ER Stress and Its Functional Link to Mitochondria: Role in Cell Survival and Death

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The endoplasmic reticulum (ER) is the primary site for synthesis and folding of secreted and membrane-bound proteins. Proteins are translocated into ER lumen in an unfolded state and require protein chaperones and catalysts of protein folding to assist in proper folding. Properly folded proteins traffic from the ER to the Golgi apparatus; misfolded proteins are targeted to degradation. Unfolded protein response (UPR) is a highly regulated intracellular signaling pathway that prevents accumulation of misfolded proteins in the ER lumen. UPR provides an adaptive mechanism by which cells can augment protein folding and processing capacities of the ER. If protein misfolding is not resolved, the UPR triggers apoptotic cascades. Although the molecular mechanisms underlying ER stress-induced apoptosis are not completely understood, increasing evidence suggests that ER and mitochondria cooperate to signal cell death. Mitochondria and ER form structural and functional networks (mitochondria-associated ER membranes [MAMs]) essential to maintain cellular homeostasis and determine cell fate under various pathophysiological conditions. Regulated Ca^{2+} transfer from the ER to the mitochondria is important in maintaining control of pro-survival/prodeath pathways. We discuss the signaling/communication between the ER and mitochondria and focus on the role of the mitochondrial permeability transition pore in these complex processes.

The ER is an elaborate membranous network present in all eukaryotic cells and responsible for many homeostatic responses that include folding and maturation of newly synthesized secretory and transmembrane proteins (Kleizen and Braakman 2004). In addition, this organelle is also the site of cholesterol and steroid biosynthesis, lipid biosynthesis, assembly of core-asparagine linked oligosaccharides, and membrane and secreted protein biosynthesis. Newly synthesized proteins require proper

folding within the ER lumen prior to trafficking to specific destinations in the cell. These cellular processes are initiated when nascent polypeptide chains emerge in ER lumen, where post-translational modifications such as N-linked glycosylation, and intra- and intermolecular disulfide bond formation facilitate the folding of polypeptides to form specific tertiary and quaternary structures for proper protein function (Molinari 2007). Although the amino acid sequence of the protein determines many

Editors: Richard I. Morimoto, Dennis Selkoe, and Jeff Kelly

Additional Perspectives on Protein Homeostasis available at www.cshperspectives.org

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Cite this article as *Cold Spring Harb Perspect Biol* 2011;3:a004424

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of these precise processes, numerous proteins, including chaperones and enzymes, aid in proper protein biosynthesis and folding. The key chaperones and folding sensors in the ER include the peptide-binding proteins BiP and GRP94, the lectins calnexin and calreticulin, and the thiol-disulfide oxidoreductases such as protein disulfide isomerase (PDI) and Erp57. The chaperone machinery collectively cooperates to prevent protein misfolding, aberrant interactions, and aggregation. The quality of protein folding is precisely monitored by an ER quality control system that only allows properly folded proteins to be transported to the Golgi compartment and directs misfolded proteins for ER-associated degradation (ERAD) by the 26S proteasome or for degradation through autophagy (Ma and Hendershot 2004; Kincaid and Cooper 2007).

Protein folding in the ER is very sensitive to extracellular stimuli and insults, and intracellular processes that alter Ca^{2+} homeostasis, redox status, and energy (sugar/glucose) stores. The ER is the central site for Ca^{2+} storage and homeostasis within the cell. The ER couples its quality control machinery to the storage and utilization of Ca^{2+} . Alterations in intraluminal Ca^{2+} can cause protein misfolding because both protein folding reactions and protein chaperone functions require high levels of calcium. Under conditions that compromise ER function, particularly the accumulation of newly synthesized unfolded proteins, the organelle signals activation of an elaborate adaptive process known as the unfolded protein response (UPR) (Ron and Walter 2007). Appropriate adaptation to misfolded protein accumulation in the ER lumen requires regulation at all levels of gene expression including transcription, translation, translocation into the ER lumen, and ERAD. Coordinate regulation of all these processes is required to restore proper protein folding and ER homeostasis (Mori et al. 1993; Patil and Walter 2001; Kaufman 2002; Schroder and Kaufman 2005; Wu and Kaufman 2006). Finally, chronic activation of UPR signaling eventually induces an apoptotic (programmed cell death) response. We will briefly discuss below the various signaling arms of the UPR

as relevant to cell survival and adaptation and ER stress as it relates to apoptosis and cellular demise.

