

Metabolic signaling functions of ER–mitochondria contact sites: role in metabolic diseases

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Abstract

Beyond the maintenance of cellular homeostasis and the determination of cell fate, ER–mitochondria contact sites, defined as mitochondria-associated membranes (MAM), start to emerge as an important signaling hub that integrates nutrient and hormonal stimuli and adapts cellular metabolism. Here, we summarize the established structural and functional features of MAM and mainly focus on the latest breakthroughs highlighting a crucial role of organelle crosstalk in the control of metabolic homeostasis. Lastly, we discuss recent studies that have revealed the importance of MAM in not only metabolic diseases but also in other pathologies with disrupted metabolism, shedding light on potential common molecular mechanisms and leading hopefully to novel treatment strategies.

Key Words

- ▶ organelle communication
- ▶ mitochondria-associated membranes
- ▶ calcium signaling
- ▶ metabolic homeostasis
- ▶ insulin resistance
- ▶ type 2 diabetes mellitus
- ▶ PP2A

Journal of Molecular Endocrinology
(2017) **58**, R87–R106

Introduction

Cellular metabolism is closely regulated and compartmentalized within distinct subcellular organelles. Mitochondria and endoplasmic reticulum (ER) play a crucial role in these processes, as their structure and function are dynamically regulated by nutritional and environmental cues, influencing energy metabolism. Particularly, both organelles are nutrient and energy sensors (Mandl *et al.* 2009, Gao *et al.* 2014), allowing the adaptation of cellular metabolism according to nutritional status. Moreover, both ER and mitochondria have recently emerged as crucial regulators of the innate immune response to both pathogens and cell stress (Martinon 2012, Lartigue & Faustin 2013), thus further controlling the metabolic adaptations in function of immune modifications. In this context, the liver is a key organ integrating both nutrient and immune signals, as it is the first organ reached by nutrients and bacterial components of the gut during

food intake, highlighting the importance of the gut–liver axis in the adaptation to nutritional environment changes (Chassaing *et al.* 2014). However, excess of nutrients or intestinal microbiota modifications (i.e. altered microbiota population and/or altered gut permeability) could be a source of pro-inflammatory agonists that could alter hepatic metabolism. Therefore, a precise integration of both metabolic and inflammatory pathways is essential for the adaptations of hepatic metabolism to environment, but more generally for the regulation of whole-body metabolism. In agreement, mitochondrial dysfunction and ER stress have been largely and independently associated with metabolic diseases, such as obesity, type 2 diabetes mellitus (T2DM) (Chang *et al.* 2015, Rieusset 2015, Salvado *et al.* 2015, Wang *et al.* 2015, Hasnain *et al.* 2016) and non-alcoholic fatty liver diseases (NAFLD) (Begriche *et al.* 2013, Takaki *et al.* 2014, Ashraf

& Sheikh 2015). Moreover, the strong interplay between the two organelles and immune signaling (Hummasti & Hotamisligil 2010, Chaudhari *et al.* 2014) further highlights their involvement in the progression toward metabolic diseases. Therefore, mitochondria and ER play an important role in metabolic homeostasis; yet, the precise mechanisms are still unclear.

An interesting and underestimated point is that both organelles are not independent but rather interconnected intracellular organelles, sharing structural and functional interactions allowing reciprocal regulations. The close contacts between ER and mitochondria, known as mitochondria-associated endoplasmic reticulum membranes (MAM), shelter various proteins with different functions and play a pivotal role in different established functions such as calcium (Ca²⁺) signaling, lipid transport, mitochondrial functions and cell survival (Giorgi *et al.* 2015b). However, recent data shed light on additional roles of MAM in nutrient and hormonal signaling, highlighting an emerging role of MAM in the control of metabolic homeostasis. Consequently, it is a close step to suggest that ER–mitochondria miscommunication could have a role in metabolic diseases.

Here, we review the established structural and functional features of ER–mitochondria interactions and discuss the important considerations to take into account when we analyze these domains. Particularly, we will focus on the emerging role of MAM in nutrient and hormonal signaling, highlighting a key role of organelle crosstalk to maintain metabolic homeostasis. Lastly, we will discuss the potential role of ER–mitochondria miscommunication in metabolic diseases.

Structural and functional features of MAM

To be as clear and complete as possible, we will start this review with an overview of the established components and functions of MAM, with a particular attention concerning their roles in cellular homeostasis. Furthermore, we will discuss the important features to take into account when analyzing MAM integrity and function.

Overview of molecular determinants of MAM

The physical interaction between ER and mitochondria was suggested in 1959 (Copeland & Dalton 1959) and for a long time, such observations have been suspected to be artifacts due to fixation. Then, MAM fractions were isolated in 1990 (Vance 1990) and further visualized in living cells using fluorescent proteins at the end of the

90s (Rizzuto *et al.* 1998). Since then, our knowledge on these contact sites and their molecular determinants of MAM has evolved with the improvement of imaging and analytic techniques, such as electron microscopy and electron tomography. Particularly, observation of MAM by wide-field digital 3D deconvolution microscope indicates that approximately 20% of the mitochondrial surface is in direct contact with the ER (Rizzuto *et al.* 1998, Friedman *et al.* 2011). The contact sites between the ER and mitochondria have been measured to be 10–30 nm wide (Csordas *et al.* 2006). A combined observation by electron microscopy and high-resolution three-dimensional electron tomography shows the presence of physical linkers between the two organelles (Friedman *et al.* 2011). Indeed, the two membranes of each organelle are closely apposed but do not fuse and thus each organelle maintains its identity. Proteins expressed at both membranes interact together, either directly or indirectly, thus forming multiprotein-tethering complexes. Interestingly, these proteins at the MAM interface have great varying functions, highlighting the crucial role of MAM in cellular homeostasis. All the proteins described so far as being localized at MAM interface in the literature are summarized in Fig. 1.

MAMs were firstly described as enriched in phospholipid (PL) enzymes such as phosphatidylserine (PS) synthase 1 and 2. Other lipid biosynthetic enzymes such as glycerol 3-phosphate acyltransferase, acylCoA synthase 4, diacylglycerol acyltransferase 2 and the microsomal triacylglycerol transfer protein are localized at MAM, regulating lipid, triacylglycerol and lipoprotein synthesis (for review, Vance 2014).

Another well-known protein complex identified at MAM interface is the Ca²⁺ channeling complex made of the inositol triphosphate receptor (IP3R) at the ER, the molecular chaperone glucose-regulated protein 75 (GRP75) and the voltage-dependent anion channel (VDAC) at the outer mitochondrial membrane. GRP75 was shown to be necessary and sufficient for the stimulatory effect of the IP3R on mitochondrial Ca²⁺ uptake. Silencing of *Grp75* abolishes the functional coupling between IP3R1 and VDAC (Szabadkai *et al.* 2006, Tubbs *et al.* 2014), thereby reducing mitochondrial Ca²⁺ uptake (Szabadkai *et al.* 2006). Recently, another mitochondrial actor was shown to interact with and regulate this Ca²⁺ channeling complex, namely cyclophilin D (CYPD), a mitochondrial matrix protein which belongs to the family of the peptidyl-prolyl cis–trans isomerases and regulates the opening of the mitochondrial permeability transition pore in stressed conditions, particularly during myocardial

