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Oxidative Protein Folding in the Endoplasmic Reticulum: Tight Links to the Mitochondria-Associated Membrane (MAM)

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Abstract

The production of secretory proteins at the ER (endoplasmic reticulum) depends on a ready supply of energy and metabolites as well as the close monitoring of the chemical conditions that favor oxidative protein folding. ER oxidoreductases and chaperones fold nascent proteins into their export-competent three-dimensional structure. Interference with these protein folding enzymes leads to the accumulation of unfolded proteins within the ER lumen, causing an acute organellar stress that triggers the UPR (unfolded protein response). The UPR increases the transcription of ER chaperones commensurate with the load of newly synthesized proteins and can protect the cell from ER stress. Persistant stress, however, can force the UPR to commit cells to undergo apoptotic cell death, which requires the emptying of ER calcium stores.

Conversely, a continuous ebb and flow of calcium occurs between the ER and mitochondria during resting conditions on a domain of the ER that forms close contacts with mitochondria, the MAM (mitochondria-associated membrane). On the MAM, ER folding chaperones such as calnexin and calreticulin and oxidoreductases such as ERp44, ERp57 and Ero1α regulate calcium flux from the ER through reversible, calcium and redox-dependent interactions with IP3Rs (inositol 1,4,5-trisphophate receptors) and with SERCAs (sarcoplasmic/endoplasmic reticulum calcium ATPases). During apoptosis progression and depending on the identity of the ER chaperone and oxidoreductase, these interactions increase or decrease, suggesting that the extent of MAM targeting of ER chaperones and oxidoreductases could shift the readout of ER-mitochondria calcium exchange from housekeeping to apoptotic. However, little is known about the cytosolic factors that mediate the on/off interactions between ER chaperones and oxidoreductases with ER calcium channels and pumps. One candidate regulator is the multi-functional molecule PACS-2 (phosphofurin acidic cluster sorting protein-2). Recent studies suggest that PACS-2 mediates localization of a mobile pool of calnexin to the MAM in addition to regulating homeostatic ER calcium signaling as well as MAM integrity. Together, these findings suggest cytosolic, membrane and lumenal proteins combine to form a two-way switch that determines the rate of protein secretion by providing ions and metabolites and that appears to participate in the pro-apoptotic ER-mitochondria calcium transfer.

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Keywords

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1. Introduction

In many cell types, the ER is the most expansive cellular organelle, encompassing close to 50% of the total membrane in liver cells and close to 20% of the total volume of insulin secreting pancreatic β cells [1,2]. The expansive size of the ER membrane reflects the manifold functions associated with this organelle, including secretory protein folding, calcium signaling and lipid synthesis and phagocytosis [3–8]. To physically accommodate these diverse functions, the continuous ER membrane system is composed of several domains that can be distinguished microscopically and isolated biochemically [9]. Among others, these domains include the rER (rough ER), the tER (transitional ER), the peripheral or cortical ER (also called PAM, plasma membrane-associated membrane) and the MAM [10]. The rER is characterized by the presence of ribosomes and translocons, which form a complex that mediates protein translocation, protein folding and protein degradation [10]. Following their folding, secretory proteins made within the rER translocate to the tER, which orchestrates COPII-mediated transport to the Golgi complex [11]. The tER can also contain ribosomes, but is distinguished from the rER by the presence of membrane buds and tubules, which contain cargo destined for protein secretion [12]. Other ER domains, however, are void of ribosomes. One such domain is the PAM, which provides ER-synthesized lipids to the plasma membrane and mediates capacitative calcium entry from the extracellular space [13]. The MAM forms close contacts with mitochondria, supports the transfer of essential lipids from the ER to mitochondria and regulates calcium exchange between these two organelles [14,15].

