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Review

ER-to-mitochondria miscommunication and metabolic diseases

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ABSTRACT

Eukaryotic cells contain a variety of subcellular organelles, each of which performs unique tasks. Thus follows that in order to coordinate these different intracellular functions, a highly dynamic system of communication must exist between the various compartments. Direct endoplasmic reticulum (ER)–mitochondria communication is facilitated by the physical interaction of their membranes in dedicated structural domains known as mitochondria-associated membranes (MAMs), which facilitate calcium (Ca^{2+}) and lipid transfer between organelles and also act as platforms for signaling. Numerous studies have demonstrated the importance of MAM in ensuring correct function of both organelles, and recently MAMs have been implicated in the genesis of various human diseases. Here, we review the salient structural features of interorganellar communication via MAM and discuss the most common experimental techniques employed to assess functionality of these domains. Finally, we will highlight the contribution of MAM to a variety of cellular functions and consider the potential role of MAM in the genesis of metabolic diseases. In doing so, the importance for cell functions of maintaining appropriate communication between ER and mitochondria will be emphasized.

1. Introduction

Eukaryotic cells are characterized by a high degree of compartmentalization of biological and biochemical functions within specialized membrane-bound organelles [1–3]. These cellular compartments are often further partitioned into subdomains, thus providing a mechanism to segregate specific processes into different regions within the same organelle. Although this division is essential for separating potentially incompatible activities, regulated integration of cellular physiology depends upon effective cross-talk and functional coordination between multiple organelles [4–6]. Such inter-organelle communication is frequently achieved by direct physical contact between organellar membranes and the necessary interactions are often highly regulated as well as dynamic in time and space [4–7]. One of the best characterized such inter-organellar communication sites is the connection between the endoplasmic reticulum (ER) and mitochondria. The first evidence for the existence of sites of physical interaction between these membranes came from electron microscopy studies over 50 years ago [8]. Rather interestingly, ER–mitochondria contacts were only isolated some 30 years later, by means of subcellular fractionation using Percoll density gradients [9,10]. This early evidence for the existence of physical ER–mitochondria interactions led to the genesis of the term MAMs, standing for Mitochondria-associated ER membranes [11]. Ever since their discovery, the importance of these contact sites in organelle cross-talk has been confirmed using numerous approaches (Fig. 1) [4–6,12].

ER–mitochondria contact sites permit reciprocal regulation of function in both organelles, thereby impacting various cellular activities, including energy metabolism, Ca^{2+} handling [13], lipid homeostasis [9] as well as regulation of cell death and survival [4–7]. In this review, we highlight the role of a number of proteins important in regulating the ER–mitochondria interface, as well as key experimental approaches used to study these inter-organellar contact sites and their physiological function. We will also discuss how alterations in ER-to-mitochondria communication contribute to the pathogenesis of major metabolic diseases.

2. ER–mitochondria coupling

ER and mitochondria communicate through close physical juxtapositioning of the two membranes, with distances between the two

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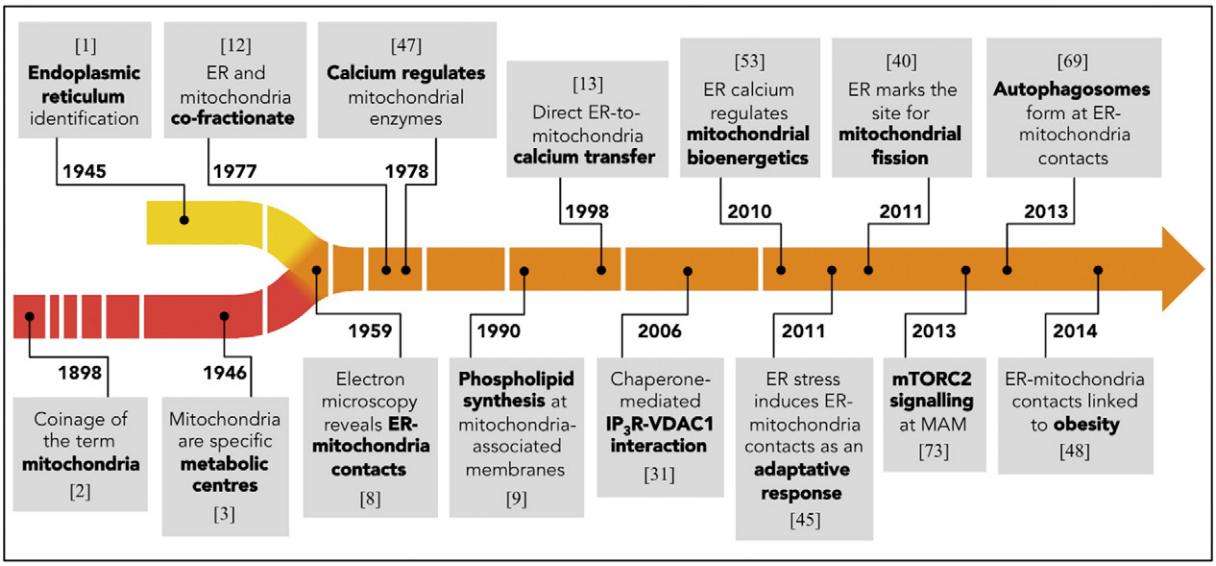

Fig. 1. ER–mitochondria contacts research timeline. Since the identification of ER–mitochondria contacts using electron microscopy, many aspects of their functional roles have been uncovered. Alterations in lipid metabolism, Ca^{2+} homeostasis and stress responses, as well as the development of metabolic diseases represent the perhaps best-characterized consequences attributed to cross-talk between both organelles.

ranging from 10 to 25 nm. Despite the close proximity of the two organelles, their membranes do not fuse, thus preserving their identity and functionality [4,5,14]. Domain-specific tethering structures help to establish and maintain the MAM, which can be either stable or transient [15]. At the molecular level, these tethering structures are composed of proteins and lipids, residing in the outer mitochondrial membrane