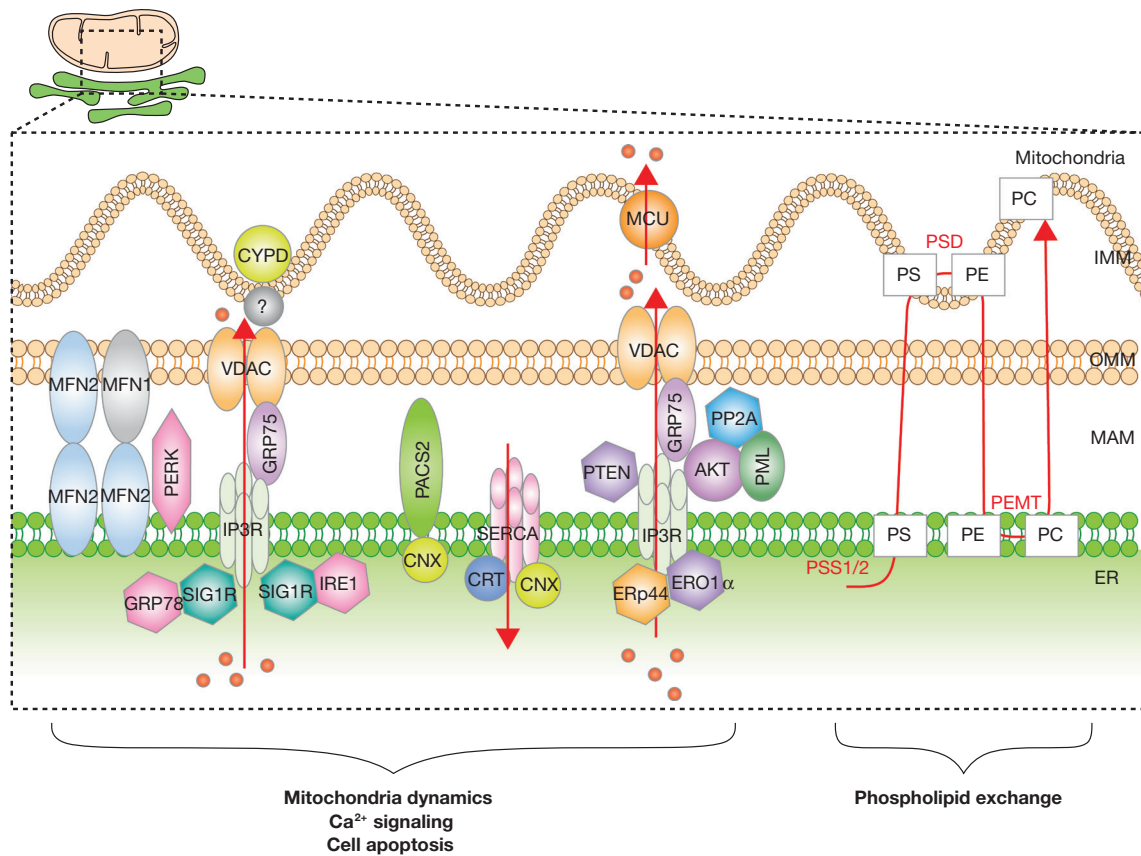


Figure 1

Schematic representation of ER–mitochondria contact sites and the established cellular signaling. Several proteins implicated in mitochondria dynamics, Ca^{2+} signaling, cellular apoptosis or phospholipid exchange at MAM interface are depicted. In yellow mitochondria and in green the ER, the red arrows correspond to calcium flux. ER, endoplasmic reticulum; IMM, inner mitochondrial membrane; MAM, mitochondria-associated membranes; OMM, outer mitochondrial membrane.

ischemia–reperfusion injury (Alam *et al.* 2015). CYPD was further identified at MAM interface, forming a high molecular complex with the IP3R–Grp75–VDAC complex in both cardiomyocytes and hepatocytes and regulating inter-organelle Ca^{2+} exchange (Paillard *et al.* 2013, Tubbs *et al.* 2014, Rieusset *et al.* 2016). It is likely that unknown interacting partners allow CypD to span mitochondrial membranes and interact with this complex.

Proteins involved in mitochondrial dynamics are also part of MAM. In fact, mitofusin 2 (MFN2), a mitochondrial protein controlling organelle fusion, was shown to be localized at both the outer mitochondrial membrane (OMM) and at the ER, their binding forming a complex which tethers the ER to mitochondria (de Brito & Scorrano 2008). Mfn2 tethers regulate Ca^{2+} homeostasis at MAM as its genetic invalidation disrupted ER–mitochondria Ca^{2+} transfer (de Brito & Scorrano 2008, Naon *et al.* 2016). In agreement, the mitochondrial ubiquitin ligase MITOL was reported to regulate ER–mitochondria interactions through

MFN2 ubiquitination (Sugiura *et al.* 2013). However, this widely accepted concept was recently challenged as both increased ER–mitochondria interactions and increased Ca^{2+} transfer were observed in *Mfn2* knockout (KO) cells (Cosson *et al.* 2012, Filadi *et al.* 2015). The discrepancy between studies was suggested to be only apparent and related to analytical artifacts (Filadi *et al.* 2015). Additive studies are therefore required to clarify the precise role of MFN2 at MAM. Furthermore, ER–mitochondria contacts and Ca^{2+} signals are also critical for mitochondrial fission (Friedman *et al.* 2011, Korobova *et al.* 2013).

Phosphofurin acidic cluster-sorting proteins 2 (PACS2) is a multifunctional homeostatic regulator playing an important role in the trafficking of ion channels between secretory pathway compartments (Kottgen *et al.* 2005). PACS2 was identified at ER–mitochondria interface where it plays a role in sorting proteins on the ER and mitochondria (Simmen *et al.* 2005). PACS2 depletion disrupts mitochondria structure and ER homeostasis,

leading to stress-mediated uncoupling of the organelles and to the inhibition of Ca^{2+} signal transmission (Simmen *et al.* 2005). Conversely, overexpression of PACS2 was shown to increase ER–mitochondria interaction and Ca^{2+} exchange in liver (Arruda *et al.* 2014), confirming that the presence of PACS2 is sufficient to control MAM integrity and function. Furthermore, PACS2 was shown to be phosphorylated on Ser437 by AKT and to control TRAIL-induced apoptosis (Aslan *et al.* 2009), but the involvement of MAM in this process is still unclear. Lastly, PACS2 regulates the activity of calnexin (CNX), an ER chaperone compartmentalized in MAM and modulating local Ca^{2+} by regulating the activity of sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA). In physiological conditions, more than 80% of CNX localize to the ER, mostly in MAM. In fact, *Pacs2* knockdown causes a redistribution of CNX away from the ER, to the plasma membrane, therefore affecting mitochondrial and ER Ca^{2+} homeostasis (Rizzuto *et al.* 2012).

Hayashi and coworkers identified the ER-resident sigma 1 receptor (SIG1R) as a novel ‘ligand-operated’ chaperone that specifically targets MAM (Hayashi & Su 2007). They found that SIG1R forms a complex with another ER chaperone, the 78 kDa glucose-regulated protein (GRP78, also known as BIP). SIG1R/GRP78 complex forms a Ca^{2+} -sensitive chaperone machinery and extends Ca^{2+} signaling from the ER into mitochondria by stabilizing IP3R at MAM. Furthermore, SIG1R is upregulated and redistributed by ER stress. Depletion of ER Ca^{2+} triggers SIG1R to dissociate from GRP78 to bind to IP3Rs. More recently, Hayashi’s group further showed that Sig-1R at MAM can stabilize the inositol-requiring protein 1 (IRE1) and enhance the cellular survival by prolonging the activation of the IRE1 signaling pathway (Mori *et al.* 2013). Taken together, these results suggest that SIG1R has a pivotal role at MAM interface in IP3R-mediated mitochondrial Ca^{2+} influx and cellular survival after ER stress.

In addition, the promyelocytic leukemia (PML) tumor suppressor also controls cell survival by regulating Ca^{2+} signaling at MAM interface. Indeed, Giorgi and coworkers analyzed PML intracellular localization by cell fractionation and identified the presence of PML in both ER and MAM fractions (Giorgi *et al.* 2010). The presence of PML at MAM was shown to control protein phosphatase 2A (PP2A)- and AKT-dependent modulation of IP3R phosphorylation and in turn IP3R-mediated Ca^{2+} release from ER. Loss of *Pml* resulted in a decreased Ca^{2+} release from the ER and a subsequent lower Ca^{2+} influx into mitochondria. More recently, the same group further

demonstrated that PML inhibited autophagy at MAM interface in a Ca^{2+} -dependent manner (Missiroli *et al.* 2016). Loss of *Pml* therefore activates autophagy and promotes cell survival upon stress. Therefore, the authors suggested that blocking autophagy should restore the activity of chemotherapy in PML-downregulated tumors.

Beyond these established actors at MAM interface, recent proteomic analyses have increased our knowledge on molecular components of MAM, highlighting new cellular functions. Until now, three studies have analyzed protein content of MAM fraction by proteomic analyses (Poston *et al.* 2011, Zhang *et al.* 2012, Sala-Vila *et al.* 2016). Poston and coworkers have identified 250 proteins in MAM in NG108/105 cells (a cholinergic neuroblastoma–glioma hybrid cell line) and confirmed the presence of the above-mentioned proteins. Interestingly, the numerous other proteins found at MAM interface seem, primarily, to be involved in metabolic and protein processing activities. Zhang and coworkers also performed a proteomic analysis of the MAM proteome in uninfected and HCMV-infected human foreskin fibroblasts and showed that HCMV infection modulated 991 proteins at MAM interface, selectively modulating MAM functional activities. Lastly, Sala-Vila and coworkers recently reported a proteomic analysis of MAM from mouse liver and identified calveolin 1 (CAV1) as an important component of this subdomain controlling its relative cholesterol content. Differential proteomics analysis between MAM from wild-type (wt) and CAV1-deficient mice suggests that CAV1 participates in intracellular steroid and lipoprotein metabolism-related processes at MAM.

