Published in final edited form as:

Curr Mol Med. 2013 February ; 13(2): 317–329.

Cell Death and Survival Through the Endoplasmic Reticulum-Mitochondrial Axis

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Abstract

The endoplasmic reticulum has a central role in biosynthesis of a variety of proteins and lipids. Mitochondria generate ATP, synthesize and process numerous metabolites, and are key regulators of cell death. The architectures of endoplasmic reticulum and mitochondria change continually *via* the process of membrane fusion, fission, elongation, degradation, and renewal. These structural changes correlate with important changes in organellar function. Both organelles are capable of moving along the cytoskeleton, thus changing their cellular distribution. Numerous studies have demonstrated coordination and communication between mitochondria and endoplasmic reticulum. A focal point for these interactions is a zone of close contact between them known as the mitochondrial-associated endoplasmic reticulum membrane (MAM), which serves as a signaling juncture that facilitates calcium and lipid transfer between organelles. Here we review the emerging data on how communication between endoplasmic reticulum and mitochondria can modulate organelle function and determine cellular fate.

Keywords

Cell death; endoplasmic reticulum; metabolism; mitochondria

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

1. ORGANELLE FUNCTION

The endoplasmic reticulum (ER) is an organelle whose primary function is protein synthesis and folding [1]. One third of cellular proteins are synthesized by ER-associated ribosomes, particularly those destined for secretion [2]. In the ER lumen, nascent polypeptides are modified with N-linked glycans and made accessible to proteins involved in proper protein folding and quality control [3, 4]. In addition, the ER is the main site for biosynthesis of a variety of lipids such as phospholipids, cholesterol, and ceramides, which are transported to other organelles and cellular membranes *via* vesicles of the secretory pathway [5].

ER is composed of a series of continuous membranous structures that are organized into subdomains that include the rough ER, smooth ER, transitional ER and the nuclear envelope [6]. The architecture of the ER changes continually according to cellular demand through the processes of fusion, fission, elongation and membrane degradation [6]. Structural differences correlate with differences in ER function [6]. The rough ER is mainly laminal and associated with polyribosomes for protein synthesis. The smooth ER is primarily composed of tubular structures. This is the site of phospholipid biosynthesis and is the chief point of contact with other organelles [6]. Changes in ER membrane curvature and structure are facilitated by several proteins, including GTPases and reticulons [6]. For a detailed view of ER structure and related processes, readers are referred to a recent excellent review by Park & Blackstone [6].

Mitochondria are essential for ATP production by oxidative phosphorylation and the synthesis of many important metabolites. Mitochondria are composed of an outer mitochondrial membrane (OMM) and an inner mitochondrial membrane (IMM) [7]. The OMM is highly permeable allowing small molecules to pass freely. The IMM is impermeable to most small molecules and ions, thus the pH and ion characteristics of the intermembrane space reflects that of the cytoplasm, whereas the ionic composition of the space inside the IMM, called the matrix, is distinct from that of the cytoplasm. The concentration of specific ions may be higher or lower than the cytoplasmic concentration as a consequence of pumps and exchangers in the IMM. For instance, the mitochondrial matrix acts as an important store of intracellular calcium. The IMM contains the electron transport complexes I–IV (ETC) of the oxidative phosphorylation system (OXPHOS) and is deeply folded into cristae increasing surface area [8]. The matrix contains many enzymes including those of the tricarboxylic acid (TCA) cycle and β -oxidation that generate NADH, which acts as an electron donor for OXPHOS [9, 10]. Electrons are passed from NADH to oxygen, the electron acceptor, through the ETC. In the process, protons are shuttled across the IMM to the intermembrane space generating an electrochemical gradient, whose potential energy is then used by complex V in the IMM to generate ATP. The IMM potential (ψ_m) also serves as an important signaling mechanism, especially during degradation of damaged mitochondria (mitophagy) and cell survival decisions.

Mitochondria are often depicted as discrete isolated entities scattered in the cytoplasm. However, similar to the ER, they actually form a dynamic interconnected network. Mitochondrial morphology is controlled by coordinated fusion and fission events, the

appropriate balance of which is essential to maintain mitochondrial stability and function [11].

ER and mitochondria play key roles in sensing and reacting to cellular stress. Moreover, an increasing number of studies have demonstrated coordination and communication between these two organelle networks in several cellular processes. This interplay influences both spatial and functional organization of the organelles and ultimately can determine cell survival.

1.1. Endoplasmic Reticulum is a Fundamental Sensor of Cellular Stress

The best characterized ER signaling pathway activated in response to stress is the unfolded protein response (UPR). The UPR is activated by the accumulation of misfolded proteins in the ER lumen, caused either by an increase in protein synthesis, outpacing protein processing or by deficiencies in protein folding, trafficking and degradation [12, 13]. In addition to intraorganellar signals, numerous factors external to the ER can induce UPR. For instance, physiological levels of post-prandial lipid and glucose concentrations have been shown to induce acute ER stress, highlighting the metabolic and physiological importance of this pathway [14].

