal respiration [45]. They also show perturbed responses to ER stress including an abnormal increase in ROS and defects in mtDNA biogenesis [8–10]. The regulation of intrinsic apoptosis is also impaired in PERK-deficient cells. PERK$^{-/-}$ cells are hypersensitized to apoptosis induced by the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor thapsigargin or the inhibitor of N-linked glycosylation tunicamycin [39,43,46], while these same cells are more resistant to apoptosis induced by ER-derived ROS [43]. Disrupting downstream PERK signaling also sensitizes mitochondria to stress *in vivo*. For example, conditional knockin of the nonphosphorylatable serine 51 to alanine (S51A) eIF2α mutant (eIF2α^{S51A}; a mutant resistant to ER stress-induced, PERK-dependent eIF2α phosphorylation [33]; Figure 1) into the pancreas leads to mitochondrial damage in pancreatic β cells – a cell type highly dependent on UPR signaling due to maintain high

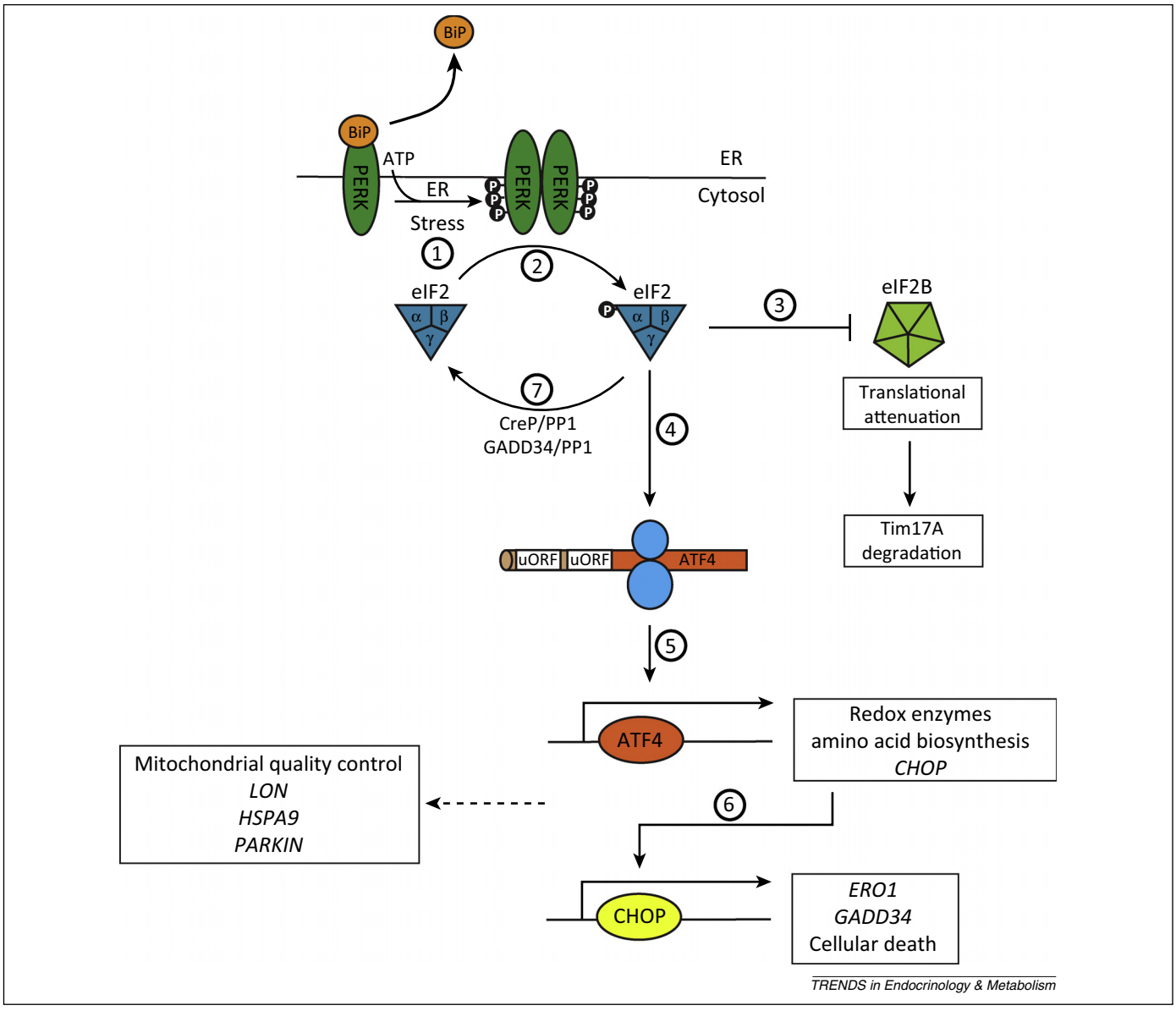

Figure 1. PERK-dependent signaling through eIF2α phosphorylation. In response to ER stress, the ER Hsp70 chaperone BiP dissociates from PERK, inducing PERK autophosphorylation and dimerization (step 1). Active PERK selectively phosphorylates the α subunit of eIF2 (eIF2α) (step 2). Phosphorylated eIF2α inhibits the eIF2B GTP exchange factor, resulting in global translation attenuation (step 3). This results in the downstream degradation of the core TIM23 subunit Tim17A and reduced mitochondrial protein import. Alternatively, certain stress-responsive genes such as the transcription factor ATF4 are selectively translated during conditions of eIF2α phosphorylation through upstream open reading frames (uORFs) localized to the 5′ UTR, allowing synthesis of these proteins during stress (step 4). Active ATF4 induces expression of cellular proteostasis genes involved in cellular redox and amino acid biosynthesis, as well as additional stress-responsive transcription factors such as CHOP (step 5). CHOP in turn is involved in the induction