Established cellular functions of MAM

Until now, the most described functions of ER–mitochondria interactions are lipid biosynthesis, Ca^{2+} transfer and response to cellular stress, key cellular processes that are involved in life and death events. These functions have been largely reviewed (Marchi *et al.* 2014, Naon & Scorrano 2014, van Vliet *et al.* 2014, Giorgi *et al.* 2015a) and will not be described in detail here.

Briefly, the close juxtaposition between ER and mitochondria has been firstly implicated in the exchange of PL between organelles (Vance 1990). PL synthesis is usually restricted to ER. Therefore, they must be transported from ER to the membranes of other organelles. One mechanism of PL transport between ER and mitochondria is via membrane contact sites. Indeed, PS is synthesized in ER of mammalian cells by the exchange of serine for the choline or ethanolamine head-groups of phosphatidylcholine

or phosphatidylethanolamine (PE) by PS synthase-1 and PS synthase-2, which are enriched at MAM. Then, newly made PS is transferred into mitochondria through MAM, where it is decarboxylated to PE via PS decarboxylase in mitochondrial inner membrane. PE is also produced at MAM by acylation of lyso-PE by lyso-PE acyltransferase.

Later, another important function was discovered: a quasi-synaptic mechanism of Ca^{2+} transmission (Rizzuto *et al.* 1998). The ER was known for a long time as the main Ca^{2+} store of the cell, whereas mitochondria were also shown to accumulate Ca^{2+} . When the basal concentration of cytosolic Ca^{2+} rises (typically ~100 nM), the mitochondrial Ca^{2+} concentrations can increase almost simultaneously to as much as 10 mM (Rizzuto & Pozzan 2006). This was an unexpected observation by Rizzuto and coworkers (1993, 1998) considering the low affinity to Ca^{2+} of the mitochondrial Ca^{2+} uniporter. Moreover, Ca^{2+} released by the ER into the cytosol in response to IP_3 is transferred to mitochondria much more efficiently than cytosolic Ca^{2+} elevations induced by leakage of Ca^{2+} from this organelle (Rizzuto *et al.* 1993, Hajnoczky *et al.* 1995). These observations led to the proposal by Rizzuto and Pozzan of the existence of close contact points between ER and mitochondria, enriched in IP_3R and R_YR . Therefore, the release of high Ca^{2+} concentrations at contact sites between the two organelles leads to the formation of microdomains of high Ca^{2+} concentration that are crucial for efficient Ca^{2+} uptake by mitochondria (Rizzuto *et al.* 1993, 1998). One of the main functions of Ca^{2+} uptake by mitochondria is to activate intra-mitochondrial dehydrogenases (Eriksson *et al.* 1998), leading to the activation of mitochondrial metabolism and energy production.

The movement of Ca^{2+} between the ER and mitochondria is an essential component of the cell survival processes. When MAMs are disrupted, the release of Ca^{2+} from the ER mediated by IP_3R is suppressed and ATP production and cell survival are reduced (Rowland & Voeltz 2012). Similarly, apoptosis is attenuated in cells that are partially depleted of IP_3Rs (Jayaraman & Marks 1997). At the opposite, apoptosis occurs when excess Ca^{2+} flow out of the ER into mitochondria via the IP_3R , which are highly concentrated in MAM (Rizzuto *et al.* 1993, 1998). The flow of Ca^{2+} from the ER into mitochondria promotes the oligomerization of BAX (a pro-apoptotic protein of OMM) and causes permeabilization of OMM. In consequence, cytochrome c is released into the cytosol where the caspase cascade is activated and ultimately, apoptosis is induced (Scorrano *et al.* 2003). The Ca^{2+} -mediated activation of the mitochondrial fission

protein, DRP1, also stimulates BAX oligomerization and increases apoptosis (Hoppins & Nunnari 2012). Apoptosis is also induced upon depletion of PACS2, the MAM-associated multifunctional sorting protein. Depletion of *Pacs2* dissociated the ER from mitochondria and induced extensive mitochondrial fragmentation (Simmen *et al.* 2005). In agreement, when mitochondrial fusion is increased, apoptosis is attenuated (Frank *et al.* 2001). Taken together, the disruption of MAM and the corresponding increase in mitochondrial fission/fragmentation are associated with the induction of apoptosis.

Important structural features to take into account when analyzing MAM

Beyond the molecular determinants of MAM, several important structural features have to be considered when we talk about MAM, including the frequency of organelle contact, the spacing between both organelles and the contact size/volume, as all these parameters could be extremely variable from one study to another, depending on the cellular context and the technology used to study MAM.

Frequency It was initially suggested that 80% of mitochondria are in contact with ER in the liver (Montisano *et al.* 1982). Later, more sophisticated analytical techniques revised downward this frequency and found that around 20% of mitochondria are in contact with ER at least in HeLa cells (Rizzuto *et al.* 1998). However, MAMs are very dynamic structures and the amount of mitochondria in contact with the ER can widely vary.

Spacing The distance between ER and mitochondria was originally estimated to be about 100 nm (Soltys *et al.* 1992), whereas more sophisticated approaches have shown that it can be even smaller, up to 10 nm at the smooth ER and 25 nm at the rough ER (Csordas *et al.* 2006). This close proximity of ER with the OMM supports the interaction between proteins localized on opposing membrane faces. The distance between both organelles regulates MAM function (Giacomello & Pellegrini 2016) as Ca^{2+} transfer from ER to mitochondria is optimal at 15 nm and reduced at 5 nm (Csordas *et al.* 2010). In agreement, disruption of ER–mitochondria physical interactions through invalidation of MAM proteins is often associated with loss of MAM function, such as Ca^{2+} transfer or lipid synthesis (Simmen *et al.* 2010).

Spacing Lastly, it is noteworthy that these contact zones between ER and mitochondria could be more or less distant. They can be occasional, meaning that a small proportion of mitochondria membrane is in contact with the ER (Rizzuto *et al.* 1998). Otherwise, the ER can constrict entirely mitochondria, notably for mitochondria division (Friedman *et al.* 2011). Furthermore, the length of these tethers can be dynamically regulated as it was shown that apoptotic agents are able to narrow the ER–mitochondria gap (Csordas *et al.* 2006).

ER–mitochondria contacts are variable, and it is unclear if all connections between both organelles consistently have the same protein composition. Furthermore, it seems that modulation of the expression of several proteins at the MAM interface is sufficient to destabilize ER–mitochondria interactions. This is the case for PACS2 (Simmen *et al.* 2005, Arruda *et al.* 2014), SIG1R (Hayashi & Su 2007), IP3R1 (Arruda *et al.* 2014), GRP75 (Szabadkai *et al.* 2006, Tubbs *et al.* 2014), MFN2 (de Brito & Scorrano 2008, Tubbs *et al.* 2014) and CYPD (Rieusset *et al.* 2016). Therefore, these data suggest that several proteins are involved in MAM stabilization or formation. However, no MAM protein is exclusively expressed at MAM; therefore, the modulation of their expression could have unspecific effects beyond MAM.

Altogether, these data mean that these features of MAM should be considered with the highest caution when analyzing ER–mitochondria communication in health and diseases. Nevertheless, it raises the intriguing possibility that the ER–mitochondria distance could be controlled to affect ER–mitochondria function.

New functions of MAM in cellular metabolic homeostasis

The importance of Ca²⁺ signaling and mitochondrial metabolism in various cellular signaling pathways highlights the importance of MAM for metabolic homeostasis. New functions of MAM in hormonal and nutrient signaling recently emerged, thus highlighting the dynamic regulation of ER–mitochondria interactions in function of energy state and nutrient status. Furthermore, metabolic homeostasis is dependent on proper signaling pathways from ER, such as the unfolded protein response (UPR) and the autophagy, both controlled by MAM integrity and by MAM actors. Here, we review the recent studies demonstrating that MAM could be an important hub for several signaling pathways controlling metabolic homeostasis (Fig. 2 and Table 1).

Role of MAM in energy metabolism and redox status

Metabolic homeostasis is dependent on both energy and redox status, as they control the activity of numerous metabolic enzymes. Interestingly, ER–mitochondria interactions were involved in the control of both energy metabolism and redox status, thus pointing at MAM as an important hub for metabolic homeostasis.