Our current understading of MAM structure and function has evolved over 50 years, beginning with an electron microscopy-based report in 1959 that demonstrated the close proximity of the ER with mitochondria in fish gills [16]. Subsequent studies from 1969 to 1971 reported that liver microsomes composed of ER and associated with mitochondria contained all enzymes required for the synthesis of mitochondrial phospholipids [17–19]. During the 1970's, protocols designed to isolate ER using detergent-free homogenization uncovered the tenacious association of mitochondria with heavy ER membranes [20,21], leading to the hypothesis that biochemically distinct domains of the ER mediate interaction with mitochondria [21]. However, this provocative idea did not gain support until 1990, when the *bona fide* biochemical isolation of an ER domain associated with crude mitochondria from a Percoll gradient was reported. This critical breakthrough led to the designation of this domain as the MAM and subsequently enabled further characterization of the MAM [22–24]. Also during that decade, studies demonstrated the extent and structure of the MAM by confocal and electron microscopy [25,26].

In parallel to these biochemical and microscopical analyses, seminal studies demonstrated that ER-mitochondria contacts at the MAM accommodate calcium transfer between the two organelles [27,28]. This direct transfer occurs in a "quasi-synaptic" manner without major spills into the cytosol [26,29,30]. This model is supported by the enrichment of IP3Rs on the MAM, where they mediate the bulk of calcium release from the ER [31]. It is not yet known whether this enrichment extends to the SERCA transmembrane ATPase calcium pumps [32,33], which transport two calcium ions per hydrolyzed ATP molecule into the ER [34]. Together, these data have led to the conclusion that the MAM is not only a site of lipid synthesis and transfer, but also a calcium signaling hub.

The importance of ER-mitochondria calcium signaling is most evident during the onset of apoptosis. Upon apoptosis onset, a robust transfer of calcium from the ER to the cytosol and mitochondria promotes cell death [35]. The MAM accommodates this transfer, since the disruption of the MAM, achieved by siRNA knockdown of PACS-2, results in the inhibition of ER calcium release and apoptosis onset [36]. Surprisingly however, PACS-2 knockdown also induces an unfolded protein response (UPR), typically elicited by disruption of ER oxidative protein folding [36]. Together with the observation that the MAM appears to be particularly enriched in ER folding chaperones and oxidoreductases [37–39], this domain of the ER may be functionally linked to the folding of newly synthesized proteins and might bridge the control of oxidative protein folding to ER calcium homeostasis. This review will summarize the functional ties between the MAM and ER oxidative protein folding, in addition to the more established roles of the MAM in apoptosis onset and progression.

2. Proteins regulating the composition and formation of the MAM

Recent research has focused on the description of connecting structures between the ER and mitochondria at the MAM. The *in vitro* incubation with proteinase not only detaches the ER from mitochondria, but also disrupts calcium transfer, thus suggesting these two organelles may be connected by proteinaceous tethers [40]. Since phospholipid import into mitochondria only decreases by 50% upon incubation of the mitochondria and the MAM with proteinase K, membrane-membrane association or membrane fusion between the two organelles may also occur [41]. Both lipid import into mitochondria and calcium transfer between the ER and mitochondria could depend on ubiquitination of yet to be identified substrates on the MAM: In yeast, phosphatidylserine transport to the mitochondria requires the expression of Met30p, a subunit of the SCF ubiquitin-ligase complex [42], whereas the amount of total ER calcium in mammalian cells depends on the ubiquitination of the ER stress protein Herp (homocysteine-inducible ER protein) by the expression levels of the E3 ubiquitin ligase POSH (plenty of SH3s) [43].

In yeast, the integral ER membrane protein Mmm1p, the peripheral ER membrane protein Mdm12p, and the two mitochondrial outer membrane proteins Mdm10p and Mdm34p have been demonstrated to tether the ER to mitochondria and to mediate mitochondria inheritance and movement [44,45]. Mdm12p and Mmm1p are members of the SMP proteins family, whose members are widespread amongst eukaryotic species and play roles ranging from endocytosis to lipid metabolism and mitochondrial inheritance [46]. The role of mammalian protein homologues of this protein family in the formation of the MAM is currently unclear.

However, three MAM-regulating proteins are nevertheless known in mammalian cells: mitofusin-2, GRP75 and PACS-2 (Figure 1) [36,47,48]. Mitofusin-2 is an outer membrane GTPase that stabilizes interaction between adjacent mitochondria, thus promoting their fusion [49–51]. Surprisingly, mitofusin-2 also regulates ER morphology and calcium homeostasis, and mediates tethering of the ER to mitochondria [47]. A similar bridging and calcium homeostasis function is performed by GRP75, an hsp70 homologous cytosolic chaperone, that bridges the mitochondrial voltage dependent anion channel (VDAC) to the large N-terminal IP3 binding regulatory domain of the IP3R [48].