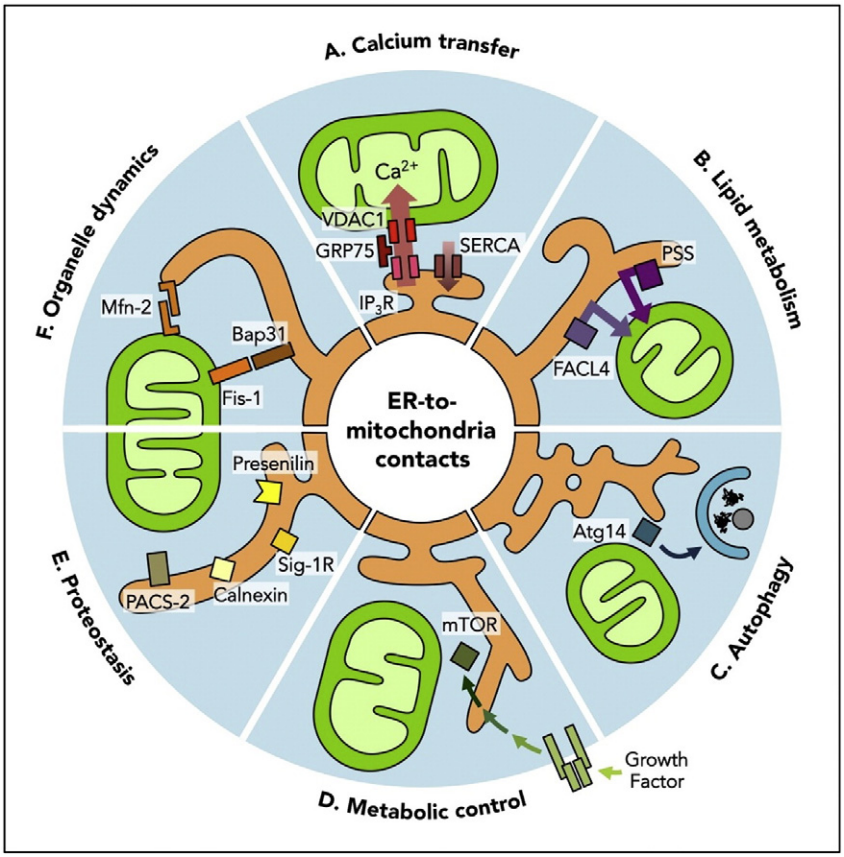

Fig. 2. ER–mitochondria contacts and cell physiology. A, Direct Ca^{2+} transfer occurs between ER and mitochondria via IP_3R and VDAC channels coupled to the cytoplasmic chaperone GRP75. The SERCA pump is also present at the ER–mitochondria interface. B, Crucial enzymes for lipid metabolism reside at the ER–mitochondria contacts, among them phosphatidylserine synthase (PSS) and fatty acid-CoA ligase 4 (FACL4). C, ER–mitochondria contacts have been shown to represent nucleation spots for autophagosomes via ATG14 enrichment. D, Metabolic regulator complex mTORC2 is present at MAM, and increases there in response to growth factor stimulation. E, ER–mitochondria contacts contain proteins involved in protein homeostasis, such as the proteases presenilins, quality control lectin calnexin and sorting factor PACS-2. F, Proteins that regulate organelle dynamics are present in MAM, such as the mitochondrial constriction GTPase Drp-1, and the mitochondrial fusion GTPase Mfn-2.

(OMM) or the ER membrane, which interact to facilitate the formation of MAM. These inter-organellar domains not only sustain communication between ER and mitochondria but also confer new properties and functions to the associated organelles (Fig. 2).

Using selective trypsin digestion, Csordás et al. initially demonstrated that the binding between ER and mitochondria is protein-dependent [14]. More recent studies then identified proteins involved in the formation of the MAM. Two independent studies using mass spectrometry each identified approximately 1000 proteins localized to the MAM (991 and 1212 proteins, respectively) [16,17]. Despite the large number of proteins identified, only 44% of them identified were common to both studies. This difference may be due, in part, to differences between cell types, i.e., human fibroblasts versus mouse brain, respectively [16,17]. In addition, the samples analyzed, though highly enriched for MAM, were most certainly contaminated with other cellular components reflecting the tissue or cell type of origin.

Mitochondria obtain most of their proteins by direct import from cytosolic ribosomes [18,19]. In addition, mitochondrial membrane biogenesis requires the import of membrane phospholipids. The ER is the main site of phospholipid biosynthesis and plays a fundamental role in intracellular vesicular trafficking pathways. Because mitochondria are not connected to classical vesicular trafficking mechanisms, they require direct lipid transfer from the ER [6]. More importantly, biosynthesis of some phospholipids depends on this communication. Phosphatidylserine (PS), synthesized at ER, is transported to mitochondria for its conversion to phosphatidylethanolamine (PE) by the mitochondrial enzyme PS decarboxylase [9,20]. Then, PE returns to the ER for conversion to phosphatidylcholine (PC), which is ultimately transferred back for mitochondrial membrane biosynthesis [6,20].

2.1. The ERMES complex in yeast

The best characterized ER–mitochondria tethering complex is the ER–mitochondria encounter structure (ERMES) identified in yeast (*Saccharomyces cerevisiae*). This complex is composed of 4 proteins: Mmm1 localized at the ER membrane, Mdm10 and Mdm34 in the OMM, and the cytosolic protein Mdm12 [21–23]. The ERMES complex has been implicated in many cellular functions, including phospholipid exchange and Ca^{2+} cross-talk between ER and mitochondria, protein import and mitochondrial genome maintenance [23].