The transfer of Ca²⁺ from ER to mitochondria is crucial for the control of mitochondria energy metabolism. Indeed, mitochondrial Ca²⁺ levels control the activity of three deshydrogenases of Krebs cycle (Denton 2009), thus influencing ATP synthesis. In agreement, increased ER–mitochondrial coupling was shown to promote mitochondrial respiration and bioenergetics during early phases of ER stress (Bravo *et al.* 2011). Conversely, genetic or pharmacological inhibition of IP3R altered mitochondrial function, lowering ATP production and triggering autophagy (Cardenas *et al.* 2010). Thus, IP3R-mediated Ca²⁺ release is important for cellular bioenergetics. Furthermore, this bioenergetic response seems important for cellular adaptation during early stage of ER stress as moderate ER stress increased ER–mitochondria interactions to increase Ca²⁺ transfer and enhance mitochondrial respiration (Bravo *et al.* 2011). However, sustained activation of ER stress impaired mitochondrial metabolism (Wang & Pantopoulos 2011), suggesting a strong link between metabolic insufficiency and ER stress-mediated apoptosis.

Some enzymes involved in ER redox regulation are found at MAM interface, such as the ER oxidoreductase 1 alpha (ERO1 α) (Gilady *et al.* 2010, Anelli *et al.* 2012, Lynes *et al.* 2012). This protein is important for the formation of disulfide bonds within newly synthesized polypeptides (Hatahet & Ruddock 2009). The localization of ERO1 α on the MAM is dependent on oxidizing conditions within the ER (Gilady *et al.* 2010) and was shown to potentiate the release of Ca²⁺ during ER stress (Li *et al.* 2009). In addition, several chaperones important for protein folding are present at MAM interface and regulate either IP3R or SERCA (Simmen *et al.* 2010). Therefore, ER oxidative protein folding is an important regulator of Ca²⁺ homeostasis and signaling.

Role of MAM in insulin signaling

Insulin is a key hormone of post-prandial metabolism controlling blood glucose levels through a signaling pathway composed of IRS-PI3K-AKT.

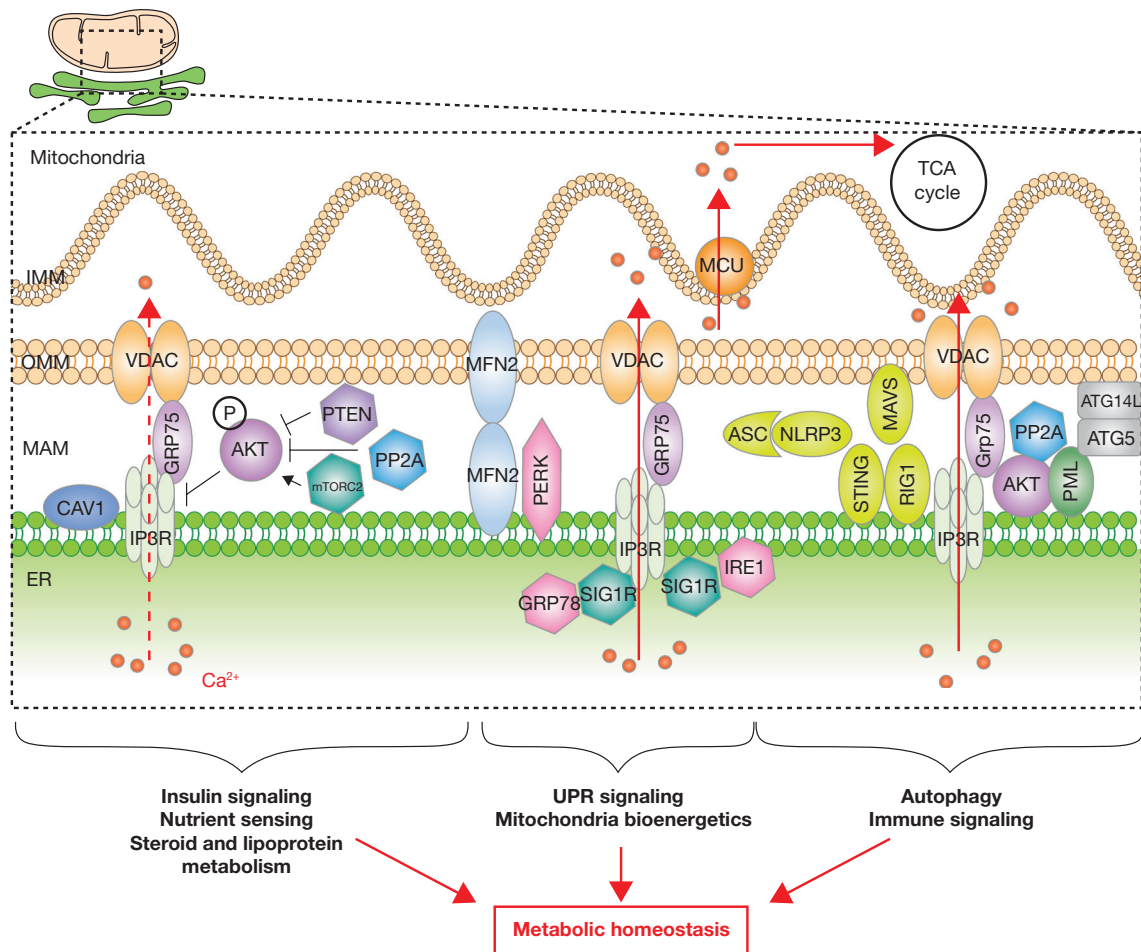


Figure 2

MAM is an important hub for several signaling pathways controlling metabolic homeostasis. The new role of MAM in insulin and nutrient signaling and in steroid and lipoprotein metabolism, as well as their role in unfolded protein response (UPR) signaling, mitochondria bioenergetics, autophagy and in immune signaling, participating to the control of metabolic homeostasis.

Several insulin signaling proteins were recently identified at MAM interface, suggesting that MAM could be an important hub for insulin signaling. Indeed, the protein kinase AKT (also called PKB) was found to interact with the PML/PP2A/IP3R complex in MAM fractions (Giorgi *et al.* 2010) and controlled ER Ca²⁺ release. The phosphorylation of IP3R by AKT reduced cellular sensitivity to apoptotic stimuli through a mechanism that involved diminished Ca²⁺ flux from the ER to mitochondria (Szado *et al.* 2008). The presence of AKT at MAM seems to regulate MAM integrity as ER–mitochondria interactions are reduced in *Akt* KO cells (Betz *et al.* 2013). Importantly, an increase of phosphorylated AKT (active form) was found in the liver of obese and diabetic mice (Tubbs *et al.* 2014), associated with a disruption of MAM, confirming an interplay between AKT and MAM integrity. However, the exact role of AKT at MAM in the control of insulin

action requires further investigations. Furthermore, mammalian target of rapamycin complex 2 (mTORC2), another kinase important for cellular metabolism, was found at MAM interface (Betz *et al.* 2013). Similar to AKT, loss of mTORC2 disrupted ER–mitochondria interactions (Betz *et al.* 2013). mTORC2 localization to MAM is stimulated by growth factors and insulin and controls AKT phosphorylation as well as the phosphorylation of its targets PACS2, IP3R and hexokinase 2 (Betz *et al.* 2013). Therefore, MAM could be considered as a hub for mTORC2–AKT signaling. Lastly, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), another protein controlling AKT activity, was identified at MAM interface (Bononi *et al.* 2013). PTEN was shown to interact with IP3R at MAM and to increase Ca²⁺ transfer from the ER to mitochondria through its protein phosphatase activity (Bononi *et al.* 2013). As this situation is associated with

Table 1 Summary of the most important proteins with metabolic signaling functions at MAM discussed in this review.