UPR SIGNALING: CELL SURVIVAL

In higher eukaryotic cells, three ER membrane-associated signal transducers sense the presence of misfolded proteins in the ER lumen and initiate adaptive responses (Fig. 1).

These transducers are two protein kinases IRE1 (inositol requiring enzyme 1) (Cox et al. 1993; Yoshida et al. 2001), PERK (PKR-like eukaryotic initiation factor 2 α kinase) (Harding et al. 2000a,b), and the transcription factor ATF6 (activating transcription factor 6) (Yoshida 2001; Lee et al. 2002; Yoshida et al. 2003; Yamamoto et al. 2007). Under normal cellular conditions in which the ER is presumably “stress-free,” the intraluminal amino-terminal domains of IRE1 and PERK and the carboxy-terminal domain of ATF6 are maintained in an inactive state by interaction with the chaperone BiP/GRP78 (Bertolotti et al. 2000; Liu et al. 2003). This model for negative regulation of the UPR by BiP is also supported by the observation that BiP overexpression prevents activation of the UPR on ER stress (Dorner et al. 1990). Whether BiP is the primary regulator of each UPR sensor is not clearly known, as simple disruption of the interactions between BiP and the UPR sensors may not result in constitutive activation (Oikawa et al. 2007). Recently, the crystal structure of the yeast Ire1p luminal domain (Credle et al. 2005) identified the existence of a deep, long MHC1-type groove in the Ire1p dimer and proposed that unfolded polypeptides directly bind Ire1p to mediate its dimerization. However, although analysis of the human IRE1 indicated a similar structure, the MHC1-type groove was not solvent-accessible (Zhou et al. 2006). In addition, the luminal domain was shown to form dimers in the absence of added polypeptide, bringing into question the requirement for peptide binding to promote dimerization. Because these structures represent static conformations, it is possible the altered conformational states may regulate both BiP and peptide binding. Therefore, it is reasonable to

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Figure 1. Signaling the unfolded protein response: Three proximal sensors IRE1, PERK, and ATF6 regulate the UPR through their respective signaling cascades. Under nonstressed conditions, BiP binds to the luminal domains of IRE1 and PERK to prevent their dimerization. On accumulation of unfolded proteins in the ER lumen, IRE1 released from BiP dimerizes to activate its kinase and RNase activities to initiate XBP1 mRNA splicing, thereby creating a potent transcriptional activator. Primary targets that require IRE1/XBP1 pathway for induction include genes encoding functions in ERAD. Similarly, ATF6 released from BiP transits to the Golgi compartment for cleavage by S1P and S2P proteases to yield a cytosolic fragment that migrates to the nucleus to further activate transcription of UPR-responsive genes. Finally, PERK released from BiP dimerizes, autophosphorylates, and phosphorylates eIF2α on Ser 51 leading to general attenuation of translational initiation. Paradoxically, eIF2α phosphorylation induces translation of ATF4 mRNA. The PERK/eIF2α/ATF4 regulatory axis also induces expression of anti-oxidative stress response genes and expression of genes encoding proteins with proapoptotic functions, such as CHOP.

speculate that BiP binding as well as peptide binding act together to regulate IRE1 dimerization. Future studies in this area should resolve this issue. It is believed that the primary trigger for release of BiP from the sensors is the accumulation of misfolded proteins. This, coupled

with other unidentified luminal events, results in oligomerization and activation of the IRE1 and PERK kinases and results in the execution of a complex and fascinating downstream intracellular signaling pathway (Bertolotti et al. 2000; Ron and Walter 2007; Aragon et al. 2009;