of additional stress-responsive genes including ER oxidase 1 (ERO1), the protein phosphatase 1 (PP1) regulatory subunit GADD34 and cell death factors (step 6). This pathway is repressed through dephosphorylation of eIF2α through the activity of PP1 mediated by the constitutively expressed regulatory subunit CReP/PPP1R15B or the stress-induced regulatory subunit GADD34/PPP1R15A (step 7). Mitochondrial quality control factors including *LON*, *HSPA9*, and *PARKIN* are also transcriptionally induced during ER stress through PERK-dependent ATF4 activation, although the specific downstream transcription factors required for their transcription are currently undefined. Adapted from [30]. Abbreviations: PERK, protein kinase RNA (PKR)-like endoplasmic reticulum kinase; ER, endoplasmic reticulum; Hsp70, heat shock protein 70; BiP, binding immunoglobulin protein; eIF2α, α subunit of eukaryotic initiation factor 2; TIM23, translocase of the inner membrane 23; ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein.

levels of insulin secretion [32]. Furthermore, PERK mutations in humans have been suggested to induce hepatic mitochondrial dysfunctions associated with the rare disease Wolcott–Rallison syndrome, although this genetic relationship requires further study [47,48].

Altered PERK activity contributes to the pathogenesis of metabolic diseases

ER and mitochondria in specialized tissues such as the pancreatic islets and liver are critical for maintaining glucose and lipid homeostasis in organisms. Metabolic disease states such as T2D and obesity have been clearly linked to both ER and mitochondria dysfunction. Although cellular studies have suggested a protective role for the PERK arm of UPR in the acute phase of ER stress, sustained activation of PERK is detrimental, promoting apoptosis and tissue inflammation [49]. In chronic metabolic disease such as diabetes associated with obesity, sustained activation of PERK may overwhelm the ability of cells to restore homeostasis and favor pathogenesis. For