Proteins	Localization	Functions	MAM interactors	References
AKT	cyt, MAM	Serine/threonine kinase Activator of insulin signaling pathway antiapoptotic function inhibits Ca ²⁺ release from ER Regulator of MAM integrity	IP3R, PACS2, HKII, PP2A, PML, PTEN, mTORC2	Szado et al. (2008) Giorgi et al. (2010) Betz et al. (2013) Tubbs et al. (2014)
mTORC2	cyt, MAM	Serine/threonine kinase Activator of Akt Activator of insulin signaling pathway Regulates mt Ca ²⁺ uptake and bioenergetics Regulator of MAM integrity	AKT, PACS2	Betz et al. (2013)
PTEN	cyt/mt/MAM	Protein and lipid phosphatase Negative regulator of AKT Negative regulator of insulin signaling pathway Regulates ER Ca ²⁺ release	AKT, IP3R	Bononi et al. (2013)
PP2A	cyt, ER, MAM	Proapoptotic function Serine/threonine phosphatase Negative regulator of AKT Negative regulator of insulin signaling pathway Involved in glucose-mediated reduction of ER–mt interactions	PML, AKT, IP3R	Giorgi et al. (2010) Theurey et al. (2016)
CYPD	mt/MAM	Regulator of mPTP opening Regulator of MAM integrity Regulator of ER–mt Ca ²⁺ transfer Loss of CYPD induces hepatic insulin resistance and protects cardiomyocytes from hypoxia/reoxygenation injury	IP3R-GRP75-VDAC complex	Paillard et al. (2013) Tubbs et al. (2014) Rieusset et al. (2016) Theurey et al. (2016)
MFN2	mt/ER/MAM	Regulator of mt fusion Regulator of liver and muscle insulin sensitivity Regulator of MAM integrity Regulator of ER–mt Ca ²⁺ transfer	MFN1, PERK	de Brito & Scorrano (2008) Cosson et al. (2012) Filadi et al. (2015) Naon et al. (2016)
GRP78	ER/MAM	ER chaperone Stabilizes IP3R at MAM	SIG1R	Sebastian et al. (2012) Hayashi & Su (2007)
IRE1	ER/MAM	Involved in unfolded protein response Is stabilized at the MAM by SIG1R when cells are under ER stress	SIG1R	Mori et al. (2013)

Proteins	Localization	Functions	MAM interactors	References
PERK	ER/MAM	Protein kinase involved in unfolded protein response Increases ER–mitochondria interactions	MFN2	Verfaillie et al. (2012) Munoz et al. (2013)
ATG14L and ATG5	cyt/MAM	Regulator of autophagy Enriched at MAM after starvation	STX17	Hamasaki et al. (2013)
MAVS	mt/MAM	Regulator of innate immune signaling	MFN2	Horner et al. (2011)
STING	ER/MAM	Regulator of innate immune signaling		Ishikawa et al. (2009)
NLRP3	mt/MAM	Regulator of inflammasome activation	ASC	Zhou et al. (2011)

cyt, cytoplasm; ER, endoplasmic reticulum; MAM, mitochondria-associated membranes; mt, mitochondria.

reduced phosphorylated AKT at MAM, PTEN could control ER–mitochondria Ca^{2+} transfer by counteracting AKT activation and AKT-mediated phosphorylation of IP3R.

Recently, MAM integrity was further shown to control directly insulin action in hepatocytes (Tubbs et al. 2014). Indeed, it was shown that experimental disruption of MAM altered insulin signaling and action in hepatocytes, whereas overexpression of MAM proteins enhanced it (Tubbs et al. 2014), demonstrating that MAM integrity controls insulin action. Similarly, pharmacological alteration of mitochondrial Ca^{2+} uptake was shown to disrupt insulin signaling in cardiomyocytes (Gutierrez et al. 2014). Conversely, loss of *Akt* or *mTorc2* disrupted MAM integrity (Betz et al. 2013), suggesting a reciprocal relationship between MAM and insulin signaling. However, the molecular mechanisms underlying this relationship are still unclear. Future investigations will determine whether MAMs control the canonical insulin signaling pathways in the cytosol or whether insulin signaling requires to transit at the MAM interface.

Role of MAM in nutrient signaling

Both ER and mitochondria are now considered as nutrient sensors allowing adaptation of cellular metabolism. Interestingly, MAM interface could also act as a nutrient-regulated hub to adapt mitochondrial metabolism.

Several recent studies suggest that MAMs are dynamically regulated by the energetic state of the cell. Indeed, ER–mitochondria contacts were shown to increase twice in length in the liver when nutrients become limiting and to re-modulate mitochondria cristae by a mechanism involving OPA1 and MFN2 (Sood et al. 2014), suggesting that MAM can help mitochondria to adapt to

metabolic transitions. In agreement, disruption of MAM inhibits starvation-induced autophagy by inhibiting the PS transfer from ER to mitochondria-derived autophagosomes (Hailey et al. 2010), supporting the role of MAM in starvation-induced processes. Conversely, our laboratory further demonstrated that MAM integrity is reduced after feeding in liver, a regulation reproduced by increasing blood glucose levels (Theurey et al. 2016). In agreement, high glucose levels reduced ER–mitochondria interactions and Ca^{2+} exchange in HuH7 cells, pointing toward glucose as a major regulator of MAM integrity in high-energy state. At the molecular level, we revealed that high glucose levels disrupted MAM integrity and function through the pentose phosphate (PP)-PP2A pathway, and subsequently induced mitochondria fragmentation and altered mitochondria respiration (Theurey et al. 2016). Altogether, these data point at MAM as a glucose sensor to adapt cellular bioenergetics, likely contributing to the adaptive fuel partitioning during nutritional transition. The capacity to switch from lipid to glucose oxidation during the transition from a fasted to a fed state is called metabolic flexibility, and metabolic inflexibility was associated with ectopic lipid accumulation and insulin resistance (Galgani et al. 2008). Therefore, the capacity of MAM to connect energy sensing to mitochondria physiology plays a crucial role for the control of glucose homeostasis.

The mechanism by which the phosphatase PP2A could control the glucose-mediated reduction of ER–mitochondria interactions is still unclear. As PP2A was shown to control AKT phosphorylation and Ca^{2+} release by IP3R at MAM interface (Giorgi et al. 2010), this regulation could depend on phosphorylation state of yet-to-be identified substrates at MAM. We suggest that

high glucose levels could reduce the presence of PP2A at MAM interface, as an increase of phosphorylated AKT (Tubbs *et al.* 2014) and an increase of the presence of mTORC2 (Betz *et al.* 2013) were observed at MAM after insulin stimulation or refeeding in liver.

Role of MAM in UPR signaling

ER plays a key role in the folding of proteins. Compromising this ER function leads to the accumulation of misfolded proteins, which triggers an adaptive ER response called the unfolded protein response (UPR). The UPR is known to upregulate chaperones, to inhibit protein synthesis, to enhance degradation of misfolded proteins and to expand the ER (Schroder 2008). ER stress is sensed predominantly by three ER transmembrane proteins, namely double-stranded RNA-activated protein kinase-like ER kinase (PERK), IRE1 α and activating transcription factor 6 (ATF6), which subsequently activate different signaling pathways aiming at promoting cell survival. Interestingly, activation of UPR also controls insulin signaling pathways (Salvado *et al.* 2015), suggesting that the control of UPR by MAM could indirectly influence metabolic homeostasis.

Firstly, several proteins of the UPR were shown to be present in MAM fractions. Indeed, both GRP78 and IRE1 were involved in interactions with SIG1R to facilitate inter-organelle signaling for survival (Hayashi & Su 2007, Mori *et al.* 2013). Another actor of the UPR found at MAM is PERK, which plays a key role in organelle tethering, controlling ROS transfer and apoptosis (Verfaillie *et al.* 2012). Loss of PERK reduced ER-mitochondria interactions and disrupted ER morphology and Ca²⁺ signaling. Furthermore, MFN2 was shown to physically interact with PERK to negatively regulate its activity (Munoz *et al.* 2013). These authors showed that the silencing of PERK partially rescued mitochondria fragmentation and aberrant mitochondrial Ca²⁺ content caused by loss of *Mfn2*. However, further studies are required to determine whether PERK-mediated effects on mitochondria are dependent on its presence at the MAM interface.

Furthermore, the initial adaptive phase of ER stress was shown to be associated with an increase of ER-mitochondria interactions (Bravo *et al.* 2011), likely to increase energy synthesis, which is required for the proper folding of proteins. Conversely, MAM integrity further controls the activation of UPR and subsequent ER stress. Indeed, the genetic invalidation of several MAM proteins, such as PACS2 (Simmen *et al.* 2005), SIG1R (Hayashi & Su 2007), MFN2 (Sebastian *et al.* 2012) or

CYPD (Rieusset *et al.* 2016) were shown to concomitantly induce MAM disruption and ER stress.

Role of MAM in autophagy

Starvation-induced autophagy is a cellular catabolic process degrading cellular constituents to generate energy in period of scarcity (Kaur & Debnath 2015). Therefore, autophagy plays an important regulatory role in metabolic homeostasis. In agreement, impaired autophagy is associated with hepatic steatosis (Lavallard & Gual 2014), whereas suppression of autophagy leads to hepatic ER stress and insulin resistance (Yang 2010).