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Korennykh et al. 2009; Li et al. 2010). Concomitantly, the third branch of the UPR is activated when ATF6 translocates to the Golgi apparatus where it is cleaved by the serine protease site-1 (S1P) and the metalloprotease site-2 protease (S2P) to generate an active transcription factor (Chen et al. 2002). Interestingly, ATF6 activation is also sensitive to the redox status of the cell and recent evidence suggests that only the reduced monomeric form of ATF6 translocates to the Golgi apparatus (Nadanaka et al. 2007). The overall effect of this tripartite UPR signaling is to attenuate the global mRNA translation and simultaneously up-regulate the expression of chaperones to improve ER folding capacity and ERAD function. The various branches of the UPR are briefly described below.

In the early 1990s, investigations in the budding yeast *S. cerevisiae* identified the ER stress-regulated kinase and endoribonuclease IRE1 that is conserved from yeast to humans. Two independent groups identified Ire1p/Ern1p as an ER transmembrane protein kinase that acts as a proximal sensor in the yeast UPR that initiates unconventional removal of a 252 base intron within the basic leucine zipper (bZIP) transcription factor Hac1p to induce expression of UPR genes (Cox et al. 1993; Mori et al. 1993). Subsequently, several groups showed that X-box binding protein-1 (*XBPI*) mRNA is the mammalian homolog of yeast Ire1p and the substrate for the endoribonuclease activity of mammalian IRE1 (Shen et al. 2001; Yoshida et al. 2001; Calton et al. 2002; Lee et al. 2002). On activation of the UPR, the endoribonuclease activity of IRE1 cleaves *XBPI* mRNA to remove a 26 base intron. This splicing reaction creates a translational frame shift to produce the active (or spliced) form of the transcription factor (XBP1s). Spliced Xbp1 is a transcriptional activator for many of the UPR target genes and, in conjunction with ATF6 α , launches a transcriptional program to produce ER protein chaperones and proteins involved in ER biogenesis and phospholipid synthesis with the net effect of expanding the folding capacity of the ER to resolve the protein-folding defect (Lee et al. 2003). Some of the

genes identified that require the IRE1/XBP1 pathway are those that encode functions involved in ERAD, such as EDEM, ERdj4, and PDI. Indeed, cells that are deficient in either IRE1 or XBP1 are defective in ERAD. Recently, the endoribonuclease activity of IRE1 was suggested to target and degrade ER-associated mRNAs as an additional mechanism to relieve the ER protein-folding load (Merksamer et al. 2008; Hollien et al. 2009).

The bZIP-containing activating transcription factor 6 (ATF6), the second arm of the UPR pathway, was identified as another regulatory protein that, like XBP1, binds the ER stress elements (ERSE-I and II), UPR elements (UPRE), and cAMP response elements (CRE) in the promoters of UPR-responsive genes (Yoshida et al. 1998). In this manner, increased expression of ERAD machinery, such as the ER degradation-enhancing α -mannosidase-like protein (EDEM), increases the clearance and degradation of misfolded proteins in the ER lumen (Kokame et al. 2001). There are two known alleles of ATF6, ATF6 α and ATF6 β , both synthesized in all tissues as ER transmembrane proteins. ATF6 α deletion sensitizes cells and animals to persistent ER stress. In vivo, this failure to recover from ER stress results in fatty liver, uncovering a potential connection between ER stress and lipid metabolism (Wu et al. 2007). It was also reported that ATF6 α interacts with CRTC2 to antagonize the ability of CREB to activate gluconeogenesis in the liver (Wang et al. 2009). The transcriptional coactivator PGC-1 α , that regulates several exercise-associated aspects of skeletal muscle function, mediates the UPR in myotubes and skeletal muscle through coactivation of ATF6 α . Efficient recovery from acute exercise is compromised in *Atf6 α* ^{-/-} mice (Wu et al. 2011). Thus, both ATF6 and XBP1, a transcriptional target of ATF6 that requires splicing by the endoribonuclease activity of IRE1, are considered as the predominant regulators of the adaptive UPR transcriptional response to resolve protein misfolding.