The role of PACS-2 in MAM tethering is complex. PACS-2 affects the composition of the MAM by mediating ER localization of the calcium release channel TRPP2 (the transient receptor potential protein 2, also known as polycystin-2) and of the chaperone calnexin, in particular on the MAM [38,52]. Since the interaction of calnexin with SERCA in the ER inhibits calcium uptake [53] and calcium can exit the ER through TRPP2, siRNA knockdown of PACS-2, associated with reduced amounts of calnexin and TRPP2 at the MAM, increases the amount of IP3R-releasable calcium in the presence of extracellular calcium (see also section

5) [36]. PACS-2 siRNA knockdown also uncouples the ER from mitochondria, suggesting PACS-2 is required for MAM stabilization or formation [36]. Interestingly, PACS-2 knockdown increases ER calcium levels, but also induces the UPR, indicative of ER stress and suggesting that the MAM could impact on the control of ER oxidative protein folding as well [36].

3. ER folding mechanisms and stress

The ER maintains an optimized environment to support the production of secretory proteins. This includes maintaining millimolar concentrations of free calcium, which is more than 1,000-fold higher than the concentration of free calcium in cytosol [54,55]. Depletion of ER calcium stalls oxidative protein folding and prevents export of secretory proteins from the ER [56,57]. The orchestration of polypeptide folding and formation of disulfide bonds requires chaperones and oxidoreductases [58]. One of the best-characterized chaperones is the luminal protein BiP/GRP78, which is an ATPase that promotes the folding of hydrophobic regions in polypeptides to the interior in a calcium-dependent manner [59,60]. Following the enzymatic action of BiP/GRP78, newly synthesized proteins interact with a pair of closely related lectins, the transmembrane protein calnexin and its luminal counterpart calreticulin, both of which require calcium for their folding activities as well [61–65]. These two proteins recognize monoglucosylated newly synthesized proteins and mediate their folding, assisted by disulfide bond formation catalyzed by the oxidoreductases protein disulfide isomerase (PDI) and ERp57 [66,67]. To ensure the exclusive export of fully folded proteins, the ER uses sophisticated and stringent quality control mechanisms that retain incompletely folded intermediates by chaperones [68]. One example is the calnexin/calreticulin cycle, where nascent secretory proteins interact in their monoglucosylated forms with the two chaperones that promote their folding. This interaction is followed by a removal of the terminal glucose sugar by glucosidase II, leading either to export from the ER for proteins that have attained their mature conformation, or to re-glucosylation of incompletely folded proteins by UDP-glucose:glycoportein glucosyltransferase (UGGT) to allow another round of enzymatic action by calnexin and calreticulin [69,70]. Interestingly, chaperones and oxidoreductases such as calnexin, BiP/GRP78 and, in particular, calreticulin not only depend on calcium for their functioning, but also buffer calcium within the ER and thus determine the amount of free calcium within the ER, essential for the functioning of ER chaperones and normally about one fifth of the total ER calcium content of 5 mM [61,71]. For calreticulin, the calcium buffering function may even be more crucial than its role in ER protein folding, since this function can not be compensated by calnexin, the related lectin chaperone of the ER [72].

Interference with chaperone-assisted protein folding, either by depletion of ATP or ER calcium, or by a block of glycosylation or generation of unfavorable redox conditions that prevent disulfide bond formation can all result in ER stress and activation of the UPR [73,74]. Thus, the block in cargo protein folding under conditions of low calcium appears to result from its manifold roles as a co-factor for chaperone-substrate interaction by calnexin and calreticulin [75,76] and BiP/GRP78 [77], and also by its role in the formation of complexes between chaperones and oxidoreductases such as between calreticulin and ERp57 [78].