The ERMES is thought to facilitate lipid exchange at the ER–mitochondria interface by establishing a zone where the distance between organelles is relatively small, thus ensuring the specificity and efficacy of the exchange reaction [6]. Three of the ERMES proteins (Mdm12, Mdm34 and Mmm1) contain a synaptotagmin-like-mitochondrial-lipid binding protein (SMP) domain. The SMP domain is related to the TULIP domain (tubular lipid-binding), which binds and transports lipids, thereby providing a structural basis to explain how lipid exchange occurs between adjacent membranes at ER–mitochondria contacts [24]. According to one study, deletion of ERMES components alters PS conversion to PC, demonstrating that the ERMES complex is required for optimal lipid exchange between cellular compartments. These defects can be partially rescued by the expression of an artificial ER–mitochondrial tether, suggesting that close membrane proximity alone can facilitate transfer [22,23]. Nevertheless, another study by Nguyen et al. shows that the deletion of ERMES proteins in yeast neither directly affects PS to PE conversion nor alters mitochondrial inheritance, because both these effects are secondary to morphological changes in mitochondria [25]. Accordingly, these observations suggest that the ERMES complex plays a structural role, linking ER to mitochondria and regulating mitochondrial morphology, without participating directly in lipid transfer [25]. Recent studies suggest that maintenance of ER–mitochondria tethering and phospholipid transfer requires, in addition to the ERMES complex, ER-shaping proteins [26] and components of the ER membrane protein complex (EMC) [27], underscoring that the regulation of this process is complex and is still not completely understood.

2.2. Tethering complexes in mammalian cells

Homologues of the core components of ERMES have not yet been identified in mammalian cells, where the ER–mitochondrial interface appears to be more complex. Many proteins and protein complexes have been implicated in ER–mitochondria tethering, as well as facilitating functional transit of metabolites and signaling molecules between both organelles. Most of these proteins are components of the MAM and contribute to their stability (Fig. 2) [28].

Mitofusins, Mfn-1 and Mfn-2, are two related GTPases that localize to the mitochondrial surface, where they participate in mitochondrial fusion. Notably, Mfn-2 is also present at the ER, specifically at MAM, where it forms heterotypic or homotypic interactions with mitochondrial Mfn-1 or Mfn-2, thus forming ER–mitochondria bridges (Fig. 2F) [29]. Accordingly, Mfn-2 silencing in embryonic fibroblasts and HeLa cells has been shown to increase the distance between ER and mitochondria [29]. Furthermore, MAM composition is altered in cells lacking Mfn-2, consistent with a loosening of the ER–mitochondria connection in the absence of Mfn-2 [29]. Mfn-2 activity at the ER–mitochondria interface is regulated by MITOL, a mitochondrial ubiquitin ligase [30]. MITOL mediates the addition of lysine 63-linked polyubiquitin chains to mitochondrial-localized Mfn-2, but not to ER-localized Mfn-2. Polyubiquitination of Mfn-2 induces oligomerization, a fundamental step in Mfn-2-dependent ER–mitochondria tethering [30].

In mammals, Ca^{2+} transfer from ER to mitochondria is facilitated by the cytosolic chaperone glucose-regulated-protein 75 (GRP75), which forms a complex with the ER transmembrane Ca^{2+} release channel, the inositol 1,4,5-trisphosphate receptor (IP_3R), and the mitochondrial porin voltage-dependent anion channel (VDAC) in the OMM [31]. This indirect interaction between IP_3R and VDAC provides the juxtapositioning required for efficient ER-to-mitochondria Ca^{2+} transfer (Fig. 2A). Once Ca^{2+} ions have passed through the OMM, they enter into the matrix through a mitochondrial Ca^{2+} uniporter (MCU). The many roles attributed to ER–mitochondria Ca^{2+} transfer will be discussed in Section **4**.

ER–mitochondria contacts are also enriched in proteins that participate in the synthesis and transport of phospholipids and glycosphingolipids [9,32]. Phosphatidylserine synthase (PSS), the enzyme responsible for generating PS in the ER was one of the first described components of MAM [9,33]. Newly synthesized PS is preferentially funneled from ER to mitochondria [10], where it is used for the synthesis of PE [6]. In mammals, PE is also transferred back to the ER for its conversion to PC, via PE methylation by the hepatic enzyme PE-N-methyltransferase [34]. This enzyme is enriched in MAM and is actually a good marker for MAM fractions in hepatocytes [6,34]. The fatty acid-CoA ligase type 4 (FACL4), which plays a key role in lipid synthesis and degradation, is also present in MAM, and is frequently used as a marker during subcellular fractionations [6]. These, and other enzymes involved in lipid homeostasis, are considered structural components of the MAM, contributing both to the stability and dynamics of this structure (Fig. 2B) [6,33]. For more details concerning lipid management at the ER–mitochondria interface, the interested reader is referred to additional literature [6].

Another protein complex that potentially aids in tethering ER to mitochondria is that formed between the integral ER membrane protein Bap31, and the mitochondrial fission protein Fis-1 in the OMM. Upon induction of apoptosis, this complex recruits and activates caspase-8, which in turn cleaves Bap31 to yield a pro-apoptotic fragment p20Bap31 [35]. Thus, this complex serves as a platform that couples ER–mitochondrial communication directly to apoptotic signaling.

The ER–mitochondria interface is also a site for processes involved in cellular proteostasis (Fig. 2E). The sorting factor, phosphofurin acidic cluster sorting protein 2 (PACS-2), is present at the MAM. Depletion of PACS-2 increases organelle distance and favors apoptosis by promoting

caspase-dependent cleavage of Bap31 [36]. Other MAM components associated with protein processing include the ER-resident chaperone sigma-1 receptor (Sig-1R) [37], various presenilin proteases [38] and calnexin, which is involved in protein quality control [39].