Interestingly, autophagosome formation was shown to occur at MAM interface (Hamasaki *et al.* 2013). The authors demonstrated that different proteins of autophagy (ATG14L and ATG5) were enriched at MAM after starvation (Hamasaki *et al.* 2013). Interestingly, the disruption of MAM by knocking down *Pacs2* or *Mfn2* decreases the number of autophagosomes, suggesting that MAM integrity is a requirement for autophagosome formation. According to this model, disruption of MAM by *Mfn2*-KO inhibits lipid transfer and starvation-induced autophagy by inhibiting the PS transfer from ER into mitochondria-derived autophagosomes (Hailey *et al.* 2010). Interestingly, two proteins that are present at MAM interface were recently shown to regulate autophagy; PML inhibits autophagy at MAM interface by a Ca²⁺-dependent mechanism (Missiroli *et al.* 2016), whereas PP2A dephosphorylates Unc-51-like autophagy activating kinase 1 and induces autophagy (Wong *et al.* 2015). Although both PML and PP2A interact together at MAM (Giorgi *et al.* 2010), it is still unclear whether the regulation of autophagy by PP2A involves its presence at MAM interface.

Role of MAM in immune signaling

Immune responses and metabolic regulation are tightly coupled, and prolonged or exacerbated inflammatory responses have been associated with metabolic diseases (Chassaing *et al.* 2014, Delzenne *et al.* 2015, Lackey & Olefsky 2016). Therefore, the recent involvement of MAM in immune signaling supports a role of MAM in metabolic homeostasis.

Several proteins involved in immune response to DNA viruses, such as the mitochondrial antiviral-signaling protein (MAVS) (Horner *et al.* 2011) or the stimulator of interferon genes (STING) (Ishikawa *et al.* 2009) were shown to be localized at MAM. Indeed,

retinoic acid-inducible gene 1 is recruited at MAM during viral infection and binds to MAVS to initiate a signaling cascade leading to the upregulation of pro-inflammatory cytokines. This process is supported by the ER–mitochondria tethering function of MFN2 (Horner *et al.* 2011). Interestingly, the hepatitis C virus NS3/4A protease, involved in the cleavage of MAVS to inhibit a strong antiviral response, was shown to target MAM (Horner *et al.* 2011), highlighting the role of MAM in the regulation of innate immune signaling.

Autophagy and inflammasome activation are intimately linked and mutually influence each other (Martins-Marques *et al.* 2015). In agreement with the role of MAM in the autophagosome formation, MAMs were also shown to be important for the inflammasome activation. Indeed, NOD-like receptor pyrin domain-containing 3 (NLRP3) protein and its adaptor ASC co-localized to the MAM fractions following the activation of the NLRP3 inflammasome by nigericin or monosodium urate (Zhou *et al.* 2011). Although mitochondrial ROS are required for the activation of inflammasome (Tschopp 2011), the exact molecular mechanisms by which MAMs regulate immune signaling are still unclear.

Role of ER–mitochondria miscommunication in metabolic diseases

Given the important role of MAM structure and function in the control of numerous signaling pathways regulating metabolic homeostasis, it is not surprising that ER–mitochondria miscommunication participates to metabolic diseases (Fig. 3).

ER–mitochondria miscommunication and insulin resistance

The potential involvement of ER–mitochondria communication in hepatic insulin resistance was firstly suggested by a study highlighting a strong relationship between ER stress and mitochondria dysfunction. Indeed, experimental mitochondrial dysfunction was shown to induce ER stress through an elevation of cytosolic free Ca²⁺ and led subsequently to aberrant insulin signaling and increased hepatic gluconeogenesis (Lim *et al.* 2009). In agreement, liver-specific KO of the mitochondrial *Mfn2* (Sebastian *et al.* 2012) or loss of the mitochondrial *CypD* (Rieusset *et al.* 2016) induced both hepatic ER stress and insulin resistance, whereas cells deficient in the three isoforms of IP3R in ER showed mitochondrial dysfunction (Cardenas *et al.* 2010), thus confirming an interplay

between ER and mitochondria dysfunction in the context of metabolic diseases.

Our group investigated the involvement of MAM disruption in hepatic insulin resistance (Tubbs *et al.* 2014). We found that MAM integrity is altered in palmitate-induced insulin-resistant HuH7 cells, as well as in liver of different models of obese and diabetic mice (*ob/ob* and high-fat and high-sucrose diet (HFHSD)-fed mice). Importantly, disruption of MAM integrity by genetic or pharmacological inhibition of CYPD induced insulin resistance in mice and disrupted insulin signaling in human primary hepatocytes. Interestingly, treatment of diabetic mice with antidiabetic drugs (HFHSD mice with rosiglitazone or *CypD*-KO mice with metformin) improved insulin sensitivity and restored organelle communication. Conversely, the rescue of MAM integrity in primary hepatocytes of *ob/ob* or HFHSD mice by adenoviral overexpression of *CypD* improved insulin action confirming a key role of ER–mitochondria miscommunication in hepatic insulin resistance (Tubbs *et al.* 2014). Other independent observations support a link between disrupted MAM and hepatic insulin resistance. For example, ER–mitochondria interactions were disrupted after cellular loss of *mTorc2* (Betz *et al.* 2013), whereas mice with liver-specific KO of rictor, a mTORC2 subunit, showed impaired glucose and lipid homeostasis (Hagiwara *et al.* 2012). Similarly, loss of *Mfn2* induced reduced ER–mitochondria interactions in pro-opiomelanocortin (POMC) neurons, whereas hepatic-specific loss of *Mfn2* in mice induced hepatic insulin resistance and altered glucose homeostasis (Sebastian *et al.* 2012). Interestingly, overexpression of the constitutive active AKT2 restored glucose fluxes and lipogenesis in mTORC2-deficient hepatocytes (Hagiwara *et al.* 2012), whereas overexpression of *Mfn2* improved HFD-induced insulin resistance (Gan *et al.* 2013), suggesting that both mTORC2-AKT and MFN2 are important for metabolic homeostasis. However, whether these effects involve modulations of MAM is still unclear.

Mechanistically, we suggest that a loss of Ca²⁺ transfer from ER to mitochondria links MAM disruption to hepatic insulin resistance. In the case of *CypD*-KO mice, the disruption of Ca²⁺ exchange between both organelles leads to organelle stress, lipid accumulation, activation of protein kinase C ϵ and alteration of insulin-stimulated AKT phosphorylation (Rieusset *et al.* 2016). Whether this mechanism can be generalized to genetic and nutritional models of obesity and T2DM has to be determined. Interestingly, ER–mitochondria contacts are also reduced in POMC neurons of HFD mice (Schneeberger *et al.* 2013),