example in diabetic *ob*/*ob* mice, ER stress appears to promote diabetic pathology including insulin insensitivity and glucose intolerance. Chemical chaperones such as 4-phenylbutyric acid (4-PBA) or tauroursodeoxycholic acid (TUDCA) alleviate ER stress by facilitating the folding of proteins in the ER. Administration of these molecules attenuates UPR signaling and restores metabolic homeostasis [50]. Further supporting a role for dysfunction in ER–mitochondrial communication in the pathology of metabolic disease, ablation of MFN2 in liver or skeletal muscle likewise leads to diabetic phenotypes. These are associated with sustained PERK activation and mishandling of glucose in these tissues [51]. Cell based studies suggest that suppression of PERK signaling either by knockdown of PERK or alleviating ER stress improve the metabolic disruptions in MFN2$^{-/-}$ cells [45]. These data suggest that although typical transient UPR signaling may be beneficial in resolving ER stress and preserving mitochondrial function, sustained activation of the UPR can promote pathophysiologic changes in metabolism and disease.

The above results suggest that PERK has a protective role in regulating mitochondrial function in the presence or absence of ER stress. Despite this evidence, it is difficult to determine whether the mitochondrial defects in PERK-deficient cells reflect a decreased capacity for PERK to directly regulate mitochondrial function or an indirect consequence of aberrant PERK signaling that impair mitochondrial function. Regardless, new results are emerging that highlight a protective role for PERK in regulating mitochondrial proteostasis and function in response to acute ER stress, which are discussed below.

The regulation of mitochondrial quality control through PERK signaling

Mitochondrial quality control pathways regulate mitochondrial proteostasis during stress to prevent the accumulation of misfolded proteins that can lead to mitochondrial dysfunction and cellular pathology. Mitochondria maintain their proteome on three distinct organizational levels: molecular, organellar, and cellular fate via apoptosis [52,53]. The capacity to differentially influence mitochondrial proteostasis through these pathways provides a mechanism to sensitively regulate mitochondrial integrity in response to the diverse extents and types of cellular insults encountered in human physiology. As discussed below, PERK directly influences mitochondrial quality control at each organizational level, suggesting that PERK signaling is a primary mechanism for regulating mitochondria proteostasis and function during ER stress (Figure 2).

PERK-dependent regulation of molecular quality control pathways

In response to moderate levels of stress, mitochondrial proteostasis is maintained by a network of quality control factors involved in mitochondrial protein import, folding, and proteolytic pathways (Box 2). PERK affects the composition and activity of these pathways through both transcriptional and post-translational mechanisms, directly increasing cellular capacity to protect the mitochondrial proteome from damage that can occur in response to ER stress (e.g., increased ROS).

PERK activation induces the downstream expression of mitochondrial quality control factors such as the matrix-localized AAA$^+$ quality control protease LON during ER stress [9,37,54] (Figure 1). Although the specific transcription factors required for the increased expression of *LON* remain to be identified, this process requires the activity of the PERK-regulated transcription factor ATF4 [54]. LON is a critical regulator of mitochondrial proteostasis that functions in many aspects of mitochondrial biology including the degradation of oxidatively damaged mitochondrial proteins [55–57], the assembly of electron transport chain complex IV [54,58], and the regulation of mtDNA transcription and replication through the degradation of the