3. Assessment of ER–mitochondria contacts

Since the first observations of ER–mitochondria contacts in the mid-twentieth century [8], various methods have been employed to evaluate physical and functional ER–mitochondria coupling (Fig. 3). The most direct approach to visualize physical ER–mitochondria interaction is by electron microscopy, which offers the resolution required to quantify the distance between membranes, the number of contacts, and the length of their interface [14]. This technique, coupled with thin tomography, has been used to provide further insight to the 3D nature of organelle contacts (for instance, ER-mediated mitochondrial constriction [40]). These experimental strategies, however, are not widely available, and thus are not well-suited for routine ER–mitochondria examination. Recently, the use of IP_3R–VDAC proximity ligation assay (PLA) has proven useful for the quantification of ER–mitochondria interactions [39]. When combined with standard fluorescence microscopy, this approach also provides a fairly simple method to analyze the cellular distribution of organelle contacts. Colocalization of recombinant targeted proteins, specific probes or immunofluorescent labels represents a third widely used strategy in organelle research, which however is inherently limited by the fact that the distance between ER and mitochondria at contact sites is below the resolution of optical systems (around 150 nm for confocal microscopes). Therefore, this approach is more a measure of organelle proximity, rather than actual ER–mitochondria contacts.

A biochemical approach to studying ER–mitochondria communication is the purification of MAM using Percoll density gradients [41]. The main objective of this method is to analyze the composition of ER

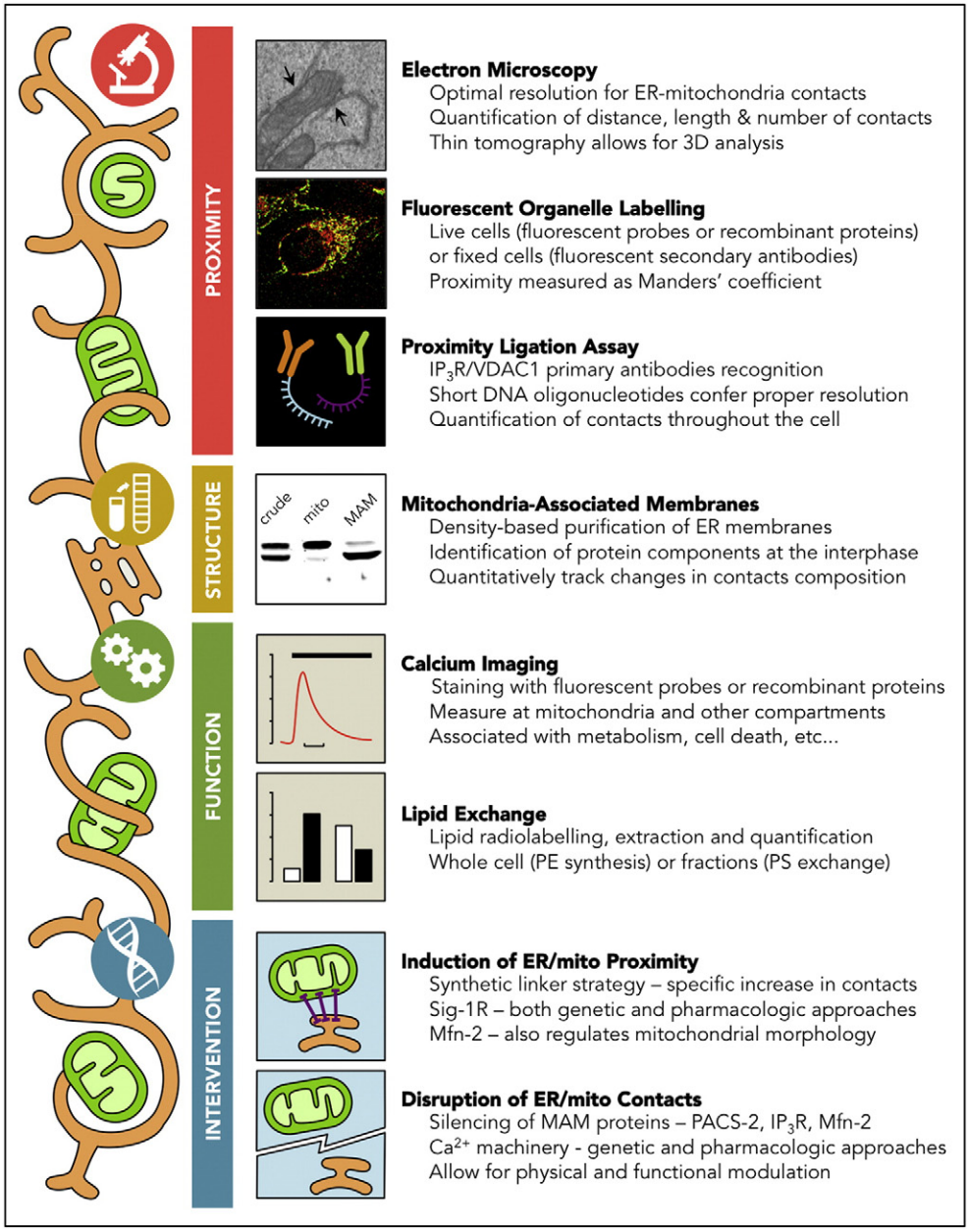

Fig. 3. ER–mitochondria communication toolkit. Currently, different strategies are available to study ER–mitochondria communication. Imaging of organelle proximity can be achieved using electron microscopy, fluorescence microscopy and the proximity ligation assay. The composition of ER–mitochondria contacts can be determined via cell fractionation, and separation of mitochondria from MAM. For functionality, Ca^{2+} transfer and lipid exchange are two quantifiable events that occur at the ER–mitochondria interface. Finally, there are some genetic and pharmacologic strategies that permit increasing or disrupting ER–mitochondria contact sites and, in doing so, assessing their importance in cell physiology.

membranes in close interaction with mitochondria. Identification of molecular entities residing at MAM, as well as how they change in response to the cellular environment, yields important information concerning the processes orchestrating ER–mitochondria cross-talk. One example of MAM plasticity is the redistribution and repurposing of the protein chaperone calnexin. In response to ER stress, calnexin moves from the ER–mitochondria interface, where it regulates Ca^{2+} homeostasis, to the ER protein folding compartment [42]. This exquisite mechanism not only contributes to restoring protein homeostasis via ER quality control, but also adjusts mitochondrial Ca^{2+} uptake, in order to engage mitochondria in the adaptive response (see Section 4 for further details).