Three major signaling pathways mediate the UPR. The upstream sensors are inositol-requiring protein 1 alpha (IRE1 α), eukaryotic translation initiation factor 2-alpha kinase (PERK) and activating transcription factor 6 (ATF6). These transmembrane ER proteins remain inactive as long as their luminal domains are bound by an ER-resident chaperone, the immunoglobulin heavy chain binding protein/78kDa glucose-regulated protein (BiP/GRP78). When misfolded proteins accumulate in the ER, BiP binds to exposed hydrophobic domains and is competed away from the sensors, allowing their activation [15] (Fig. 1A). The kinases PERK and IRE1 α are activated through oligomerization and trans-autophosphorylation. Whereas, ATF6, a transcription factor, translocates to the Golgi where it is cleaved by a protease to an active form, which then moves to the nucleus where it binds the promoters of genes upregulated by the UPR. Activated PERK phosphorylates the eukaryotic translation initiation factor 2-alpha (eIF2 α), attenuating general translation and inducing preferential translation of specific target proteins, such as the activation transcription factor 4 (ATF4). ATF4 induces expression of additional UPR response genes including the pro-apoptotic transcription factor CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP). The third member of the UPR, IRE1 α , acts by processing the mRNA of the X-box-binding protein 1 (XBP-1) such that it then encodes an active transcription factor that promotes the expression of genes involved in the reestablishment of ER homeostasis. For a more detailed description of the UPR, readers are referred to two excellent reviews [16, 17].

The ER is also involved in monitoring lipid membrane composition, helping to activate appropriate responses when lipid homeostasis is disrupted. For example, sterol levels are sensed directly by the sterol sensory domain of the sterol regulatory element-binding protein (SREBP) cleavage-activating protein (SCAP), an integral protein in the ER and nuclear envelope. In the presence of high cellular sterol concentrations SCAP retains SREBP at the ER membrane. When sterol concentrations are low, SCAP escorts SREBP to the Golgi for

proteolytic activation of SREBP as a transcription factor that promotes expression of genes involved in the cholesterol synthesis, thus restoring homeostasis [13, 14].

The ability of the ER to sense cellular stress facilitates cellular adaptation to a changing environment. In the early phase, the UPR is triggered as a compensatory mechanism to reestablish ER homeostasis and avoid cellular damage. This phase of UPR activation leads to an increase in chaperone synthesis, a general blockade of protein translation, an increase in ER volume and degradation of misfolded proteins, all of which seek to restore ER function and to reduce protein load in the ER. However, under conditions of prolonged ER stress, during the so-called late phase of the UPR, the same survival response can lead to activation of apoptotic pathways, culminating in caspase activation, mitochondrial damage and cell death [16, 17].

1.2. Mitochondrial Life and Death Decisions

In addition to pro-survival functions of ATP production and cellular metabolism, mitochondria play pivotal roles in cell death processes, during both apoptosis and necrosis. There are two different pathways to engage apoptosis: the extrinsic pathway, triggered by extracellular signals recognized by death receptors located in the plasma membrane; and the intrinsic pathway, in which internal triggers, such as DNA damage or oxidative stress, initiate the cascade of events leading to apoptosis. Mitochondria are involved both in the amplification of the external pathway and in the initiation of the intrinsic pathway. They serve as important sites of integration for anti- and pro-apoptotic signals for both pathways [18].

An initiating trigger for the intrinsic apoptotic pathway is a sudden loss of ψ_m across the IMM. As a result, several pro-apoptotic proteins are released to the cytoplasm, such as cytochrome c, apoptosis inducing factor (AIF) and second mitochondria-derived activator of caspases (Smac)/ direct IAP binding protein with low pI (Diablo) [18]. Loss of ψ_m , and the subsequent release of factors, occurs through the formation of a channel known as the mitochondrial permeability transition pore (MPTP). The exact composition and regulation of the MPTP is not fully understood but it is thought to form at sites where the IMM and OMM meet. Several proteins participate, such as the voltage dependent anion channel (VDAC), the IMM adenine nucleotide translocase (ANT), cyclophilin D in the matrix and members of the Bcl-2 family of proteins in the OMM (Fig. 1B). The Bcl-2 family has been classified as: a) anti-apoptotic, such as Bcl-2 and Bcl-XL; b) multidomain pro-apoptotic, including Bax and Bak; and c) pro-apoptotic BH3-only, such as Bid and Bim [19]. The balance between pro- and anti-apoptotic proteins determines the mitochondrial execution of the apoptotic program. Although each of these proteins can influence MPTP, emerging studies suggest that few of these proteins are absolutely required for activation of the intrinsic pathway. For a more detailed description of apoptosis, readers are referred to the following reviews [20, 21].