Activation of the third arm of the UPR is mediated through PERK, an ER-associated transmembrane serine/threonine protein kinase. On

accumulation of unfolded proteins in the ER lumen, PERK dimerization and *trans*-autophosphorylation leads to activation of the kinase function that phosphorylates the α subunit of eukaryotic translation initiation factor (eIF2 α) at Ser51. This phosphorylation attenuates mRNA translation initiation to reduce protein synthesis and the protein folding demand on the ER (Harding et al. 1999, 2000a,b, 2001; Morimoto et al. 2004). There are three additional eIF2 α kinases, PKR (double-stranded RNA-activated protein kinase), GCN2 (general control nonderepressible kinase 2), and HRI (heme-regulated inhibitor kinase) that also phosphorylate Ser51 on eIF2 α . The precise role of the individual eIF2 α kinases is somewhat unclear because a single stress can activate more than a single eIF2 α kinase. For example, PKR is also activated by ER stress and PKR can protect cells from ER stress and initiate inflammatory response signaling (Nakamura et al. 2010). In addition to translational attenuation, activation of PERK branch of the UPR also decreases transcription of several dependent genes such as that of ribosomal RNA (DuRose et al. 2009). Although phosphorylation of eIF2 α inhibits general translation initiation, it is required for the selective translation of several mRNAs. Two transcription factors that require eIF2 α phosphorylation are the activating transcription factors 4 and 5 (ATF4, ATF5). Expression profiling studies identified several genes, including those encoding amino acid biosynthesis and transport functions, antioxidative stress responses, and apoptosis genes, such as growth arrest and DNA damage 34 (GADD34) and CAAT/Enhancer binding protein (C/EBP) homologous protein (CHOP) (Harding et al. 2000; Ma et al. 2002) that require PERK, eIF2 α phosphorylation, and ATF4 (Scheuner et al. 2001; Ron 2002; Harding et al. 2003). Lack of eIF2 α phosphorylation in β cells caused a severe diabetic phenotype because of heightened and unregulated proinsulin translation, defective folding, and trafficking of ER cargo proteins, reduced expression of ER stress response and β cell-specific genes, increased oxidative damage, and apoptosis. However, glucose intolerance and β cell death in these

mice were attenuated by a diet containing antioxidants (Back et al. 2009). It seems that phosphorylation of eIF2 α coordinately attenuates mRNA translation, prevents oxidative stress, and optimizes ER protein folding to support insulin production. The finding that increased proinsulin synthesis is sufficient to cause oxidative damage in β cells may reflect events in the β cell failure associated with insulin resistance in type 2 diabetes that include decreased insulin production, loss of β cell-specific gene expression, increased expression of UPR genes, oxidative stress, and apoptosis (Huang and Tindall 2007; Laybutt et al. 2007).

ER STRESS-DEATH RESPONSE

Apparently, execution of the UPR program does not always result in successful and efficient alleviation of the ER stress and therefore under conditions of severe or prolonged stress signals, the UPR can also culminate in induction of apoptosis (Rao et al. 2004). Both mitochondrial-dependent and -independent cell death pathways likely mediate apoptosis in response to ER stress. The ER might actually serve as a site where apoptotic signals are generated and integrated to elicit the death response. Several mechanisms by which apoptotic signals are generated at the ER include: (1) pro-apoptotic Bcl-2 proteins Bak and Bax are switched on by the IRE1 α pathway leading to regulated Ca^{2+} release from the ER (Hetz et al. 2006); (2) cleavage and activation of procaspase-12; (3) IRE1-mediated activation of ASK1 (apoptosis signal-regulating kinase 1)/JNK (c-Jun amino terminal kinase); and finally, (4) PERK/eIF2 α -dependent induction of the proapoptotic transcription factor CEBP homologous protein (CHOP). CHOP is a downstream transcriptional target of ATF6 and PERK/eIF2/ATF4. CHOP is a bZIP-containing transcription factor that inhibits the expression of Bcl-2, and activates transcription of several genes that encode apoptotic functions including GADD34, DR5, and TRB3 (McCullough et al. 2001). In addition, ER stress-induced IRE1 α phosphorylation leads to recruitment of TRAF2