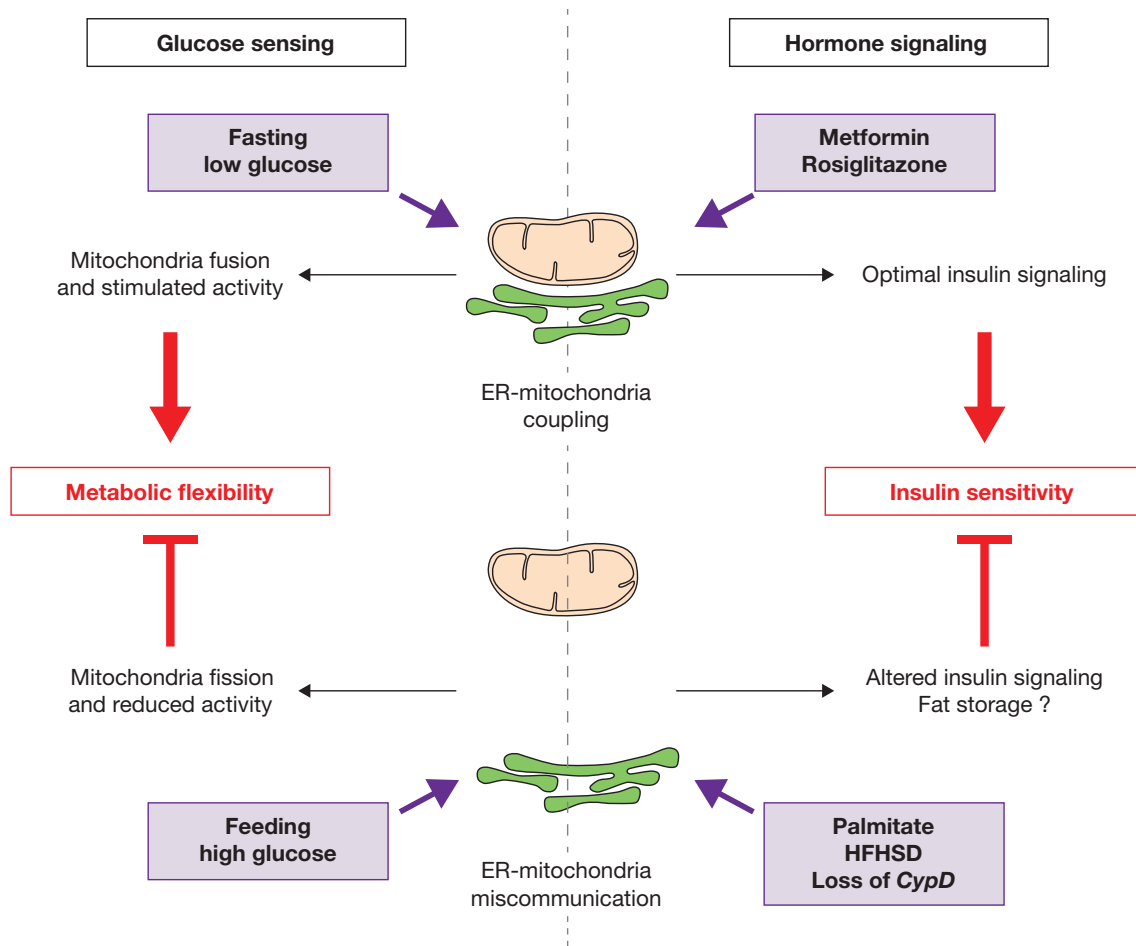


Figure 3

Role of ER–mitochondria miscommunication in metabolic diseases. At top: optimal ER–mitochondria interactions are required for metabolic flexibility (left) and insulin signaling (right). Top left: increased organelle interactions at fasting state promote mitochondria fusion and maximal oxidative capacities to predominantly oxidize lipids. Top right: antidiabetic drugs improve both insulin sensitivity and organelle interactions in diabetic mice. At the bottom: ER–mitochondria miscommunication is involved in metabolic inflexibility during nutritional transition (left) and in insulin resistance (right). Bottom left: high glucose levels reduce ER–mitochondria interactions at post-prandial state, presumably leading to the storage of excess of glucose into lipids. Bottom right: palmitate treatment, high-fat and high-sucrose diet (HFHSD) feeding or loss of cyclophilin D (*CypD*) reduces both organelle interactions and insulin sensitivity.

suggesting a miscommunication between ER and mitochondria in multiple tissues in the context of T2DM. A link between disrupted MAM and insulin resistance was also found in white adipose tissue of mice deficient in *Cisd2* (also known as WFS2), an iron-sulfur protein localized at MAM interface (Chen *et al.* 2009, Wang *et al.* 2014).

Recently, another group reported that MAM content is conversely increased in the liver of obese mice, leading to mitochondrial Ca^{2+} overload and mitochondrial dysfunction (Arruda *et al.* 2014). They further showed that reinforcing hepatic MAM by IP3R1 or PACS2 overexpression induced insulin resistance, whereas reducing the expression of these proteins in liver of obese mice improved insulin sensitivity (Arruda *et al.* 2014).

The discrepancy between studies is actually unclear but could be related to differences in mice metabolic status, environmental housing conditions or experimental analysis. Indeed, it is possible that MAMs are sensitive to a variety of environmental signals, from nutrients to pathogens. As both mitochondria (Hummasti & Hotamisligil 2010) and ER (Chaudhari *et al.* 2014) have been recently involved in the modulation of immune responses, modifications of environmental conditions, such as changes in microbiota flora and activation of immune signals, could explain the discrepancy between results. Furthermore, as no MAM proteins are specific to this subcellular compartment, we cannot exclude that modulating their expression could have non-specific effects, further participating to the discrepancy between

studies. Along these lines, reduced or excessive ER–mitochondria contacts, likely depending on the timing of the adaptive response upon a metabolic challenge, could represent a new and important mechanism contributing to hepatic mitochondrial dysfunction and insulin resistance. Future studies in which MAM will be dynamically studied are required to clarify this element.

ER–mitochondria miscommunication and metabolic inflexibility

Metabolic flexibility is the capacity of a cell to switch from lipid to glucose utilization during fasted-to-fed transition, and metabolic diseases are classically associated with metabolic inflexibility (Galgani *et al.* 2008). Importantly, we found that chronic disruption of MAM in the liver of insulin-resistant mice is associated with a loss of MAM regulation by energy state. Indeed, fasting-to-postprandial transition reduced ER–mitochondria interactions in liver of wt mice, whereas this regulation is lost in the liver of obese and diabetic mice (Theurey *et al.* 2016). Furthermore, sucrose consumption in drinking water also reduced ER–mitochondria interactions in liver of fasted wt mice, whereas this regulation is lost in the liver of *ob/ob* and *CypD*-KO mice, both characterized by chronic disruption of MAM integrity, mitochondrial fission and altered mitochondrial respiration. As the regulation of ER–mitochondria interaction by glucose levels allows to control mitochondria dynamics and function and was also suggested to adapt hepatic metabolism to nutritional state, chronic disruption of MAM could participate to both hepatic metabolic inflexibility and mitochondrial dysfunction associated with hepatic insulin resistance. In line with this evidence, ER–mitochondria interactions are controlled by PP2A (Theurey *et al.* 2016), and hyperactivation of PP2A was associated with insulin resistance (Kowluru & Matti 2012). Therefore, increased PP2A activity could participate in the disruption of MAM in the liver of insulin-resistant mice. Future studies are required to understand the molecular mechanisms of MAM disruption in the context of hepatic metabolic diseases and their role in metabolic inflexibility.

ER–mitochondria miscommunication and other diseases associated with metabolic alterations

Several human pathologies exhibiting alterations of both organelle and Ca²⁺ homeostasis are associated with metabolic perturbations. This is the case of neurodegenerative diseases, such as Alzheimer's disease

(AD) (Kandimalla *et al.* 2016), viral infections (Kralj *et al.* 2016), Wolfram syndrome (Boutzios *et al.* 2011) or some cancers (Klil-Drori *et al.* 2016). Interestingly, ER–mitochondria miscommunication has recently been suggested or reported in these pathologies. However, it is still unclear whether MAM alterations in these pathologies contribute to the metabolic phenotype of patients.

In AD, high levels of β -amyloid plaques are observed, which are generated by the proteolytic cleavage of amyloid precursor protein by presenilin-1 and -2, components of the γ -secretase complex (Area-Gomez & Schon 2016). The molecular mechanism underlying the physiopathology of AD is rather unclear, but AD has interestingly been associated with altered metabolism, altered Ca²⁺ homeostasis and mitochondrial dysfunction. Consistent with these alterations, several evidence suggested that MAMs may play a role in neurodegenerative diseases, including AD, as recently highlighted by several reviews (Volgyi *et al.* 2015, Area-Gomez & Schon 2016, Joshi *et al.* 2016, Krols *et al.* 2016, Paillusson *et al.* 2016). Indeed, presenilins are enriched at MAM (Area-Gomez *et al.* 2009). Furthermore, ER–mitochondria interactions and MAM functionality (cholesteryl ester synthesis and PL transfer) are increased in fibroblasts from AD patients, in fibroblasts from mouse models of AD, as well as in cell expressing gain-of-function mutation of presenilin (Area-Gomez *et al.* 2012, Area-Gomez & Schon 2016). However, whether ER–mitochondria hyperconnectivity participates to the AD-associated alterations of lipid, Ca²⁺ and mitochondria, as well as to the pathology itself, requires further investigations. Although it remains a challenge to clearly demonstrate the existence of a causal relationship between MAM and AD, these preliminary data are rather exciting.

Concerning viral infections, several viral proteins were shown to localize to MAM (for reviews, see Williamson & Colberg-Poley 2009, Colberg-Poley *et al.* 2015, de Armas-Rillo *et al.* 2016). This is the case for the human cytomegalovirus exon 1 protein and viral mitochondria-localized inhibitor of apoptosis (Bozidis *et al.* 2010, Zhang *et al.* 2011, 2013), for the hepatitis C virus core protein (Williamson & Colberg-Poley 2009, Horner *et al.* 2011, 2015), for the human immunodeficiency virus protein-R (Huang *et al.* 2012) and for the dengue virus (Chatel-Chaix *et al.* 2016). The localization of some viral proteins at MAM was reported either to induce changes in the abundance of cellular proteins at MAM (Bozidis *et al.* 2010, Zhang *et al.* 2011, 2013) or to disrupt ER–mitochondria interactions in host cells (Huang *et al.* 2012, Chatel-Chaix *et al.* 2016). Affecting MAM structure

and function may enable viruses to reprogram cellular metabolism. Therefore, further studies deciphering how this subcellular domain might be manipulated are required to improve anticancer therapies.