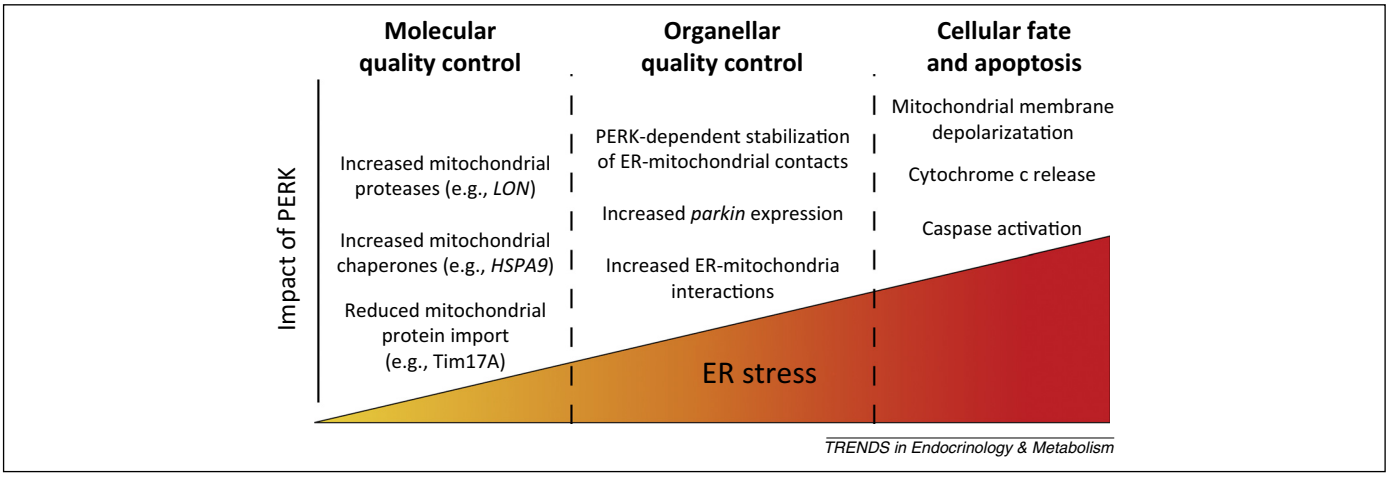

Figure 2. PERK activity regulates mitochondrial quality control on the molecular, organellar, and cellular level. In response to increasing levels of stress, cells regulate mitochondria on three levels mediated by molecular, organellar, and cellular quality control pathways. PERK activity directly influences each of these levels of quality control regulation. In response to mild levels of ER stress, PERK adapts molecular quality control pathways through the transcriptional induction of mitochondrial proteases (e.g., *LON*) and chaperones (e.g., *HSPA9*) downstream of the PERK-regulated transcription factor ATF4. PERK activation also adapts mitochondrial protein import activity through the degradation of the core TIM23 subunit Tim17A. In response to more moderate levels of ER stress, PERK influences mitochondrial quality control on the organellar level. PERK directly stabilizes ER–mitochondrial contacts and promotes formation of ER–mitochondrial interactions through the transcriptional upregulation of *Parkin*. In response to severe stresses, PERK is directly involved in the activation of mitochondrial-derived intrinsic apoptotic signaling through both transcriptional and post-translational mechanisms that induce mitochondrial membrane depolarization, cytochrome *c* release, and caspase activation. Abbreviations: PERK, protein kinase RNA (PKR)-like endoplasmic reticulum kinase; ER, endoplasmic reticulum; TIM23, translocase of the inner membrane 23; ATF4, activating transcription factor 4.

Box 2. Molecular mitochondrial quality control pathways

The vast majority of the mitochondrial proteome is encoded by the nuclear genome. These proteins are synthesized on cytosolic ribosomes and are directed to mitochondria by mitochondrial-targeting sequences (MTS) localized to the N terminus or internally within the polypeptide sequence (reviewed in [60,71]). These targeting sequences direct nuclear-encoded mitochondrial proteins to the translocase of the outer mitochondrial membrane (TOM) complex, which facilitates translocation across the outer mitochondrial membrane into the intermembrane space (IMS) (Figure I). In the IMS, polypeptides are sorted to specific multisubunit translocases and pathways that facilitate targeting to different intramitochondrial environments. For example, the 2/3 of the mitochondrial proteome targeted to the mitochondrial matrix as soluble proteins or single-pass inner membrane proteins are directed to the translocase of the inner membrane 23 (TIM23) by N-terminal targeting sequences. TIM23 facilitates translocation across the inner membrane into the mitochondrial matrix in a process dependent on the ATP-dependent activity of the mitochondrial HSP70 HSPA9/mortalin/Grp75 in the presequence associated motor (PAM) complex and the electrochemical gradient across the inner mitochondrial membrane. Once localized, the N-terminal targeting sequence is removed by mitochondrial processing peptidase (MPP), releasing the mature polypeptide to engage colocalized mitochondrial chaperoning pathways including the mitochondrial HSP70 chaperoning pathway [HSPA9 (mortalin)/DNAJA3 (Tid1)/GRPEL1], the HSP60 chaperonin (HSP60/HSP10), and the mitochondrial HSP90-like chaperone TRAP1 of the mitochondrial matrix [97]. The interactions with these chaperones and folding factors facilitate the proper folding of mitochondrial proteins into their functional conformation. Alternatively, proteins unable to fold into functional conformations or those that are damaged or misfolded during stress are degraded by ATP-dependent mitochondrial quality control proteases localized throughout mitochondria including the soluble matrix proteins LON and CLPP/CLPX and the inner membrane proteases AFG3L2 and paraplegin (with active sites directed towards the mitochondrial matrix) and YME1L (with active sites directed towards the IMS) [52].