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(tumor necrosis factor receptor-associated factor 2) and ASK1 to the cytosolic leaflet of ER membrane (Kawamori et al. 2003). Simultaneous activation of the PERK and IRE1 pathways also impacts NF- κ B-IKk signaling pathway during ER stress by either activation of IKk or degradation of the p65 subunit (Hu et al. 2006).

The mechanism of ER stress-induced apoptosis through Bak and Bax, that localize to both ER and mitochondria, has been shown to be associated with release of ER calcium with concomitant increase of mitochondrial calcium (Nutt et al. 2002). The Ca^{2+} released from the ER enters the mitochondria leading to depolarization of the inner membrane, cytochrome c release, and activation of the Apaf-1 (apoptosis protease-activating factor 1)/procaspase-9-regulated apoptosis pathway. The mechanism by which ER stress is directly coupled to activation of caspases, particularly caspase 12, which was initially characterized by Nakagawa and Yuan (2000), remains somewhat elusive. The significance of ER stress-associated caspase 12 activation remains enigmatic because a functional caspase 12 is not conserved in humans.

Finally, analysis of gene-deleted mice has provided additional insight into ER stress-induced apoptosis. Cells from Apaf-1-deficient mice are susceptible to ER stress-induced apoptosis, indicating the existence of non-mitochondrial cell death pathways. Similarly, Bak/Bax double knockout, *Caspase-12*^{-/-} and *Chop*^{-/-} MEFs all show partial resistance to ER stress-induced apoptosis, further supporting the idea that they facilitate the apoptotic response on ER stress. Although, caspase-12-deficient and CHOP-deficient mice show no developmental defects, they display protection to genetically imposed or environmentally imposed ER stress. There are studies that indicate that ER stress-induced apoptosis may have a mitochondrial component (Deniaud et al. 2008). Overall, it seems PERK serves as a critical control point that determines commitment to cell death or promotes survival (Rutkowski et al. 2006; Ron and Walter 2007). However, there remain critical gaps in our

understanding of the ability of individual UPR initiators to recognize and respond to various forms of ER stress and then engage distinct survival or death responses under different cellular environments.

ER-MITOCHONDRIA INTERACTIONS

The classical concept of mitochondria as the cell's powerhouse and as an isolated organelle has been profoundly challenged over the last two decades with the realization that mitochondria function within a highly dynamic integrated reticular network that is continually remodeled by both fusion and fission events. Both the ER and mitochondria are presently accepted as dynamic organelles capable of modifying their structure and function in response to changing environmental conditions. ER and mitochondria interact both physiologically and functionally, and one of the most critical aspects of this interaction is calcium signaling between the two organelles (Fig. 2). ER and mitochondria form close contacts with 20% of the mitochondrial surface in direct contact with the ER (Kornmann et al. 2009). These contacts through which the ER communicates with mitochondria are referred to as mitochondrial-associated membranes (MAM) (Vance 1990). These physical associations have pivotal roles in numerous cellular functions including Ca^{2+} signaling, lipid transport, energy metabolism, and cell survival. The ER-contiguous membranes also contain multiple phospholipids and glycosphingolipid synthesizing enzymes including long chain fatty acid-CoA ligase type 4 (FACL4) and phosphatidylserine synthase-1 (PSS-1), and support direct transfer of lipids between the ER and mitochondria (Piccini et al. 1998; Stone and Vance 2000). The interaction between the two organelles is mediated by mitochondrial shaping proteins and key chaperones including calnexin, calreticulin, ERp44, ERp57, grp75, and the sigma-1 receptor. Over the years, a number of MAM-specific proteins have been identified including many ion channel and transporter proteins (IP3 receptors [IP3R], VDAC, Ca^{2+} ATPase, etc.), ubiquitin ligases, vesicular-sorting proteins,