The functional impact of ER–mitochondria communication on lipid exchange and Ca^{2+} transfer has been analyzed in both cell lines and isolated tissues. Lipid exchange is quantified primarily as PS-to-PE conversion, or as mitochondrial PS content [9]. These strategies require radioisotopes to label phospholipids, which can then be identified and quantified by thin layer chromatography [9] or mass spectroscopy [43,44]. Ca^{2+} transfer is more widely used and is typically measured in living cells as increases in mitochondrial Ca^{2+} elicited in response to IP_3-generating agents that provoke Ca^{2+} release from the ER (e.g. histamine). For this purpose, both recombinant targeted protein sensors [45] as well as fluorescent chemical indicators [46] have been used. Interestingly, a pericam-based inducible OMM-ER linker has been employed to directly measure Ca^{2+} microdomains at the ER–mitochondria interface [47].

Finally, to demonstrate the importance of the ER–mitochondria contacts in modulating cellular responses, several strategies have been devised to alter the nature of ER–mitochondrial interactions. To artificially tighten organelle physical association, a synthetic linker targeted simultaneously to the OMM and the ER has been used both in vitro [14] and in vivo [48]. Similarly, an inducible linker has also been developed, consisting of an OMM-targeted FKBP (FK506 binding protein) and an ER-targeted FRB (FKBP12-rapamycin binding domain), that rapidly dimerize in response to rapamycin [47]. This strategy, however, does not create new organelle contacts, but rather increases the contact surface at sites where ER and mitochondria are already in close apposition. In terms of endogenous targets, Sig-1R is a candidate target for altering ER–mitochondria interactions, either through pharmacological modulation [49] or via overexpression [50]. Mfn-2 overexpression has also been used to increase ER–mitochondria contacts [51,52]; however, the fact that Mfn-2 also participates in mitochondrial fusion complicates the interpretation of such experiments. Strategies to disrupt or decrease organelle interaction have primarily focussed on silencing the expression of relevant proteins, such as PACS-2 [48], IP_3R [48] and Mfn-2 [45]. Likewise, disruption of functional coupling has been accomplished through genetic or pharmacological inhibition of the ER-to-mitochondria Ca^{2+} handling axis by targeting the IP_3R, VDAC, or MCU.

4. ER–mitochondria control of cell function

4.1. Mitochondrial Ca^{2+} in mitochondrial metabolism and cell death

Understanding the mechanisms controlling communication between ER and mitochondria is important because it impacts on a wide spectrum of cellular fates, ranging from pro-survival metabolic responses to cell death. ER-to-mitochondria Ca^{2+} transfer through the MAM is a central mechanism in both processes, despite the divergent outcomes. A moderate increase in ER–mitochondria contacts can help cells to adapt to stress conditions that require enhanced metabolic output [24]. Moreover, constitutive, basal transfer of Ca^{2+} from ER to mitochondria, through MAM, is essential for maintaining cellular bioenergetics [53]. When this basal transfer is abrogated, cells activate the "self-eating" survival mechanism of autophagy to ensure an adequate energy supply [53]. Ca^{2+} concentration in the mitochondrial matrix acts as a rheostat for metabolism and oxidative phosphorylation [54–56] by increasing the activity of key catabolic enzymes, such as the pyruvate dehydrogenase complex (PDC), isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. The last two enzymes are directly regulated by Ca^{2+} [57,58], while the regulation of the PDC is more complex. PDC, which catalyzes the conversion of pyruvate into acetyl-CoA, is inhibited by phosphorylation. Ca^{2+} controls this process by activating the pyruvate dehydrogenase phosphatase [59] and inhibiting the pyruvate dehydrogenase kinase (PDK) [60], which together results in dephosphorylation and activation of PDC. Moreover, Ca^{2+} can directly activate mitochondrial ATP synthase, thus increasing the production of ATP [56,61].

On the other hand, mitochondrial Ca^{2+} overload sensitizes mitochondria to apoptotic stimuli [62]. High matrix Ca^{2+} levels are associated with the activation of the permeability transition pore (PTP). PTP opening results in the dissipation of the mitochondrial potential, swelling of the organelle and release of pro-apoptotic factors, such as cytochrome C, triggering programmed cell death [62–64]. Thus, if ER–mitochondria connections are too widespread and prolonged in time, the initially beneficial effects for the cell can convert into pro-apoptotic stimuli. For a more detailed review of this dual regulation, the interested reader is referred to [28]. For a better understanding of the role of MAM in cell death, see also [65].

4.2. ER–mitochondria contact sites and autophagy

As alluded to earlier, ER–mitochondria contact sites are involved in regulating autophagic activity. Macroautophagy is a self-eating process, conserved in all eukaryotes, by which cells engulf cytoplasmic contents and organelles within double-membrane vesicles, called autophagosomes, which then fuse with lysosomes for degradation. Macroautophagy, the molecular basis of cellular quality control, participates in degrading damaged or useless components, and is also employed as an adaptive response to nutrient deprivation [62,63,66]. The origin of the isolation membrane for the formation of autophagosomes has been a matter of debate for many years [66]. In yeast, a membranous structure, called the omegasome, arises from a specialized domain of the ER [67]. However, the exact origin of the isolation membrane in yeast appears to be dependent on the nature of autophagy. While so-called non-selective autophagosomes originate from the omegasome, the isolation membrane for selective autophagy of mitochondria is derived from the ERMES complex at ER–mitochondrial contact sites [68]. In mammalian cells, various organelles have been proposed as candidates that give rise to the isolation membrane, including the ER, the ER-Golgi intermediate compartment, mitochondria, the Golgi apparatus, plasma membrane and recycling endosomes [66]. Although the exact origin continues to be an issue of debate, there is strong evidence that isolation membranes can emerge from MAM. To that end, Hamasaki et al. demonstrated that some elements of the autophagic machinery, including Atg14, accumulate in MAM under conditions of starvation (Fig. 2C) [69]. Additionally, disruption of MAM, by knocking down PACS-2 or Mfn-2, can attenuate the formation of autophagosomes [69].