The UPR is controlled by the action of several ER-localized transmembrane stress sensors, Ire1, PERK and ATF6 [73,74]. The monitoring of ER oxidative protein folding by the stress sensors is highly fine-tuned. The main mechanism that controls the signaling of the stress sensors depends on their ability to dimerize upon the accumulation of unfolded proteins. The chaperone BiP/GRP78 is a central regulator of this effect, since its increasing association with unfolded proteins under conditions of ER stress results in its decreased ability to interact with the stress sensors, but eventually also results in the accumulation of “naked” unfolded proteins that are not bound to this chaperone. Accumulation of unfolded proteins within the ER induces

the transmembrane stress sensors to activate UPR signaling by different mechanisms. For instance monomeric ATF6 is transported to the Golgi complex [79], where it is cleaved by S1P/SKI-1 (site-1 protease/subtilisin kexin isozyme 1) and S2P [80,81]. Cleaved ATF6 then translocates to the nucleus, where it activates transcription of genes encoding BiP/GRP78, and XBP1 [82,83]. Conversely, Ire1 and PERK require oligomerization at the ER, which exposes and triggers their kinase activity [84–86]. Initially, the UPR utilizes all three sensor proteins to boost pro-survival signaling. Activated Ire1 becomes an endonuclease, which splices the XBP1 mRNA in mammalian cells, resulting in the expression of the active XBP1 transcription factor that promotes the production of ER chaperones and oxidoreductases [87,88]. Actived PERK counteracts ER stress by phosphorylating eIF2α, leading to translational arrest and thus reducing the ER protein load [89,90]. These responses to acute ER stress are intended to reset ER homeostasis by reducing the ER protein load, while increasing factors that combat ER stress (e.g., chaperones).

4. ER stress and calcium signaling at the MAM

Prolonged ER stress, lasting typically more than 4h, can induce apoptosis. Under these conditions, the kinase domain of oligomerized Ire1 bind TRAF2 (tumor necrosis factor receptor-associated factor 2), an adaptor protein that bridges Ire1 to the protein kinase ASK1, leading to the activation of the pro-apoptotic kinase JNK [91]. Additionally, ATF6, the PERK target ATF4, and XBP1 induce CHOP, the CCAAT/enhancer-binding protein (C/EBP) homologous protein [92,93]. CHOP is a transcription factor that promotes apoptosis by repressing Bcl2 transcription and promoting Bim transcription [94,95].

Paralleling the modulation of UPR signaling, the readout of ER-mitochondria calcium exchange also undergoes dramatic changes when ER stress persists. Under homeostatic conditions, there is a continuous ebb and flow of calcium between the ER and mitochondria [3], mediated on the ER face by MAM-localized IP3Rs that passively release puffs of calcium into calcium hot spots in the cytosol [30,96–99]. Mitochondria take up these local high amounts of calcium in a quasi-synaptic manner through a gated and highly selective calcium uniporter [100–102]. Within the mitochondria, increased calcium levels promote the activity of ATP synthase [103,104]. Calcium efflux from mitochondria is likely mediated via an as yet unidentified Na/Ca exchanger [102]. The released calcium is retrieved to the ER by SERCA calcium pumps, completing the cycle.

A sustained ER stress of more than 24h or the induction of apoptosis result in the release of ER calcium at the MAM that triggers loss of mitochondrial membrane potential to promote apoptosis [105,106]. Under these conditions, cytochrome c gradually translocates from mitochondria to the ER, where it binds the IP3R [107,108]. This binding abolishes the calcium-mediated inhibition of IP3-associated calcium release and results in a feed-forward amplification of the ER calcium release in early apoptosis. Blocking this interaction inhibits the progression of apoptosis [109,110], whereas allowing it leads to increased release of cytochrome c and a robust transfer of calcium from the ER to the cytosol and mitochondria to promote cell death [35]. Whereas the IP3R type 3 (IP3R3) apparently is the preferential calcium channel for apoptotic signal transmission from the ER to mitochondria, modulating the IP3R type 1 (IP3R1) activity also interferes with apoptotic signaling [111–114]. Moreover, calcium chelation or disruption of IP3R by pharmacological inhibiton can efficiently block apoptosis onset [109,115]. Given the localization of IP3Rs to MAMs [32,99,114], the quantity and quality of this ER microdomain appears to regulate the strength and speed of cell death. Consistent with this hypothesis, proteins that mediate MAM composition and integrity have key roles in the onset of apoptosis.