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Figure 2. MAMs support the lipid transfer from ER to mitochondria through enzymes FACL4 and PSS-1. MAMs are enriched in chaperones like sigma-1Rs and they colocalize with BiP and IP3R. A multifunctional sorting protein that controls the ER-mitochondria axis Mitochondrial chaperone grp75 is a link between ER Ca^{2+} -release channel IP3R and isoform of VDAC.

electron transport chain proteins, and mitochondrial fusion proteins. Most of these proteins are ER proteins with only a few belonging to the mitochondria such as the VDAC and the uniporters. The mitochondrial-shaping proteins that are involved in modulating these two organelles are Dynamin-related protein-1 (DRP1) (Smirnova et al. 2001) and mitofusin 1 and 2 (Mfn-1 and dMfn2) that regulate mitochondrial fission and fusion, respectively (Chen et al. 2003). The molecular machinery mediating fusion and fission events are very intricate requiring the independent but coordinated processing of both the outer and inner mitochondrial membranes. These proteins, including DRP1, Mfn1, and Mfn2, were originally identified in yeast but many of these genes have orthologs in mammals and belong to a large GTPase protein family. The vesicular-sorting protein, PACS-2 (phosphor-acidic cluster sorting protein 2) is a multifunctional sorting protein that controls the ER-mitochondria

axis and the role of this axis in cellular homeostasis and apoptosis (Simmen et al. 2005). PACS-2 is required for the intimate association of mitochondria with ER. PACS-2 depletion results in mitochondrial fragmentation and uncouples this organelle from ER indicating that PACS-2 might be involved in ER protein folding and Ca^{2+} homeostasis (Szabadkai et al. 2004).

Ca^{2+} SIGNALING AT THE MAM

An increasing body of evidence unequivocally suggests that the function of the ER is intimately connected with that of the mitochondria with Ca^{2+} signaling being at the hub of this interorganelle interaction. Mitochondria play significant roles in shaping the Ca^{2+} signal released from the ER. Under normal physiological conditions, the bulk of the Ca^{2+} resides within the ER lumen and, during cellular events requiring a Ca^{2+} signal, a small bolus is released into

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the cytoplasm only to be resequenced later and with a small proportion crossing the outer mitochondrial membrane. The ER Ca^{2+} functions both as a reservoir and simultaneously controls the activity of chaperones responsible for protein folding and processing (Rizzuto and Pozzan 2006). A great deal of recent evidence also shows that Ca^{2+} uptake into the mitochondria is controlled by specific proteins residing at the outer and inner mitochondrial membranes interface, namely the voltage-dependent anion channel (VDAC) and the Ca^{2+} uniporter (Duchen and Szabadkai 2010) and with mitochondrial Ca^{2+} being expelled by antiporters in an exchange process for either Na^+ or H^+ . Thus, the antiporter and the exchanger maintain mitochondrial membrane potential and optimal Ca^{2+} concentrations in the mitochondria. At the same time, important cellular processes that connect apoptosis to ER-mitochondria interactions is manifested when alterations in Ca^{2+} homeostatic mechanisms result in massive and/or a prolonged mitochondrial Ca^{2+} overload.

The most important molecular component of Ca^{2+} handling machinery of the ER is represented by the IP_3Rs that are primarily clustered in the MAM regions where ER is closely juxtaposed to the mitochondria and thereby delineating these zones as primary subcellular microdomains of Ca^{2+} transfer from the ER to the mitochondria (Rizzuto et al. 1998). The release of Ca^{2+} from ER stores by IP_3Rs has implications in numerous models of apoptosis as deletion of IP_3R gene by genetic ablation or antisense strategy increases resistance to apoptosis (Blackshaw et al. 2000). There are three isoforms of the IP_3R gene and recent data shows that the type 3 gene ($\text{IP}_3\text{R-3}$) localized to the MAM plays a selective role in apoptosis induction by selectively transmitting apoptotic Ca^{2+} signals into mitochondria, whereas the type 1 gene ($\text{IP}_3\text{R-1}$) preferentially mediates cytosolic Ca^{2+} mobilization (Mendes et al. 2005). Finally, a fascinating aspect of this interaction is the finding that in response to survival signals, Akt interacts and phosphorylates IP_3Rs , significantly reducing their Ca^{2+} release activity (Szado et al. 2008).