In contrast to apoptosis, catastrophic loss of ATP leading to necrosis classically has been understood as a spontaneous and unregulated form of cell death. However, several recent studies suggest that in some cases necrosis could be effectively regulated by mechanisms involving reactive oxygen species (ROS), calcium influx and opening of the MPTP [22]. For

example, in an animal model of heart failure, inhibition of MPTP protects cardiomyocytes from necrotic cell death, underlying the importance of mitochondria in cardiac necrosis [23]. Thus mitochondria play a role in multiple cell death pathways with the opening of the MPTP as a central trigger.

2. ER-MITOCHONDRIAL COMMUNICATION

ER and mitochondria each undergo constant remodeling of their respective architectures in response to cellular demand. Furthermore, both organelles have a dynamic capacity for intracellular motility, being able to change their distribution in the cell by moving along the cytoskeleton [24, 25]. This is important for cellular physiology, because it facilitates the positioning of organelles relative to one another and defines preferential sites of interaction. In recent years, the importance of motility and remodeling to ER-mitochondrial communication has become apparent.

2.1. ER-Mitochondrial Dynamics

ER-mitochondrial coupling is influenced by distribution and position of the organelles. Studies in neurons have demonstrated that ceramide induction of calcium transfer between ER and mitochondria is associated with organelle clustering at the centrosomal region [26]. This movement is regulated by cyclin-dependent kinase 5 (CDK5), through the microtubule-associated protein Tau. Accordingly, a mutation in Tau, rendering it unable to be phosphorylated by CDK5, inhibits organelle clustering and reduces calcium transfer. In a recent study, motor neurons in transgenic mice expressing a hyperphosphorylated Tau mutant showed increased ER-mitochondria coupling, corroborating *in vivo* the importance of the CDK5-Tau axis in the regulation of organelle topology [27].

Work from our laboratory has also demonstrated the involvement of the microtubule cytoskeleton in facilitating ER-mitochondria interactions [28]. Induction of ER stress in HeLa cells produces a global redistribution of ER and mitochondria towards the perinuclear region. This displacement depends on microtubule network integrity, as it is inhibited by nocodazole. Treatment of cells with cytochalasin B has no effect on organelle subcellular position, suggesting that actin fibers are not involved. We have also shown that organelle redistribution in response to ER stress is associated with an increase in ER-mitochondrial contacts at the perinuclear region. This agrees with earlier studies showing that ER and mitochondria, trafficking along acetylated microtubules, come in close association when approaching the perinuclear region [29]. The importance of this close organelle localization to programmed cell death is discussed in detail elsewhere [30].

The zone of close contact between ER and mitochondria is called the mitochondrial-associated ER membrane (MAM). It was originally thought to be simply a sign of ER contamination in mitochondrial fractionations, but pioneering studies led to its recognition as the interface between mitochondria and ER [31]. It may comprise as much as 20% of the OMM, and is held together by tethering proteins. Important proteins participate in and modulate this spatial interaction (Fig. 1C), specifically those of the calcium-handling machinery. The cytoplasmic isoform of the chaperone GRP75, for instance, forms a physical link between the calcium channels IP3R at the ER surface and VDAC at the OMM [32]. The

calcium-binding ER chaperone calnexin is also enriched at the ER-mitochondria interphase [32, 33]. This protein modulates the activity of the SERCA2b pump, supporting the concept of differential calcium handling at the ER-mitochondria interphase. Several other proteins are also present at the MAM, such as mitochondria-shaping proteins (Mitofusin-2), chaperones (sigma-1 receptor, oxidoreductases), sorting proteins (PACS-2), and other enzymes, many of which are summarized in Table 1. The composition of proteins at the MAM is regulated by the GTPase Rab32, which cofractionates with perinuclear membranes, identifying the juxtannuclear zone as a hot spot for ER-mitochondria communication [34].

2.2. Calcium Connection

Calcium is one of the most common and highly regulated second messengers. It is involved in diverse cellular processes, including exocytosis, muscle contraction, metabolism, gene transcription, proliferation and apoptosis. Under resting conditions cytoplasmic calcium levels are low, in the range of 30 nM, and tightly controlled by several membrane channels, pumps and exchangers [35]. ER is the main intracellular calcium reservoir. Calcium is released from the ER to the cytoplasm by two channels, the ryanodine receptor (RyR) and the inositol triphosphate receptor (IP3R). Calcium is returned to the ER primarily by the activity of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) [35].

Mitochondria can also store and release calcium. Calcium carries out various functions in mitochondria. One of the best-characterized roles for mitochondrial calcium is the activation of several dehydrogenases in the citric acid cycle, leading to an increase in oxidative phosphorylation and ATP synthesis. Four mitochondrial dehydrogenases are regulated by calcium. Pyruvate dehydrogenase [36], isocitrate dehydrogenase [37], and oxoglutarate dehydrogenase [36] are located in the mitochondrial matrix. Glycerol-3-phosphate dehydrogenase [38] is located in the inter-membrane space. Pyruvate dehydrogenase is of particular interest, because it is a key step coupling glycolysis with glucose oxidation. Consequently, it is highly regulated. Phosphorylation of pyruvate dehydrogenase reduces its activity [39]. Whereas, dephosphorylation by calcium-activated pyruvate dehydrogenase phosphatase restores activity [40]. Through these mechanisms calcium transfer to mitochondria plays a critical role in the regulation of cell metabolism. Preventing calcium entry into mitochondria reduces oxidative phosphorylation and compromises cell energetics [41].