Overexpression of mitofusin-2 or GRP75 inhibits apoptosis [116–119]. However, it is currently unclear whether this function stems from an influence on calcium exchange between the ER and mitochondria during either resting conditions or apoptosis onset. PACS-2 plays multiple roles in apoptosis: First, its expression is required for the ER localization of TRPP2 and for maintaining contacts between the ER and mitochondria that accommodate pro-apoptotic calcium release [36]. Second, PACS-2 is required for extrinsic and intrinsic apoptosis induction in virus-infected or transformed cells at the level of Bid translocation to the mitochondria [36,120,121]. Phosphorylation of PACS-2 Ser_{437} by Akt1 regulates the ability of PACS-2 to switch between its roles in homeostasis and apoptosis. Akt1 phosphorylation of PACS-2 promotes its binding to 14-3-3 proteins and its role in the ER homeostasis, whereas dephosphorylation of PACS-2 Ser_{437} triggered by death ligands promotes apoptotic Bid activation at mitochondria [120,122,123].

Proteins of the Bcl2 family also regulate the death-promoting calcium transfer from the ER to mitochondria. Overexpression of anti-apoptotic Bcl2 reduces the ER calcium content by increasing the calcium permeability of the ER [124], a characteristic of this organelle that may depend on Bcl2 itself or the translocon [125]. Similarly, Bcl-XL binds to the IP3R and promotes its ability to release calcium [126]. Furthermore, ER-targeting of the pro-apoptotic BH3-only protein Nix increases ER calcium content and promotes the opening of the mitochondrial permeability transition pore [127]. Recently, an interesting link between ER-localized Bax and Bak and ER stress signaling has been discovered, since Ire1 signaling requires expression of these two Bcl2 family proteins [128]. The ER-localized Bax inhibitor 1 (BI-1) opposes this role of Bax and Bak by inhibiting Ire1 and blocking ER oxidative protein folding, while at the same time reducing the overall calcium content of the ER [129–131].

5. MAM-localized chaperones and oxidoreductases modulate calcium exchange with mitochondria

ER oxidative protein folding itself may be another important regulator of calcium homeostasis and signaling, since numerous ER chaperones and oxidoreductases localize to the MAM and associate with the IP3R and SERCA. For instance, ERK1-phosphorylation of calnexin on Ser_{563} blocks calcium uptake by SERCA2b [53,132,133]. However, prolonged ER stress or ER calcium depletion lead to calnexin dephosphorylation and a removal of calnexin from the MAM, thus removing the calnexin-dependent clamp on SERCA activity [38,53,134]. PACS-2 binds to calnexin, when this SERCA regulator is dephosphorylated on $Ser_{534, 544}$, two protein kinase CK2 target sites, which can boost the role of Ser_{563}-phosphorylated calnexin in its folding and retention activities close to the translocon [132,133,135]. Thus, PACS-2 may modulate the extent of calnexin/SERCA interaction and its associated regulatory mechanism [38,132]. The thioredoxin-related oxidoreductases ERp57 and ERp44 appear to modulate ER calcium signaling in a similar way. ERp57 interacts with ER-facing cysteines of SERCA2b under oxidizing ER conditions, thus inhibiting SERCA2b calcium pumping, whereas ERp44 interacts with lumenal cysteines of the IP3R1 to inhibit calcium transfer to mitochondria when ER conditions are reducing [113,136].

The structure of the MAM directly determines the extent of this chaperone and oxidoreductase-regulated calcium flux, as observed following PACS-2 knockdown, which leads to an increase in ER calcium or in the absence of mitofusin-2, which results in lower ER calcium, but increased calcium levels within mitochondria [36,47]. Additionally, the amount of calcium on the ER cytosolic face and within the ER lumen dictates the activity of both the IP3R and SERCA; high cytosolic calcium levels block the IP3R opening, whereas high concentrations of calcium within the ER lumen block SERCA [96,137].