MOLECULAR CHAPERONES AT THE MAM

Both Ca^{2+} -binding and glucose-regulated chaperones are abundantly found on the membranes as well as lumens of both ER and mitochondria. These chaperones serve as constitutive ER Ca^{2+} pools and also facilitate proper protein folding in a Ca^{2+} -dependent manner. Some of these chaperones also couple to and regulate the activities of specific Ca^{2+} channels. A novel chaperone that specifically targets the MAM is the sigma-1R receptor, a ligand-operated Ca^{2+} -sensitive ER chaperone that colocalizes with IP_3R at the MAM. Sigma 1Rs form a Ca^{2+} -sensitive machinery or complex at the MAM along with GRP78/BiP, and are now believed to prolong calcium signaling from the ER to mitochondria by stabilizing IP_3R at the MAMs (Hayashi and Su 2007). The IP_3 receptors are vulnerable to ubiquitylation and proteasomal degradation on stimulation by IP_3 , and thus stabilization of the IP_3 receptors during intracellular signaling by the Sigma 1R is critical to maintain proper Ca^{2+} signaling both in the cytosol and in the mitochondria. Another important chaperone found at the MAM is the Grp75, and a recent study showed that cytosolic Grp75 tethers the ligand-binding domain of the IP_3 receptors to VDAC1. The mitochondrial chaperone Grp75 regulates IP_3R -mediated mitochondrial Ca^{2+} signaling (Szabadkai et al. 2006). Isoform 1 of VDAC is physically linked to the ER Ca^{2+} release channel IP_3R through Grp75, highlighting chaperone-mediated conformational coupling between the IP_3R and the mitochondrial Ca^{2+} uptake machinery. ER chaperones calnexin and calreticulin are also compartmentalized at MAM (Hayashi and Su 2007; Myhill et al. 2008). In addition to providing buffering capacity in ER, calreticulin inhibits the IP_3 receptor-mediated Ca^{2+} signaling by using its high-affinity, low-capacity, Ca^{2+} -binding domain (Camacho and Lechleiter 1995). Calnexin can regulate the Ca^{2+} ATPase activity via protein-protein interaction (Roderick et al. 2000). Another ER chaperone ERp57 can work in conjunction with calreticulin and facilitate in regulating the activity of Ca^{2+} -ATPase (Li and

Camacho 2004). ERp44 chaperone can inhibit type I IP3 receptors in a planar lipid bilayer system thereby modulating the IP3 receptor signaling (Anelli et al. 2003).

ER Ca^{2+} AND MITOCHONDRIAL PERMEABILITY TRANSITION

From the previous sections, it is clear that the function of the ER is intimately connected with that of the mitochondria, and a key process that links these ER-mitochondria interactions is the control of Ca^{2+} signaling. The mitochondria have a unique structure that contains two membranes that separates four distinct compartments, the outer mitochondrial membrane (OMM), the inner mitochondrial membrane (IMM), the space between these two membranes, and finally the matrix. The recent discovery that a massive and/or a prolonged influx of calcium into mitochondria can lead to the formation and opening of a large high-conductivity pore in the IMM, known as the mitochondrial permeability transition pore (PTP), a channel or “uniporter” driven by a large electrochemical gradient is critical for maintaining IMM stability. Mitochondrial Ca^{2+} overload and cellular redox status are considered the most potent inducers of permeability transition. The molecular identity of the PTP is not very clear but it seems to be comprised of the voltage-dependent anion channel (VDAC) localized in the OMM, the adenine nucleotide translocase (ANT) in the IMM, and cyclophilin D (a peptidyl-prolyl isomerase) localized in the matrix. Under normal physiological conditions, the opening and closing of PTP controls the homeostasis of mitochondria and regulates the matrix volume, Ca^{2+} flux, and the redox equilibrium and matrix pH. Under various pathophysiological conditions, permeabilization of the OMM can result in the release into the cytosol of a series of pro-apoptotic proteins such as cytochrome c, apoptosis-inducing factor (AIF), and smac/Diablo resulting in the demise of the cell through the execution of the apoptotic program involving proteases and nucleases. Recent studies have shown that the mitochondrial PTP plays a significant role in