Another important process regulated by mitochondrial calcium is the opening of the mitochondrial calcium-activated potassium channels [42]. These channels have been shown to protect cardiomyocytes against ischemic injury [43, 44], although the mechanism involved is not fully understood. The OMM, initially considered a permeable barrier, participates in mitochondrial calcium uptake [45, 46]. Calcium crosses the OMM through the low affinity channel VDAC, whose activity is regulated by chaperones [47], NADH levels [48] and Bcl-2 family proteins [49]. Calcium traverses the IMM through the mitochondrial calcium uniporter (mCU), driven by the ψ_{mt} [50]. Transport across the IMM is coupled with accumulation of inorganic phosphate. Both phosphate and calcium can be stored in the matrix as osmotically inactive precipitates. Release back into the cytosol can occur slowly through a calcium sodium exchanger (NCE) or in exchange for hydrogen ions

through a $\text{Ca}^{2+}/\text{H}^{+}$ exchanger [51]. Catastrophic loss of calcium occurs during opening of the MPTP. Initially, control of mitochondrial calcium homeostasis was not considered an important factor in total cellular calcium control due to *in vitro* studies describing the low calcium affinity of the mitochondrial uniporter [52]. However, using specific targeted recombinant probes, Rizzuto *et al.* showed that the rate of mitochondrial calcium uptake from the ER was similar rate to that observed by the cytosol [53] and helped establish the concept of microdomains of high calcium concentration around the ER [54]. Localization of mitochondria in close contact with ER has been verified directly both by fluorescent labeling [55] and electronic microscopy [56]. Calcium levels within the ER-mitochondria interphase have been determined to be as high as 9 mM [57, 58], highlighting the importance of distance as a key factor regulating calcium transfer between these two organelles.

ER-mitochondrial contacts facilitate calcium transfer and are in turn regulated by calcium. A truncated isoform of SERCA1 (S1T) is a calcium-pump defective variant of SERCA that is localized to ER-mitochondria microdomains and acts as a leakage channel [59]. S1T expression is induced by ER stress and leads to a reduction in mitochondrial motility and an increase in ER-mitochondrial contacts [60]. Local elevations in calcium diminish mitochondria motility by disrupting the interaction between kinesin motors and GTPases in the OMM, thus retaining mitochondria in opposition to sites of calcium release [61, 62]. This mechanism increases both local cytosolic calcium buffering capacity and delivery of ATP.

2.3. Lipid Connection

Mitochondria do not participate in classical vesicular trafficking, and thus they are not thought to interchange membrane and proteins easily with other organelles. However, mitochondria readily import phospholipids from the ER [63]. Lipid exchange between mitochondria and ER is thought to occur at specific contact sites that facilitate direct transfer from one bilayer to the other by flipping or other mechanisms, particularly in the MAM which is enriched in enzymes involved in lipid biosynthesis [64, 65] (Fig. 1D). This exchange of phospholipids not only provides a fundamental building block for mitochondrial membranes it also delivers phosphatidylserine synthesized in the ER to the IMM for decarboxylation to phosphatidylethanolamine. Recently, evidence of a protein bridge between ER and mitochondria came were described in yeast. The use of an artificial membrane-tethering protein construct lead to the identification of four proteins forming a complex called the ER-mitochondria encounter structure (ERMES) [66]. In yeast, ERMES is composed of Mdm12 (an OMM-associated protein), which associates with Mmm1 (an ER membrane protein), Mdm10 and Mdm24 [67]. Cells lacking individual subunits of this complex have lower levels of mitochondrial phospholipids [67]. This suggests that the ERMES complex could act as an MAM homologue in yeast, but the presence of a similar structure in mammals is yet to be confirmed. Interestingly, all members of ERMES were previously reported to be required for mitochondrial DNA (mtDNA) replication and morphology [68–70]. Furthermore, Mmm1 colocalizes with mtDNA nucleoids [71], and ERMES-deficient cells lose their mtDNA [69], supporting a model in which ER-mitochondrial interactions are fundamental components of mtDNA replication and /or maintenance. In addition, Mdm10 participates in the sorting and assembly machinery (SAM)

complex that mediates the insertion of β -barrel proteins to the OMM [70]. The presence of Mdm10 in both the ERMES and SAM could help regulate the accumulation of mitochondrial membrane phospholipids and proteins during mitochondrial biogenesis and turnover.