Besides the MAM structure itself, this calcium transfer could additionally require the formation of a glycosphingolipid-enriched microdomain fraction of MAMs [138], possibly to confine the chaperoning activity of sigma-1 receptors on IP3R3 to the MAM [139]. This chaperoning activity of sigma-1 receptors inhibits proteasomal degradation of the channel [112]. By doing so, the sigma-1 receptor maintains ER-mitochondria calcium signaling that is active during periods of extended ER stress. A DRM-like lipid composition of the MAM might also be required for the formation of a complex of erlin-1 and erlin-2 (ER lipid raft-associated proteins, also known as SPFH1/2, proteins with similarities to stomatin, prohibitin, flotillin, and HflC/K) that degrades IP3Rs by ERAD [140–142].

Together, these findings demonstrate that MAM formation, and MAM quality, together with the regulated enrichment of select chaperones and oxidoreductases control ER calcium homeostasis and signaling with mitochondria.

6. Role of ATP for the ER-mitochondria interaction and the MAM

The exchange of calcium between the ER and mitochondria has functions that go beyond the maintenance of homeostasis in the two organelles. High cytosolic calcium impedes mitochondria movement along microtubules, at sites of IP3R-mediated calcium release [143]. This suggests that mitochondria may coalesce near IP3R-enriched MAMs, allowing mitochondria to supply fuel to the ER, one of the major sites of ATP consumption in the cell [144,145]. Numerous activities associated with the ER consume ATP, in particular the import of calcium by SERCA [146,147], mRNA translation [148], protein translocation [149], the folding of newly synthesized polypeptides (see below), ERAD [150,151] and the transfer of lipids into mitochondria [152]. However, because many of these mechanisms are interdependent, it has been challenging to determine how much ATP is used for each process. In particular, it appears difficult to assess the individual consumption specifically used for ER oxidative protein folding, although it is clear that the correct functioning of many ER chaperones such as BiP/GRP78, GRP94 and calnexin depends on a constant supply of ATP [153–155].

Glucose has long been viewed as the chief energy provider for ER oxidative protein folding, because lack of glucose can induce ER stress [156,157] and ongoing glycolysis contributes to ER homeostasis [158]. Many of these studies, however, were performed using tumor cell lines cultured in media containing high glucose, exacerbating the abnormally high contribution of glycolysis of these transformed cells to the energy landscape [159]. However, most cell types, including the commonly used HeLa cells, still produce up to 50% of their total ATP supply within mitochondria when glucose is low [104,160–162]. Consistent with this observation, even in laboratory culture settings, the inhibition of the mitochondrial electron transport chain with Antimycin A leads to the induction of a UPR in MDCK cells and inhibition of ER-mitochondria calcium flux with CGP 37157 blocks production of secretory proteins at the ER level in CHO cells [163,164]. The disruption of the MAM and induction of the UPR by PACS-2 knockdown highlights the role of the MAM for oxidative protein folding in the ER [36]. Accordingly, the number of tight contacts between the ER and mitochondria double under conditions of ER stress, although future experiments will have to determine whether ER-stressed cells aim to increase metabolite exchange between the two organelles or whether this observation is explained by pro-apoptotic calcium exchange [40].

Overexpression of mitochondrial adenine nucleotide translocase (ANT) increases ATP export from mitochondria and reduces ER-mitochondria calcium flux, suggesting transfer of ATP from mitochondria influences ER lumenal calcium homeostasis [165]. This finding raises the question as to how ATP is imported into the ER, which is a particularly relevant question for ER oxidative protein folding. One candidate ATP importer associates with ER membranes

[166]. Confirming this finding, partial purification showed that an ADP/ATP antiporter of 56 kDa mediates ATP import into the ER in rat liver [167,168], but it is not known to which ER microdomain these candidate transport proteins are localized. Thus, like calcium, ATP production in mitochondria and its consumption in the ER may influence mitochondrial activity or proximity.