4.3. ER–mitochondria contact sites and ER stress

Disruption of normal ER function can lead to the accumulation of misfolded or unfolded proteins in the ER lumen. The unfolded protein response (UPR) is an adaptive cellular response to ER stress that restores ER homeostasis by increasing synthesis of chaperones, inhibiting general protein translation, enhancing degradation of misfolded proteins, and increasing total ER volume [70]. This response also includes changes in cellular metabolism to provide metabolic support for cellular adaptation. We have shown that in early stages of ER stress, there is a microtubule-dependent redistribution of the ER and the mitochondrial network towards the perinuclear area of the cell and an increase in ER–mitochondrial contacts in this region [45]. This reorganization is

associated with an increase in mitochondrial Ca^{2+} uptake, mitochondrial metabolism and ATP production [45]. Disruption of ER–mitochondria contacts or blockage of Ca^{2+} transfer increases cell death in response to ER stress, suggesting that this reorganization favors cell survival [45,70]. Other studies have shown that the MAM-resident chaperone Sig-1R plays an important role in regulating ER–mitochondria contacts during ER stress. Sig-1R helps to increase the efficiency of Ca^{2+} transfer to mitochondria and ATP production promoting cell survival during conditions of ER stress [28,50]. Sig-1R helps to stabilize IRE-1α, a main sensor of ER stress, at the MAM, allowing the correct folding, dimerization, and prolonged activation of IRE-1α supporting cell survival [37]. Although ER–mitochondria contacts potentially aid in generating an adaptive response in early stages of ER stress, one may also speculate that these same organelle contacts ultimately promote cell death, if stress is maintained for extended periods of time, by producing mitochondrial Ca^{2+} overload and apoptosis.

Mfn-2 has also been associated with the ER stress response. Induction of ER stress increases Mfn-2 levels [71] and, otherwise, Mfn-2 deficiency can promote ER stress [71,72] further highlighting the importance of MAM function in the ER stress response.

4.4. ER–mitochondria contact sites and cellular signaling

In addition to its role in regulating cell metabolism, autophagy and ER stress, ER–mitochondria contact sites also act as scaffolding hubs for cell signaling. One of the best examples for MAM involvement is the insulin signaling pathway, where MAM integrity seems to be fundamental for effective signal transduction [39]. Several components of insulin signaling (and other growth factor pathways) are enriched in MAM. The protein kinase Akt localizes at the ER–mitochondria interface [39,73,74] where it phosphorylates IP_3R, thus reducing Ca^{2+} release and preventing apoptosis [75,76]. Mammalian target of rapamycin complex 2 (mTORC2), another component of the insulin signaling pathway, is known to localize to ER membranes, where it interacts directly with ribosomes [77]. A more recent study shows that this interaction takes place at ER–mitochondria contact sites and increases in response to growth factor stimulation (Fig. 2D) [73]. Moreover, mTORC2 is necessary to maintain MAM integrity and mitochondrial function [73]. PTEN, a known tumor suppressor, is also enriched at MAM, where it sensitizes cells to apoptosis by dephosphorylating IP_3R and restoring Ca^{2+} release [78]. Promyelocytic leukemia protein (PML), another tumor suppressor, also localizes to these contact sites and modulates sensitivity to apoptosis by sequestering the phosphatase PP2A together with Akt and IP_3R, and regulating Akt phosphorylation and Ca^{2+} release by IP_3R [74].

IP_3R phosphorylation by Akt, in addition to regulating sensitivity to apoptosis by preventing mitochondrial Ca^{2+} overload, also provides negative feedback to the insulin signaling cascade. Recently we have shown that mitochondrial Ca^{2+} uptake is crucial for effective insulin signaling in skeletal muscle cells [79] and cardiac myocytes [80]. Pharmacological inhibition of mitochondrial Ca^{2+} uptake reduces insulin-dependent Akt phosphorylation, Glucose transporter 4 (GLUT4) membrane translocation and glucose uptake in these cells [79,80].

Tubbs et al. [39] recently described an important connection between MAM integrity and insulin signaling in hepatocytes. Disruption of MAM, by silencing structural proteins, impairs insulin signaling in hepatocytes. Conversely, overexpression of MAM proteins enhances insulin signaling. In vitro treatment of hepatocytes under lipotoxic conditions, which are commonly associated with insulin desensitization, reduces MAM content. This effect is also seen in vivo, in both genetic and diet-induced diabetic mice, which exhibit a reduction in hepatocyte ER–mitochondria interactions. The pharmacologic rescue of insulin sensitivity restores these interactions, highlighting the reciprocal regulation of both processes [39]. Nonetheless, the nature of the molecular mechanism that links MAM integrity to insulin signaling remains unknown.

5. ER–mitochondria contacts in metabolic diseases

Over the past few years, various pathologies have been associated with alterations in MAM composition and function. Here, we will focus on the role of ER–mitochondria communication in the pathogenesis of metabolism-related diseases. For a more extensive review on the role of MAM in redox-related pathologies and neurodegenerative disorders, see [6,65,81].

5.1. Changes in ER–mitochondria contacts as a mechanism for obesity-associated diseases

The last few decades have been characterized by a growing epidemic of obesity, today representing a serious public health concern worldwide. Research has shown that mitochondrial dysfunction and ER stress play key roles in the pathophysiology of obesity-related comorbidities, such as insulin resistance and type 2 diabetes mellitus (T2DM).