3. PATHOPHYSIOLOGICAL IMPLICATIONS OF THE ER-MITOCHONDRIA INTERPLAY

3.1. ER-Directed Mitochondrial Cell Death

ER-mitochondrial communication during apoptosis has been documented extensively. A large calcium influx into mitochondria from the ER can promote cell death, but the magnitude and outcome of the response is modulated by the presence of various Bcl-2 family proteins on either organelle [72, 73] (Fig. 2A). The antiapoptotic members Bcl-2 and Bcl-XL inhibit calcium delivery [74–76], whereas the pro-apoptotic members Bax/Bak, Puma and Bik stimulate delivery [77–79]. Bax and Bak localize to both mitochondria and ER [80], and their over-expression promotes apoptotic calcium mobilization [78]. Accordingly, Bax/Bak double KO cells are highly resistant to calcium-dependent death stimuli [81]. Restoration of ER-targeted Bak in these cells is sufficient to restore apoptosis sensitivity [82], suggesting that ER-localized Bak signaling provides a trigger for mitochondrial-dependent cell death [82].

Upon induction of apoptosis, MPTP opening occurs, the mitochondrial network fragments and cytochrome-c is released activating downstream caspases. Among the targets of caspases is BAP31 an ER membrane protein. Cleavage of BAP31 generates a pro-apoptotic p20 fragment that remains at the ER membrane and is necessary for the stimulation of mitochondrial calcium uptake from the ER and subsequent fragmentation of the mitochondrial network [83] (Fig. 2A). Exogenous inducers of apoptosis promote a physical contact between BAP31 and the OMM fission protein Fis1. This interaction is required for the activation of caspase-8 and generation of the p20 fragment, demonstrating a bi-directional communication between the two organelles and a pattern of feedback amplification [84]. Consistent with this, cell death caused by Fis1 over-expression is dependent on an ER calcium transmission [85].

BH3-only members of the Bcl-2 protein family play a prominent role in the ER-mitochondrial death axis, although, surprisingly, their modes of action appear to be diverse [86]. Activation of BIK, an ER BH3-only protein, results in mitochondrial fragmentation *via* activation of Drp-1, an important regulator of mitochondrial dynamics [87]. Bid, another BH3-only ER protein, is bound to PACS-2, an ER sorting protein that appears to be required for ER-mitochondria coupling [77]. During induction of apoptosis by sustained ER stress, PACS-2 participates in the trafficking of Bid to mitochondria, where it is cleaved by caspases, generating activated truncated Bid (tBid), which promotes cytochrome-c release and cell death [88]. tBid also facilitates the insertion of pro-apoptotic Bax/Bak into the OMM [88]. Interestingly, depletion of PACS-2 by siRNA leads to mitochondrial fragmentation but confers protection from staurosporine-induced apoptosis *via* the intrinsic

pathway highlighting the need for ER-mitochondrial communication in the induction of apoptosis [88].

As mentioned above, apoptosis is engaged when severe, prolonged ER stress overrides the UPR adaptive response, however the exact signals involved are not fully understood. The classic UPR PERK target gene CHOP is pro-apoptotic both suppressing the transcription of *Bcl-2* gene [89] and stimulating *Bim* and *Bax* expression [90, 91]. Recent reports show that CHOP also increases *Ero1 α* expression, which stimulates calcium release from IP3R, favoring the apoptotic calcium transfer [92]. The privileged localization of *Ero1 α* at MAM makes it an excellent candidate for a key regulator of Ca^{2+} fluxes between organelles [93]. Another mediator of ER stress induced apoptosis is activation of the c-Jun N-terminal kinase (JNK) IRE pathway. JNK can phosphorylate and inactivate anti-apoptotic *Bcl-2*. In addition, as discussed earlier, calcium release from the ER during the late phases of ER stress would inhibit mitochondrial movement, thus increasing organelle coupling [52].

ER-mitochondrial crosstalk has also been implicated in the regulation of necrosis. Nix and BNIP3, for example, are highly homologous BH3-only-like proteins residing at both the OMM and ER. Nix can stimulate dual autonomous death pathways depending on its localization. At mitochondria, Nix activates Bax/Bak- and caspase-dependent apoptosis, whereas ER-targeted Nix activates Bax/Bak-independent MPTP and IMM-dependent necrosis [94]. Likewise, homodimerization and mitochondrial membrane insertion of BNIP3 can result in the loss of membrane potential and activation of Bax/Bak and depending on the cell type and conditions result in an apoptotic or necrotic (caspase independent) type cell death [95, 96].

3.2. ER-Directed Mitochondrial Metabolism

As described above, ER-mitochondrial interactions contribute to several aspects of organelle function and metabolism. A fundamental determinant of this interaction is the distance between the two organelles, which is controlled by their movements along the cytoskeleton [28, 97]. The late phase of prolonged ER stress arrests mitochondrial movement in the proximity of ER calcium release, promoting apoptosis [60]. However, the consequences of changes in ER-mitochondria coupling during the early adaptative phases of ER stress are not as well understood. Our recent studies have shown that the increased association between ER and mitochondria is initially an adaptative response to ER stress that contributes to cell survival [28] (Fig. 2B). We demonstrated an increase in several parameters of mitochondrial bioenergetics during the early stages of ER stress including ATP production, reductive potential, oxygen consumption rate, and mitochondrial membrane potential. These increases were dependent on movement of mitochondria toward the perinuclear region and increased coupling between ER and mitochondrial networks. Interestingly, the association of these two organelles and the metabolic changes were inhibited by decreasing abundance of the mitochondria-ER tethering protein *Mfn2*, suggesting that a physical interaction of the two organelles is necessary to coordinate the response. We showed that the increase in metabolism is greatest in the perinuclear zone, where ER-mitochondria coupling is most extensive. This is consistent with studies demonstrating that ER and mitochondria trafficking along acetylated microtubules promotes ER-mitochondrial contacts