ischemia reperfusion injury in the heart as well as after myocardial infarction leading to breakdown of mitochondria and necrotic cell death (Halestrap 2010). The permeabilization of the OMM is also determined primarily by an interaction between the pro-Bax and Bak and antiapoptotic Bcl-2 family members (Welch et al. 2009). Intriguingly, the MAM has emerged as a key point in the regulation of mitochondrial Ca^{2+} and the redox equilibrium, functioning as a central hub of cellular signaling. The two abundant MAM-associated ion channels, the IP3R and the VDAC resident on the OMM, primarily mediate Ca^{2+} transfer between the two organelles and ultimately determining Ca^{2+} load (Fig. 3). Both these channels also function as redox sensors, and several proteins including regulators of autophagy (Beclin-1) and apoptosis (Bcl-2 and Bad) cluster around this core platform of Ca^{2+} channels. Antiapoptotic Bcl-2 members (Bcl-2 and Bcl2-XL) have been suggested to exert their effect by suppressing Ca^{2+} transfer from the ER to mitochondria. Overexpression of Bcl-2 decreases ER luminal Ca^{2+} , thereby inhibiting Ca^{2+} - and oxidative stress-mediated cell death (Pinton et al. 2000). On the other hand, studies have shown that knockdown of the proapoptotic members Bax and Bak increases the interaction of Bcl-2 with type-1 IP3Rs and promotes both the phosphorylation of the IP3R and constitutive Ca^{2+} leak through the IP3Rs (Oakes et al. 2005). Thus, Bcl-2 family members regulate IP3R-1 phosphorylation to control the rate of ER Ca^{2+} release and in a way regulate cell fate by determining the probability of opening the mitochondrial PTP.

FUTURE DIRECTIONS

There has been tremendous progress over the past two decades in comprehending the mechanisms underlying ER stress-dependent UPR activation. The cellular processes linking protein folding, oxidative stress, and ER stress are tightly linked, and how aberrations in this signaling network communicate to the mitochondria to regulate cell death or survival is a

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Figure 3. ER-mitochondria cross talk is mediated by protein misfolding within the ER, which results in release of calcium from the intracellular stores into the cytosol through IP3Rs. Ca^{2+} has a critical role in this ER and mitochondrial cross talk. Ca^{2+} released from ER is taken up by mitochondria and results in calcium overload and induces depolarization of permeability transition pore (PTP) and induces apoptotic stimuli to release caspases.

fascinating emerging area of investigation. Future studies are required to understand how these stresses affect protein folding, misfolding, and secretion in vivo. These studies should identify under what physiological and pathological states these pathways are activated in vivo and how they finally influence disease outcome. A coherent understanding of the ER-mitochondria cross talk and nexus will certainly aid in the development of specific therapeutic

strategies to treat diseases associated with protein misfolding and inflammation such as obesity, diabetes, and neurodegeneration, as well as those associated with aging.

ACKNOWLEDGMENTS

The authors appreciate the work of Janet L. Mitchell in preparing this article and Jennifer Harley in assisting with the illustrations.

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Portions of this work were supported by NIH grants DK042394, HL052173, and HL057346 (R.J.K.). Additionally, J.D.M. is supported by AHA grant 10SDG2610338.

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Cold Spring Harb Perspect Biol 2011; doi: 10.1101/cshperspect.a004424 originally published online August 2, 2011

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