The importance of these quality control pathways is evident, as mutations in genes encoding many of these quality control factors including *HSP60*, *SPG7*, and *AFG3L2* predispose individuals to numerous diseases including many neurodegenerative disorders [53]. Furthermore, the expression of these proteins is highly regulated during stress through stress-responsive signaling pathways such as the mitochondrial unfolded protein response (UPR^{mt}) – a stress-responsive signaling pathway that induces mitochondrial proteostasis genes following mitochondrial matrix stress [73]. Thus, through the activity of these mitochondrial proteostasis pathways and through their regulation by stress-responsive signaling pathways such as the UPR^{mt} and the PERK arm of the UPR mitochondrial proteostasis is maintained in response to a wide range of pathologic insults, preventing the aberrant accumulation of misfolded proteins within mitochondria that can disrupt mitochondrial function.

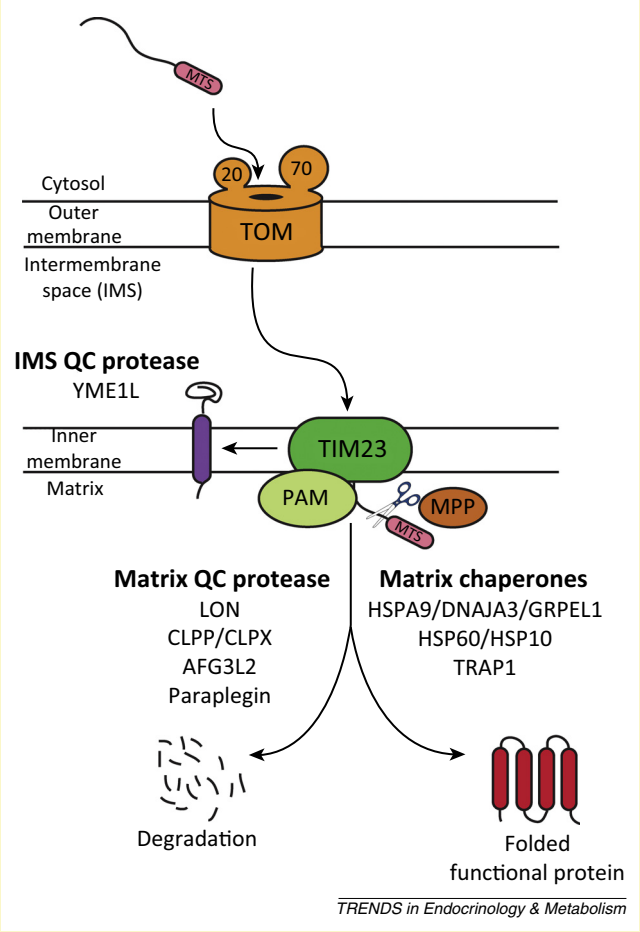

Figure I. Illustration showing the molecular pathways responsible for the import, folding, and proteolysis of nuclear-encoded mitochondrial proteins targeted to the mitochondrial matrix. Mitochondrial matrix-targeted proteins and single-pass inner membrane proteins are directed to the TOM–TIM23 import pathway for translocation across the outer and inner mitochondrial membranes in a mechanism dependent on the ATPase activity of HSPA9 in the PAM complex and the electrochemical gradient across the outer and inner mitochondrial membranes. Following translocation, the N-terminal targeting sequence is removed by MPP, releasing the mature polypeptide to interact with mitochondrial chaperones that facilitate folding into a functional conformation. Proteins unable to fold or those unable to maintain their folded conformation are degraded by the activity of mitochondrial quality control proteases. Abbreviations: TOM, translocase of the outer membrane; TIM23, translocase of the inner membrane 23; MPP, mitochondrial processing peptidase; PAM, presequence associated motor.

mitochondrial transcription factor TFAM [59]. Overexpression of LON and LON variants has been shown to prevent mitochondrial dysfunction in response to Brefeldin A-induced ER stress [54], suggesting that PERK-dependent increases in LON protect the mitochondrial proteome during ER stress.