predominantly in the perinuclear region [29]. Accordingly, the physiological importance of ER-mitochondria coupling has also been addressed in brown adipose tissue in which specific ER regions form a bud-like structure that is in close apposition to mitochondria [98]. These specific structures have not been observed in other tissues and may be involved in thermogenic mechanisms unique to brown adipose tissue, however, the exact role and regulation of these contact sites has not yet been elucidated.

3.3. ER-Directed Mitochondrial Quality Control

Mitochondrial populations are continually turned over and renewed in both mitotic and post-mitotic cells. Quality control mechanisms act to identify and selectively eliminate damaged mitochondria *via* autophagy (hereafter referred to as mitophagy) [99]. In addition to requiring the machinery involved in general autophagy and lysosomal degradation mitophagy is also dependent on a number of mitophagy-specific proteins (Fig. 2C). Interestingly, mutations in a number of these genes result in autosomal-recessive juvenile Parkinson disease (AR-JP) in humans. For instance, the *PARK2* gene, responsible for 50% of identified AR-JP mutations, encodes Parkin, an ubiquitin ligase selectively recruited to dysfunctional mitochondria with low ψ_{mt} . Ubiquitination of an OMM protein, or proteins, is thought to tag the mitochondrion for degradation [100]. Loss of Parkin function leads to ER stress-induced apoptosis of dopaminergic neurons [101]. Likewise, activation of the UPR in response to ER stress increases Parkin expression. Furthermore, as discussed earlier, ER stress can promote mitochondrial damage and vice versa illustrating the potential for extensive ER-mitochondrial crosstalk involved in the pathology of Parkinson disease. The cytoprotective effect of Parkin is independent of proteasomal degradation of its specific substrates, suggesting instead that it may prevent ER stress-induced mitochondrial damage through elimination of dysfunctional mitochondria *via* mitophagy [102]. The importance of the ER-stress-mitophagy axis is being recognized in an increasing number of human disorders. In α 1-antitrypsin deficiency, a mutation in α 1-antitrypsin gene leads to aggregation of the protein in the ER. Increased mitochondrial damage and mitophagy has been described in the livers of patients with α 1-antitrypsin deficiency and in genetic models of the disease [103].

BNIP3 and NIX have also been linked to mitophagy. OMM localized NIX contains a WXXL-like motif facing the cytosol, that acts as a selective autophagy receptor by binding to LC3/GABARAP proteins, which are required for the growth of autophagosomal membranes [104] (Fig. 2C). BNIP3 can also induce mitophagy and may function in a similar way to NIX, although the mechanisms are less clear [105]. Insight to the seemingly conflicting models of cell death by these proteins will come from a better understanding of the relationship of mitophagy in these pathways.

3.4. ER-Mitochondrial Cooperation

ER and mitochondria respond coordinately in a variety of scenarios, such as arsenic poisoning [106], cholesterol overloading [107], protein aggregation [108] and neurodegenerative disorders [109]. Under these conditions oxidative stress is a common factor providing extensive opportunities for ER-mitochondrial crosstalk, as both organelles participate in maintaining, initiating and responding to changes in redox state [110, 111].

For example, the UPR is engaged upon oxidative stress and is necessary to prevent and protect against mitochondrial dysfunction [112, 113]. One avenue through which this occurs is through the Ire1/Xbp1 pathway increasing expression of antioxidant machinery, such as catalase, thus preserving mitochondrial function. Oxidative stress not only disrupts protein folding in the ER, but can promote intramitochondrial aggregation of proteins of the respiratory chain [114]. Cleavage and clearance of misfolded mitochondrial proteins is carried out in part by the mitochondrial protease Lon. Expression of Lon is under the control of ER signaling providing a mechanism through which ER stress can enhance proper assembly of respiratory complexes [115] (Fig. 2D).

ER signaling impacts mitochondrial physiology *via* numerous avenues. In contrast, the reverse role of mitochondria as a modulator of UPR and ER function has only recently been addressed. Adenylate kinase 2, a mitochondrial enzyme involved in nucleotide metabolism in the intermembrane space was shown to be required for the induction of the UPR in secretory cells [116] (Fig. 2D). Importantly, mutations in adenylate kinase 2 have been reported to cause reticular dysgenesis, a rare and extremely severe immunodeficiency [117]. This disease is characterized by the lack of lymphocytes in peripheral blood, due to a defect in myeloid differentiation, a highly UPR-dependent process. Another example of the mitochondria-to-ER feedback involves the mitochondrial adaptor protein Trap1 (TNF receptor-associated protein 1), which has a pro-apoptotic role and is required for proper UPR activation and transduction [118] (Fig. 2D).