7. The MAM and the regulation of ER redox

The discovery that ER oxidative protein folding and mitochondrial oxidative phosphorylation compete for oxygen further supports a link between ER and mitochondria metabolism [169]. The ER enzyme that is responsible for oxygen consumption in yeast is Ero1p, a glycosylated membrane-associated flavoenzyme localized to the lumenal side of the ER [170]. Ero1p forms an electron transport chain within the ER lumen using the thioredoxin-related enzyme PDI and oxygen [171]. Ero1p uses molecular oxygen to oxidize PDI, which can then catalyze the formation of disulfide bonds within newly synthesized polypeptides [172]. The human Ero1 paralogs, Ero1-Lα and Ero1-Lβ catalyze the same reaction in human cells [173,174]. Whereas Ero1-Lα shows a widespread distribution, Ero1-Lβ is mostly found in tissues that secrete large amounts of protein, suggesting that Ero1-Lβ specifically increases the efficiency of the ER secretion machinery [173–175]. The transcription of Ero1-Lα increases upon ER stress and hypoxia, whereas the transcription of Ero1-Lβ is exclusively under the control of ER stress [173,176,177], although the significance of this different regulation is currently unclear.

Similar to oxidative phosphorylation within mitochondria, the formation of disulfide bonds by Ero1-Lα and Ero1-Lβ leads to production of ER-localized hydrogen peroxide (H_2O_2), making the ER the cellular organelle with the highest content of H_2O_2 [178]. High levels of this ROS, which occurs under ER stress [129,179–181], lead to the inactivation of SERCA by S-glutathionylation [182] and the activation of IP3R by oxidation, increasing the level of calcium on the cytosolic face of the ER [183]. In addition, H_2O_2 also evokes an increase of mitochondrial calcium [184]. Thus, ROS production by Ero1 proteins may provide an additional mechanism for the ER to attract mitochondria under conditions of ER stress. Consistent with this hypothesis, Ero1-Lα is highly enriched on the MAM [39], where it potentiates the release of calcium during ER stress [185]. It remains to be demonstrated, however, whether Ero1-Lα directly links the control of ER redox to the functioning and formation of the MAM as well.

All Ero1 proteins require flavin adenine dinucleotide (FAD) as a co-factor to remain in their active state and depend on free FAD levels within the ER [169,186,187]. FAD is made from riboflavin by FAD synthetase in the mitochondrial matrix and is converted to its reduced form ($FADH_2$) by the tricarboxylic acid cycle and oxidative decarboxylation [188,189]. In yeast, flavin carrier proteins import FAD into the ER, but they have not yet been detected in metazoans [190,191]. FAD metabolism within the mitochondria is thought to control the redox homeostasis of the ER, since deficiency of riboflavin impairs oxidative folding and secretion [192,193]. Together, these findings suggest multiple ties link the ER and the mitochondrial metabolism (Figure 1). The dependence of ER oxidative protein folding on the exchange of calcium, ATP and metabolites such as FAD with mitochondria may explain why ER chaperones and oxidoreductases regulate multiple aspects of the MAM. The coming years will lead to the identification of more MAM components and MAM regulating proteins that integrate the many interactions between the ER and the mitochondria. Of particular interest in this context appears the identification of ATP and ROS sensing and transporting proteins, which are currently unknown in most organisms.

8. Conclusions and Perspective

Critical progress has been made in the description of an apparently surprising link between ER oxidative protein folding and a domain of the ER called the MAM. Numerous ER chaperones are involved in the regulation of the function of this structure that controls cell metabolism and apoptosis. These findings suggest that proteins of the MAM and ER chaperones that regulate MAM signaling could play important roles in diseases where normal cellular life cycles are compromised. This could be relevant in tumor tissue. For instance, low levels of the ER calcium pump SERCA2b are associated with squamous cell carcinoma [194–196]. Similarly, increased levels of the sigma-1 receptor are found in breast cancer cell lines and are known to counteract ER stress response and apoptosis induction [197]. Furthermore, numerous members of ER oxidoreductases and chaperones (including calnexin and ERp57) show abnormal expression levels in tumors [198,199]. Not surprisingly, mitofusin-2 markedly suppresses cell proliferation of tumor cells when overexpressed [200] and PACS-2, frequently absent from sporadic colorectal cancer cells [201], critically determines the outcome of TRAIL-mediated apoptosis [120]. Given that the MAM performs such important roles for cellular survival and apoptosis, interference with its natural function using small molecule inhibitors could tip the balance of life or death into a desired direction. These approaches have to await the discovery of more MAM sorting mechanisms and a more comprehensive description of the MAM composition and function.