Other mitochondrial quality control factors such as the HSP70 ATP-dependent chaperone HSPA9/GRP75/mortalin are also induced downstream of PERK during ER stress through a mechanism dependent on ATF4 [9,54] (Figure 1). HSPA9 is involved in several protective mitochondrial proteostasis functions including the import of newly synthesized proteins into mitochondria [60,61], the refolding of misfolded or aggregated proteins within the mitochondrial matrix [62], and the cytosolic linking of ER–mitochondrial contacts through interactions with inositol trisphosphate receptor (IP_3R), a membrane glycoprotein Ca^{2+} channel activated by inositol trisphosphate, and voltage-dependent anion-selective channel protein 1 (VDAC1), a major component of the outer mitochondrial membrane [63]. Again, overexpression of HSPA9 attenuates ROS and increases cellular viability during ER stress induced by glucose deprivation, suggesting that PERK-dependent increases in HSPA9 protect mitochondrial function during ER stress [64]. Similarly, HSPA9 overexpression attenuates cell toxicity induced by proteotoxic Aβ involved in Alzheimer's disease [65,66], whereas HSPA9 knockdown sensitizes cells to Aβ toxicity [65,67]. The importance for HSPA9 regulation is also evident *in vivo*, where alterations in HSPA9 post-translational modifications and/or protein

levels have been found in patients with neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease [68].

PERK also influences mitochondrial proteostasis through translational attenuation. The core Tim17A subunit of the translocase of the inner membrane 23 (TIM23) import complex is selectively degraded following translational attenuation induced by eIF2α phosphorylation [69]. TIM23 is responsible for importing mitochondrial proteins across the inner mitochondria membrane (Box 2). Adapting TIM23-dependent import, such as through Tim17A degradation, has been proposed to promote mitochondrial proteostasis through multiple mechanisms [69]. Reduced TIM23 import will decrease mitochondrial accumulation of newly synthesized, unfolded proteins that challenge mitochondrial proteostasis pathways, reducing the folding load on mitochondrial chaperones and proteases and freeing these factors to protect the integrity of the established mitochondrial proteome – a protective mechanism similar to that afforded to the ER environment by PERK-dependent translational attenuation. Adapting TIM23 subunit composition could also alter the selectivity of mitochondrial protein import, providing a mechanism to directly adapt mitochondrial proteome composition and function during ER stress, a mechanism similar to that mediated by post-translational phosphorylation of the translocase of the outer membrane (TOM) [70,71]. Finally, attenuation of TIM23-dependent import provides a mechanism for the activation of stress-responsive transcription factors such as the mammalian homolog of *Caenorhabditis elegans* ATFS-1 (activating transcription factor associated with stress-1) – a transcription factor involved in the upregulation of mitochondrial proteostasis genes whose activation requires stress-dependent reductions in mitochondrial protein import [72,73].

The capacity for PERK to regulate mitochondrial proteostasis pathways during ER stress displays distinct parallels to the role for the UPR in regulating ER proteostasis pathways involved in ER protein folding, trafficking, and degradation. These results demonstrate an emerging role for UPR signaling in coordinating the regulation of ER and mitochondrial environments during ER stress through PERK signaling. Similarly, other eIF2α kinases have also been shown to influence mitochondrial quality control in response to stresses including inflammation, mitochondrial dysfunction, and lipotoxicity [74–76]. This indicates that eIF2α phosphorylation is a general mechanism to protect mitochondria during conditions of stress. As new transcriptional and proteomic approaches are being applied to study the impact of stress on cellular physiology, additional mitochondrial proteostasis factors will likely be identified to be transcriptionally or post-translationally regulated through PERK or other eIF2α kinases, further defining the intricate role for this pathway in regulating mitochondria proteostasis environments in response to pathologic insults.