4. EMERGING TOPICS IN ER-MITOCHONDRIAL COMMUNICATION

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Tau hyperphosphorylation and formation of protein aggregation of amyloid- β (A β) peptide. PS1 and PS2 form part of the γ -secretase complex that generates A β proteolytically. A recent report demonstrates that PS2 over-expression increases the physical interaction between ER and mitochondria and subsequent calcium transfer. This effect is enhanced by familial AD PS2 mutations [119]. The increase in calcium transfer may be the result of a direct interaction between PS2 mutants and IP3R on the ER [120]. Apart from the defects in calcium homeostasis and mitochondrial dysfunction, AD patients show altered lipid metabolism, another MAM-related process. This suggests that A β deposition and Tau hyperphosphorylation may be secondary consequences of perturbing MAM functions at the ER-mitochondrial interface, and not the primary cause of the disease, leading to the so called "MAM hypothesis" of AD linking the etiology of a disease to the ER-mitochondria axis [121].

Also present at the MAM are the glycosphingolipid biosynthetic enzymes that make possible the synthesis gangliosides [122] (Table 1). GM1 ganglioside is a sialic acid-containing glycosphingolipid that is present in cell membranes playing important roles in neuronal differentiation and development. Studies report protective effects of GM1 in neurodegenerative disorders, such as AD [123]. Indeed, GM1 forms a complex with A β in AD brains, although it is still controversial whether this interaction induces a nontoxic α -helix or a pathogenic β -sheet structure [122, 124]. Nevertheless, several previously unrelated events in the pathology of AD seem to converge at MAM alterations. In patients with

Parkinson's disease, treatment with GM1 ganglioside produces a significant relief of symptoms [125], which can be explained by an interaction between GM1 and α -synuclein that inhibits α -synuclein aggregation [126]. Further studies are needed to clarify whether α -synuclein is associated with MAM, as it is possible that some protein folding and processing takes places at MAM.

Some cell types may be more susceptible to alterations in the ER-mitochondria communication. For example, cardiac myocytes are extremely rich in mitochondria due to a high demand for ATP. As striated muscle they also possess an extensive network of sarcoplasmic reticulum (SR), a specialized form of ER. Diverse forms of cardiac stress or injury have been shown to alter SR-mitochondria interactions. Such changes have been implicated in the misregulation of excitation-contraction coupling, declines in ATP production, and altered calcium handling that participates in the genesis and establishment of cardiac arrhythmias [127].

New examples and aspects of ER-mitochondria crosstalk are continually emerging. For example, the complex Cdc48/Npl4/Ufd1 is required for retro-translocation of unfolded proteins from the ER lumen to the cytosol, and subsequent targeting for proteasomal ER-associated degradation (ERAD). Under oxidative stress, the mitochondrial protein Vms1 favors the relocation of the complex Cdc48/Npl4/Ufd1, leading to proteasomal degradation of mitochondrial proteins [128]. Absence of Vms1 produces mitochondrial dysfunction and, eventually, cell death due to the accumulation of misfolded proteins inside mitochondria [128]. How this common pathway of protein degradation impacts ER-mitochondrial communication remains unknown. In the same way, autophagy, senescence, vesicular trafficking, inflammation and mitosis, are venues in which inter-organelle communication is only starting to be explored.

5. CONCLUDING REMARKS

Diverse lines of study demonstrate that ER and mitochondria are closely integrated both in regard to space and function, allowing them to coordinate complex cellular responses ranging from stress adaptation to cell death. The ER-mitochondrial axis is emerging as a fundamental component of the physiopathology of diseases (summarized in Table 2).

A better understanding of interorganellar communication will allow the development of new strategies to concomitantly manipulate mitochondrial and ER physiology, potentially serving as the basis for new therapies targeted toward neurodegenerative and myodegenerative diseases, where protein misfolding and mitochondrial dysfunction constitute the foundations of pathology.

Acknowledgments

This research was funded in part by the National Institutes of Health (HL072016, and HL097768 to B.A.R.), the American Heart Association (0655202Y to B.A.R.), FONDECYT 1120212 (to S.L.), FONDECYT 3120220 (to C.Q.) and ANILLO ACT 1111 (to S.L.). R.B.-S., A.E.R, J.K., and C.L.-C. are recipients of PhD and MSc CONICYT Fellowships Chile, respectively.