Mitochondrial dysfunction in various metabolically-relevant cell types and tissues mediates several deleterious effects associated with obesity and/or T2DM. This is supported by an association between mitochondrial dysfunction and reduced insulin sensitivity, as well as decreased adipocyte secretion of the anti-inflammatory, insulin-sensitizing hormone adiponectin [82] and impaired β-cell function in the pancreas [83] (Fig. 4). In mouse models, hepatic mitochondrial dysfunction is linked to impaired liver adiponectin signaling and non-alcoholic steatohepatitis in response to high fat diet (HFD) [84]. Mitochondrial dysfunction has also been associated with a reduced ability of skeletal muscle to respond to insulin, likely exacerbating ectopic lipid accumulation and whole body insulin resistance [85].

Impaired mitochondrial function is in turn related with ER stress, which is recognized as an independent contributor to impaired insulin production in pancreatic β cells and insulin resistance [86], in addition to decreased human adipocyte adiponectin production, leptin resistance and elevated inflammation [87,88]. Until very recently, obesity-related research has viewed ER stress and mitochondrial dysfunction as independent events that are increased in obesity and associated metabolic diseases [89–91]. However, as discussed in previous sections, these two organelles are intimately connected and physically linked via MAM, making them functionally interdependent: mitochondria dysfunction is expected to affect the ER and vice versa. In support of this notion, experiments impairing mitochondrial function have shown to also activate ER stress in 3T3L1 adipocytes [92]. In vivo, mice on a HFD (with the ensuing obesity and metabolic syndrome phenotype) show signs of both mitochondrial dysfunction and ER stress in the liver and skeletal muscle [93]. On the other hand, silencing of Mfn-2 (see Section 2) leads to ER stress and reduced insulin signaling in the skeletal muscle and liver, and greater susceptibility to developing insulin resistance [72].

A recent study showed that obesity in mice is accompanied by marked reorganization of MAM in the liver, with increased ER–mitochondria contacts and simultaneous mitochondrial Ca^{2+} overload accompanied by mitochondrial dysfunction [48]. Animals with artificially-induced ER–mitochondria interactions and fed a HFD showed increased hepatic lipid accumulation with impaired glucose homeostasis and insulin sensitivity [48]. Interestingly, down-regulating ER–mitochondria tethering (PACS-2) or Ca^{2+} transport (IP_3R), improved both cellular stress and glucose metabolism in obese mice. These observations suggest that ER–mitochondrial coupling in obesity may play a pathophysiological role in insulin resistance and T2DM [48]. However, not all studies agree on the deleterious effects of ER–mitochondria contacts, suggesting that there may be tissue- or context-specific roles for the MAM. Ablation of Mfn-2 in pro-opiomelanocortin (POMC) neurons in the hypothalamus (key in appetite regulation, with an anorexigenic effect) resulted in loss of ER–mitochondria contacts, ER stress-induced leptin resistance, hyperphagia, reduced energy expenditure and obesity [90]. Moreover,

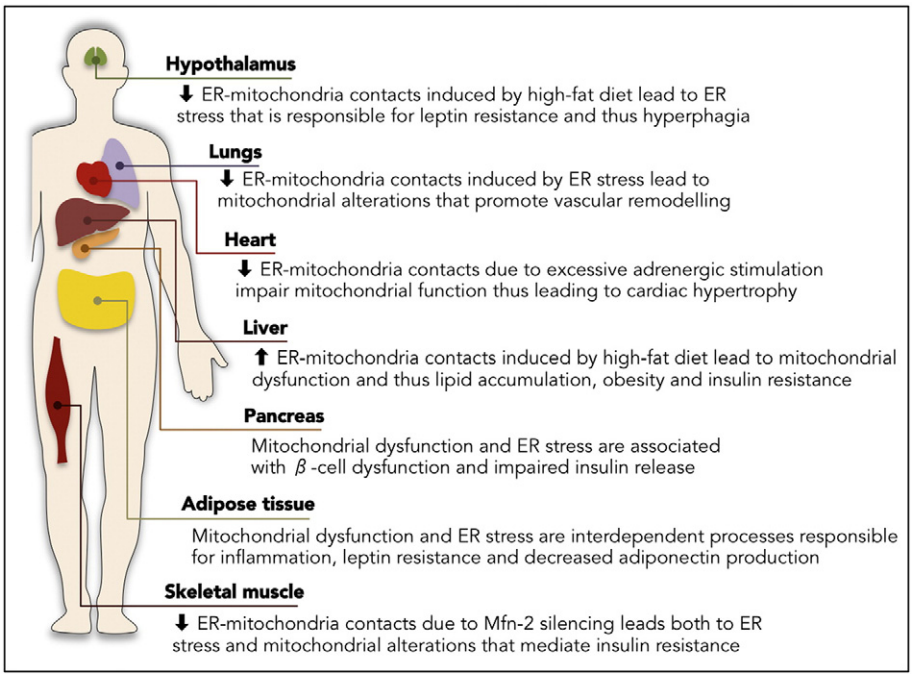

Fig. 4. Atlas of ER–mitochondria contacts and metabolic diseases. Recent evidence suggests that altered ER-to-mitochondria communication is associated with obesity, insulin resistance, cardiac hypertrophy and pulmonary artery hypertension in different tissues.

POMC neurons in HFD-induced obese mice showed less ER–mitochondria contacts when compared to lean controls [90] (Fig. 4). Time-course studies in these mice established that down-regulation of Mfn-2 expression in the hypothalamus was induced as early as four days on a HFD, and was maintained throughout the observation period. Overexpression of Mfn-2 improved the altered parameters, such as body weight, body fat, plasma leptin and food intake, as well as attenuating markers of ER stress.