PERK-dependent regulation of organellar quality control

Global mitochondrial dysfunction induced by stress can be further corrected through organellar quality control pathways involved in mitochondrial fusion and fission processes (reviewed in [77,78]). Mitochondrial fusion can rescue global defects in specific mitochondrial pathways (e.g., electron transport chain) through content mixing, allowing restoration of pathway integrity in the absence of new protein synthesis. Mitochondrial fusion proteins can also promote protective interactions with other organelles such as the ER, facilitating the exchange of metabolites that can stimulate mitochondria function. Alternatively, mitochondrial fission allows cells to segregate dysfunctional mitochondria (often identified by depolarization of the mitochondrial membrane). Once segregated, these mitochondria can be targeted to organellar degradation by the lysosome in a process referred to as mitophagy.

Above, we discuss the structural role for PERK in stabilizing ER–mitochondrial contacts, reflecting a protective role for PERK in mitochondrial organellar quality control. During ER stress, PERK signaling can also regulate organellar mitochondrial quality control through the transcriptional upregulation of the E3 ligase Parkin through the downstream activation of the PERK-regulated transcription factor ATF4 [79,80]. Parkin overexpression increases ER–mitochondrial interactions favoring interorganellar Ca^{2+} exchange and mitochondrial bioenergetics [81]. Conversely, depletion of Parkin decreases ER–mitochondrial contacts, indicating a defect in ER–mitochondrial tethering [81]. Parkin overexpression also attenuates ER stress-induced cell death, suggesting a protective role for PERK-dependent Parkin induction [79–82]. Parkin has many functions that could be protective during ER stress including the ubiquitination of specific substrates to target them to proteasomal degradation, removal of damaged mitochondria through mitophagy, and nondegradative functions that promote cellular physiology during stress [83]. Although the specific contributions of PERK-dependent increases in Parkin expression on ER–mitochondrial or mitochondrial organellar quality control remain to be further established, the capacity for cells to increase Parkin during conditions of ER stress provides an additional level of mitochondrial quality control to promote mitochondria proteostasis and function.

PERK-dependent regulation of cellular fate

In response to cellular insults too severe to be corrected by adaptive responses such as those described above, cellular quality control pathways initiate apoptotic signaling and programmed cell death (reviewed in [7,84]). Severe ER stress has been connected to multiple cell death cascades including calpain activation, caspase-12 activation, and most prominently mitochondrial initiation of intrinsic apoptosis. In this latter process, ER stress leads to MPTP opening, membrane depolarization, and release of cytochrome *c* through a mechanism involving oligomerization of BAX and BAK, the central proapoptotic BCL-2 (B cell lymphoma 2) family proteins, on the mitochondrial outer membrane [7,85]. Free cytochrome *c* in the cytosol induces formation of the apoptosome, activation of initiator caspase-9, and subsequent activation of the executioners caspase-3/caspase-7 that mediate the cell death program.

The PERK arm of the UPR extensively connects ER stress to intrinsic apoptosis. Chronic activation of PERK by sustained ER stress induces high expression of the transcription factor CHOP downstream of ATF4. $CHOP^{-/-}$