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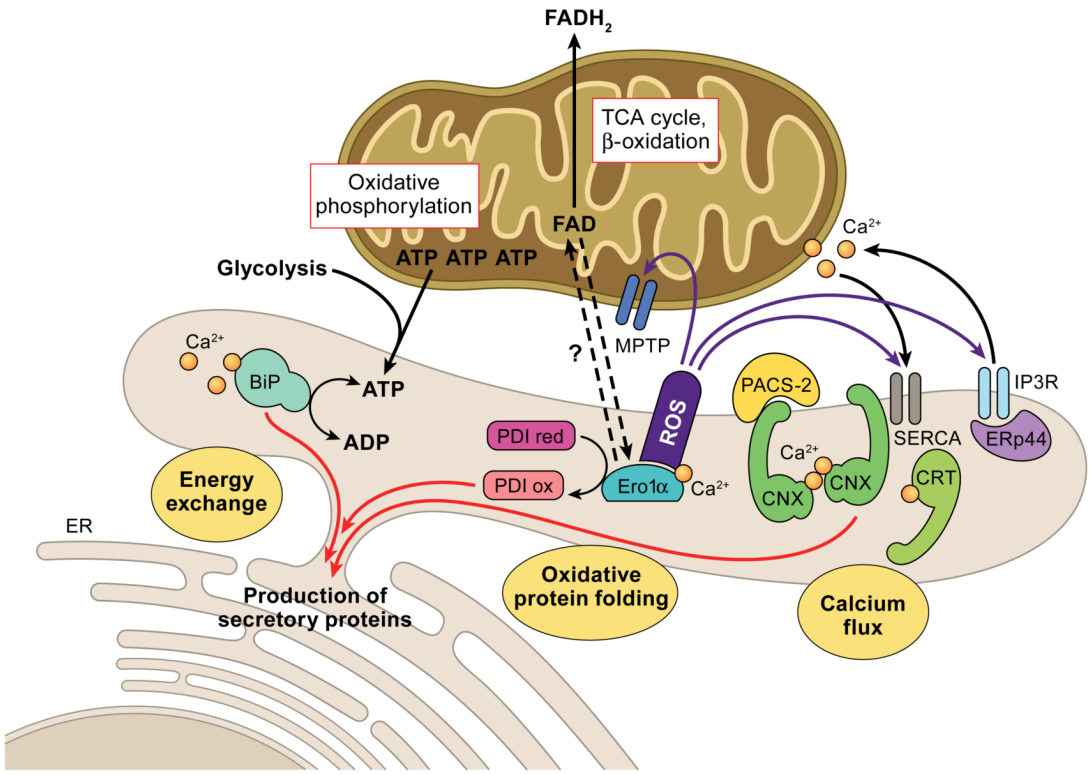

Figure 1.

Three major metabolic exchanges between the ER and mitochondria impact on ER chaperone systems that catalyze the production of secretory proteins. **1. Energy exchange.** GRP78/BiP requires ATP that is predominantly supplied by mitochondria, and to a lesser extent from glycolysis. GRP78/BiP also depends on ER calcium. **2. Oxidative protein folding.** PDI forms disulfide bonds with the help of its recharger Ero1α, which can bind calcium and FAD derived from mitochondrial metabolism. Import proteins of FAD into the ER are currently unknown in mammalian systems. ROS produced by Ero1α could directly impact on the mitochondrial permeability transition pore (MPTP), SERCA and the IP3R. **3. Calcium flux.** The calnexin/calreticulin folding cycle depends on ATP and ER calcium. Calnexin and calreticulin buffer free calcium within the ER, but also associate with SERCA to modulate calcium import. The localization of calnexin to the MAM and hence potentially its interaction with SERCA is under the control of PACS-2. The MAM-associated moiety of the oxidoreductase ERp44 interacts with the IP3R and regulates IP3R calcium release.