Cardiovascular diseases are frequently associated with obesity, metabolic syndrome, insulin resistance and T2DM. Pathological cardiac hypertrophy, often associated with changes in cardiomyocyte metabolism and insulin resistance [80,94], is the gateway to more serious conditions, such as heart failure. Using cultured rat neonatal cardiomyocytes, we demonstrated that insulin can induce Ca^{2+} release from the ER [95] and its subsequent uptake into mitochondria [80]. As described in Section 4.4, Ca^{2+} transfer from ER to mitochondria is essential for insulin signaling in cardiomyocytes [80]. The treatment of cardiomyocytes with norepinephrine, mimicking a pro-hypertrophic milieu, enhances the distance between ER and mitochondria, decreasing insulin-induced mitochondrial Ca^{2+} uptake, Akt phosphorylation and glucose entry, thereby providing evidence for insulin desensitization [80]. Blocking mitochondrial Ca^{2+} uptake is sufficient to reproduce the effects of norepinephrine, suggesting that ER–mitochondria communication is an essential component in the pathogenesis of cardiac hypertrophy and associated metabolic dysfunction (Fig. 4) [80].

Current evidence strongly suggests that ER–mitochondria interactions play key tissue- and context-specific roles in the regulation of energy metabolism and the pathophysiology of obesity-related metabolic diseases. Further deciphering the intracellular mechanisms involved in the pathogenesis of such disorders, in the perspective of developing protective strategies in metabolically relevant tissues, hold the potential of significantly reducing the deleterious consequences of obesity.

5.2. The ER–mitochondria junction in pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is a severe disease of the pulmonary circulation, characterized by excessive vascular remodeling that causes right ventricular failure, and is ultimately lethal [96]. The median survival of untreated patients is less than 3 years after diagnosis and, despite the progress in current pharmacology, survival rates remain low [97]. A key event in the development of PAH occurs in smooth muscle cells derived from pulmonary artery (PASMCs), which acquire a highly proliferative phenotype characterized by resistance to apoptosis, leading to lumen occlusion of small vessels in the pulmonary circulation, thereby causing an increase in mean pulmonary artery pressure [98].

Currently, mitochondria are recognized to play a key role in vascular remodeling observed in patients with PAH. PASMCs from these individuals undergo a metabolic shift, away from utilizing fatty acid and glucose oxidation to generate ATP, towards relying on cytoplasmic glycolysis as the main source of energy. Suppression of mitochondrial PDC and oxidative phosphorylation are crucial in the pathogenesis of PAH. These events initiate a series of changes that include increased mitochondrial membrane potential, an increased threshold for PTP opening, and resistance to apoptosis as a consequence. Additionally, the generation of mitochondria-derived reactive oxygen species and metabolic intermediates decreases. All these factors contribute to the activation of transcription factors, such as nuclear factor of activated T-cells (NFAT), hypoxia-inducible factor 1α (HIF-1α), or epigenetic mechanisms, all of which tend to stimulate cellular proliferation [99].

Severe PAH is associated with the induction of the mitochondrial metabolic shift. Likewise, viral infections [100], inflammation [101], drug or toxin exposure [102], mutations in the bone morphogenetic protein (BMP) receptor 2 [103] and hypoxia [100] have similar effects. Of note, these same factors also promote ER stress. Moreover, ER stress is known to favor metabolic remodeling of mitochondria in pulmonary arteries during PAH, due to the disruption of ER–mitochondria contacts [104].

In PASMCs, the induction of ER stress leads to the activation of the Activating transcription factor 6 (ATF6), promoting increased expression of Nogo-B, a member of the reticulon family of ER-shaping proteins [104]. Nogo-B, in turn, increases the distance between mitochondria and ER, resulting in a decrease in Ca^{2+} transfer from ER to mitochondria, therefore inhibiting Ca^{2+}-sensitive mitochondrial enzymes. This contributes to the mitochondrial metabolic remodeling, which, as

previously indicated, is critical in the pathogenesis of PAH [104,105]. The specificity of ATF6 activation and Nogo-B expression in the pulmonary circulation during evolution of PAH, makes them attractive therapeutic targets for future research.

As a result of the mitochondrial metabolic shift during PAH, a characteristic phenotype is promoted that is associated with increased mitochondrial fragmentation and an increased proliferation of PASMCs [98]. These structural changes have been associated in turn with changes in levels of proteins involved in mitochondrial dynamics that regulate both fission and fusion of the mitochondrial network. Available reports indicate that both in patients and experimental PAH models there is an increase in Drp-1 (a protein promoting mitochondrial fission) associated with decreased levels of proteins involved in the fusion process, such as Mfn-1 and Mfn-2 [106]. Mfn-2 deficiency contributes to PASMC hyperproliferation in patients with PAH and in two well-established rodent models of PAH [107].

In summary, ER–mitochondria contact sites are able to regulate processes, such as mitochondrial fragmentation and Ca^{2+} homeostasis, and their disruption may be part of the molecular and cellular mechanisms underlying several human diseases (Fig. 4).

6. Concluding remarks

The importance of the communication between ER and mitochondria is widely recognized; however, we have yet to uncover the many different facets of the exchange processes involved and their importance in cell physiology and the genesis of diseases.

Although data available on the proteins that constitute the structural link between ER and mitochondria in mammalian cells are constantly increasing, there are still many uncertainties concerning the exact composition of MAM and how it changes in response to various stimuli and cellular stress. A better understanding of the dynamic structure of MAM will allow the development of new tools and strategies to modulate ER–mitochondria contacts, thereby providing a solid basis for future targeted therapies.

Communication between organelles is just beginning to be taken into account in the study of human disease. Whereas in the past, dysfunction of an individual organelle type was generally viewed as contributing alone to a specific pathology, it is now clear that communication between organelles is widespread and that disruption of such exchange is detrimental to human health. This is particularly apparent for ER–mitochondria contacts in the pathogenesis of a growing number of diseases, and particularly those related to the so-called metabolic syndrome. However, it remains an important challenge to definitely demonstrate the existence of a causal relationship between aberrant organellar communication and the development of specific pathologies. Despite a growing body of evidence demonstrating strong associations, it is still remains unclear whether these abnormalities are a cause or a consequence of disease.

With this in mind, the hope for the next few years is that the scientific community will resolve these questions and develop a comprehensive understanding of the functional components of MAM, how they adapt to stress, and how alterations in this platform contribute to the development of human disease.

Transparency Document

The Transparency Document associated with this article can be found, in the online version.

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