cells show a significant attenuation in ER stress-induced apoptosis, implicating this transcription factor in ER stress-induced cellular death [86,87]. CHOP appears to play multiple roles in apoptosis related to the regulation of mitochondrial-derived apoptotic signaling. It can drive the transcription of proapoptotic BCL-2 protein family members such as BiM (BCL-2 interacting mediator of cell death) and PUMA (p53 upregulated modulator of apoptosis) and repress production of the prosurvival BCL-2 protein thus favoring the oligomerization of BAX/BAK at the mitochondrial outer membrane [88,89]; however, this is not observed in all cells suggesting cell type-specific signaling [37]. In addition, translational attenuation induced by PERK suppresses the activity of prosurvival MCL-1 due to its short half-life compounding the promotion of proapoptotic signaling induced by CHOP [90]. Similarly, PERK-dependent translational attenuation and ATF4 activation decreases levels of prosurvival X-linked inhibitors of apoptosis (XIAP), which directly inhibits caspase activity [91]. CHOP-dependent expression of other target genes including GADD34 and ERO1 have also been proposed to induce apoptosis through increased ROS [92]. CHOP has also been suggested to promote the expression of ATF5, which can enhance apoptotic signaling through the increased expression of NOXA, a proapoptotic BCL-2 family protein that promotes BAX/BAK-dependent apoptosis [39]. Finally, the coactivation of ATF4 and CHOP downstream of PERK is suggested to influence cell survival by increasing protein synthesis, leading to increases in ROS and depleting ATP to trigger apoptotic signaling [37].

Despite the heavy focus on CHOP, overexpression of CHOP alone is insufficient to induce apoptosis, indicating that other mechanisms must similarly contribute to PERK-dependent apoptotic signaling [37]. This could be, in part, attributed to contributions of other UPR signaling pathways, such as the IRE1-dependent recruitment of tumor necrosis factor receptor-associated factor 2 (TRAF2) and induction of c-Jun N-terminal kinase (JNK) signaling (reviewed in [7,84]). The inherent complexity of UPR-mediated apoptotic signaling likely reflects the requirement for multiple checks and balances when defining cellular fate following pathologic insults that induce ER stress; such a level of redundancy results from the dual nature of the UPR in dictating prosurvival and proapoptotic signaling in response to ER stress. Additionally, different cells likely depend on distinct pathways to influence ER stress-induced cellular death through direct modulation of mitochondrial effectors (e.g., BAK/BAX oligomerization) or through post-translational regulation of proapoptotic signals such as Ca^{2+} mobilization and ROS. Ultimately, this level of complexity underscores the importance of ER–mitochondrial coordination and PERK signaling in dictating cell fate decisions in response to pathologic insults that induce ER stress.

Concluding remarks and future perspectives

The study of ER–mitochondrial contacts and the regulatory pathways that coordinate their interorganellar functions is in its infancy. Despite significant progress in the past 5 years, we are only beginning to understand the critical regulatory role of signaling pathways in coordinating ER–mitochondrial function in the context of cellular stress. Here, we describe our current understanding of the contributions of PERK and PERK-regulated signaling on ER–mitochondrial communication and the regulation of mitochondrial proteostasis. These results demonstrate the capacity for PERK to coordinate ER and mitochondrial function in response to ER stress as part of the global UPR. In addition to the questions brought up in the context of this review (Box 3), new exciting questions are emerging as ongoing research efforts define the molecular mechanisms by which stress-responsive signaling pathways such as those regulated by PERK influence mitochondrial proteostasis and function. For example, does PERK signaling intersect with other stress-responsive signaling pathways involved in regulating mitochondrial proteostasis and function during stress (e.g., other arms of the ER UPR or the mitochondrial UPR)? Do other stress-responsive eIF2α kinases similarly influence mitochondrial proteostasis and function in response to non-ER stress based cellular insults (e.g., nutrient deprivation, oxidative stress, viral infection)? Is there a therapeutic opportunity to attenuate mitochondrial dysfunction in human disease by targeting adaptive PERK signaling pathways that promote mitochondrial proteostasis and function or in suppressing chronic signaling that may promote pathology? These are just a few examples of the exciting questions that make the study of UPR-dependent regulation of mitochondrial function an exciting area for future research.

Box 3. Outstanding questions

- What are the contributions of ER stress-dependent alterations in ER–mitochondrial signaling for the pathophysiology of human diseases?
- What are the molecular factors that interact with PERK at sites of ER–mitochondrial contact sites and how do they influence PERK-dependent regulation of mitochondrial function?
- How does PERK-dependent adaptation of mitochondrial quality control pathways influence mitochondrial proteostasis and function during conditions of ER stress?
- What are the specific molecular factors that underlie the complexity of mitochondrial-derived apoptotic signaling induced downstream of PERK?

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