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Fig. 1. ER and mitochondrial signaling cascades

(A) Accumulation of unfolded proteins in the ER lumen releases BiP and activates transmembrane sensors IRE1, PERK and ATF6, whose signaling recruits several transcription factors (XBP1, ATF4 and CHOP), leading to the activation of the Unfolded Protein Response genetic program. (B) Mitochondria perform essential metabolic reactions (Krebs cycle, ATP production, ROS generation), and are key regulators of cell death by receiving different inputs (e.g. Bcl-2 family proteins) and compartmentalizing potent inducers of apoptosis (AIF, cytochrome c and Smac/DIABLO). (C) Several proteins are compartmentalized in the Mitochondria-Associated Membranes, allowing a direct ER-mitochondria communication. Noteworthy is the participation of GRP75, forming a bridge between the Ca^{2+} channels IP3R and VDAC, while Calnexin (CNX) directly modulates the activity of SERCA2b. (D) The ER releases Ca^{2+} through specialized channels (IP3R), and pumps it back through SERCA. Mitochondria directly uptake this Ca^{2+} through a series of

channels (mCU and VDAC) driven by its transmembrane potential (ψ). ER-mitochondria communication also allows the transfer of lipids between organelles at the MAM.

Fig. 2. Life and death through the ER-mitochondria axis

(A) Severe ER stress induces pro-apoptotic signaling from the ER (IRE1 and PERK) that leads to mitochondrial Ca²⁺ overload. ER-directed mitochondrial fragmentation (via tBid and p20) precludes mitochondrial permeabilization and release of apoptotic factors (e.g. cytochrome C). (B) Non-lethal ER stress induces a microtubule-dependent spatial reorganization of ER and mitochondria that leads to enhanced organelle coupling and calcium transfer. Increased Ca²⁺ uptake produces a boost in mitochondrial bioenergetics and ATP production as an adaptive response. (C) The engagement of the UPR upon mitochondrial damage activates the mitophagy pathway, coordinating the elimination of dysfunctional organelles. (D) Disturbances in general homeostasis require a synchronic response from ER and mitochondria. While ER signaling protects mitochondrial integrity, mitochondria have important components for proper UPR signaling.

Table 1**Protein Components and Regulators of Mitochondria-Associated Membranes (MAMs)**

Components	General Function	MAM Function
IP3R type3	ER calcium channel	Ligand-gated channel that releases calcium from the ER, generating high $[Ca^{2+}]$ microdomains at the ER-mitochondrial junctions [55].
VDAC type 1	OMM calcium channel	Mediates calcium channeling to the IMS from the high $[Ca^{2+}]$ microdomain generated by the opening of the IP3R [46].
GRP75	Mitochondrial chaperone	Physically link VDAC1 and IP3R channels, allowing a positive regulation of mitochondrial calcium uptake [32].
Mfn2	Mitochondrial fusion protein	Controls ER-mitochondria interface forming homo- and hetero-complexes comprising Mfn2 at the ER and Mfn2 or Mfn1 on mitochondria [66].
Sigma-1 receptor	Ligand-activated ER chaperone	Activated by ligand or by depletion of calcium in the ER. It prevents IP3R degradation by proteasome, leading to prolonged calcium signaling to mitochondria [129].
BiP	ER chaperone	Forms a dormant complex with Sigma-1 receptor in physiological conditions [129].
Calnexin	ER quality control lectin	Enriched on the MAM [32]. Also, interacts with SERCA2b to block calcium import [130].
Rab32	GTPase	Modulates ER calcium handling and disrupts the enrichment of calnexin in the MAM [34].
PACS-2	Sorting protein	Regulates MAM composition, promoting calnexin enrichment [32]. It also determines the ER localization of polycystin-2 (PC2), which is a calcium-activated calcium release channel [131].
Ero1 α	ER oxidoreductase	Stimulates IP3R activity, being released from MAM in reducing conditions [132].
PML	Tumor suppressor	Forms complexes with IP3R, Akt, and PP2a at MAM, modulating IP3R activity by phosphorylation [133].
Presenilin-2	Aspartyl proteases	Increases the physical interaction between ER and mitochondria and the calcium uptake by mitochondria [51].
PSS 1-2	Phosphatidylserine synthesis	Promotes the partitioning of phosphatidylserine between the ER and mitochondria [134].
FACL-4	Fatty acyl-CoA ligase	Mediates formation of lipid esters required for β -oxidation in mitochondria [135].
Cer-Glc-T and SAT	Glycosphingolipids synthesis	Provides glycosphingolipids for mitochondria [136].

Table 2

Examples of Diseases with Alterations in the ER-Mitochondria Axis

Disease	Characteristics	Alterations in the ER-Mitochondria Axis
Autosomal-recessive juvenile Parkinson's disease	Mutations in Parkin, an ubiquitin ligase necessary to mitophagy	ER stress-induced apoptosis of dopaminergic neurons [100]. The Parkin expression is increased for UPR [91].
α 1-antitrypsin deficiency	α 1-antitrypsin mutation produces its aggregation in the ER	Increased mitochondrial damage and mitophagy [102].
Reticular dysgenesis	Mutations in the mitochondrial enzyme adenylate kinase 2	Adenylate kinase 2 is mitochondrial protein required for the induction of the UPR [115].
Alzheimer's disease	Tau hyperphosphorylation and formation of protein aggregates of A β peptide	Presenilin-2, which generates A β , increases the interaction and calcium transfer at MAM [108].