Wolfram syndrome (WFS) is a rare neurodegenerative and metabolic disorder associated with a shortened lifespan. Mutations in human *CISD2/WFS2*, an iron-sulfur protein localized in the ER, cause WFS (Amr *et al.* 2007). Interestingly, *CISD2* was localized at MAM (Wang *et al.* 2014), and adipocyte-specific loss of *Cisd2* is associated with a reduction of ER-mitochondria interactions, mitochondrial dysfunction and altered insulin signaling in adipose tissue (Wang *et al.* 2014). Therefore, ER-mitochondria miscommunication may play a role in metabolic alterations of WFS patients. Future studies are required to clarify this issue.

Recent evidences suggest a role of MAM in cancer disease, as this subcellular domain is a platform for several oncogenes and tumor suppressors (Marchi *et al.* 2014). The first link comes from studies on PML, a tumor suppressor frequently altered in cancers (Gurrieri *et al.* 2004). Interestingly, PML is present at the MAM interface where it controls both apoptosis (Giorgi *et al.* 2010) and autophagy (Missiroli *et al.* 2016). The proapoptotic and anti-autophagic action of PML relies on its role to transfer Ca^{2+} from ER to mitochondria, through the control of AKT-mediated IP3R phosphorylation (Giorgi *et al.* 2010). Other tumor suppressors, such as PTEN (Bononi *et al.* 2013) and p53 (Giorgi *et al.* 2015a), have also been localized at the MAM interface and regulate Ca^{2+} flux and apoptosis by regulating AKT activity. Recently, two other tumor-related proteins regulating Ca^{2+} transfer at MAM emerged as prognosis indicators in some cancers. For example, FATE1, a cancer-testis antigen localized at MAM, was recently implicated in the regulation of Ca^{2+} - and drug-dependent apoptosis in cancer cells by modulating ER-mitochondria distance. Therefore, high FATE1 expression in the tumor is a poor prognosis indicator in patients with adrenocortical carcinoma (Doghman-Bouguerra *et al.* 2016). Furthermore, the ER-localized thioredoxin-related transmembrane protein 1 (TMX1) was shown to interact with SERCA2b under oxidizing conditions in a thiol-dependent manner to decrease SERCA activity and, thus, the ER Ca^{2+} load. Therefore, cancer cells with low TMX1 exhibit increased ER Ca^{2+} , accelerated cytosolic Ca^{2+} clearance and reduced Ca^{2+} transfer to mitochondria, dampening mitochondria activity in tumor cells and favoring tumor growth (Raturi *et al.* 2016).

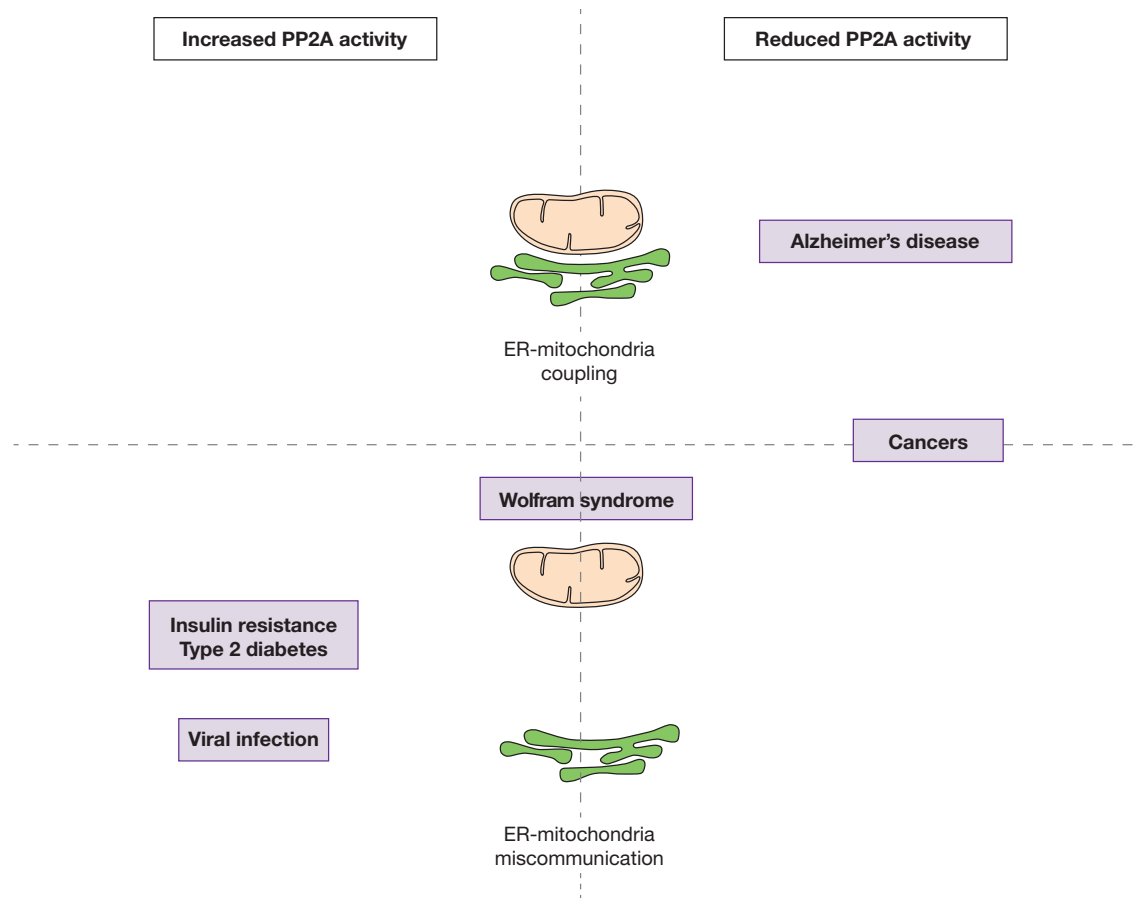
The interest of PP2A at ER-mitochondria contact sites could also have implications in these pathologies (Fig. 4).

Indeed, reduced PP2A activity has been observed in Alzheimer's disease (Sontag & Sontag 2014) and in cancers (Grech *et al.* 2016), whereas an increased expression of the catalytic subunit of PP2A has been observed after viral infection (Tsunematsu *et al.* 2016). Until now, no link has been made between PP2A and WFS. In other words, the induction of ER-mitochondria interactions is associated with reduced PP2A activity in AD, whereas the reduction of ER-mitochondria interactions is associated with PP2A hyperactivation/hyperexpression in both T2DM and viral infection. In addition, the upregulation of PP2AC after HCV infection is involved in the alteration of insulin-mediated glucose metabolism in liver (Tsunematsu *et al.* 2016), highlighting the importance of PP2A in the metabolic alterations induced by viral infection. Therefore, we suggest that the reciprocal regulation of both PP2A and MAM in these pathologies may point at an interesting relationship. Whether a different relocalization of PP2A at MAM interface participates to this antagonist regulation requires further studies. For cancers, it is still unknown whether ER-mitochondria interactions are modulated. However, the fact that PML is important for an appropriate Ca^{2+} flux in the MAM compartment by mediating the recruitment of PP2A (Giorgi *et al.* 2010), suggest a potential interplay between MAM, PP2A and cancers.

Even if all these observations are really preliminary, in the future, attention should be drawn to (i) the link between ER miscommunication and these pathologies, (ii) to the involvement of MAM in the metabolic alterations that occurs in these diseases, as well as (iii) to the role of PP2A at MAM interface.

Looking for regulatory players of the ER-mitochondria crosstalk to treat metabolic diseases?

As ER-mitochondria contact sites are dynamic and can change in size in response to nutritional and environmental cues, their targeting to improve metabolic diseases is an attractive perspective. However, the molecular mechanisms underlying how their crosstalk is regulated remains unclear. Recently, post-translational modifications, such as palmitoylation, were shown to finely localize proteins at the MAM interface (Lynes *et al.* 2012). The emerging role of PP2A at MAM (Giorgi *et al.* 2010, Theurey *et al.* 2016) suggests that protein phosphorylation could be important for MAM functionality. In agreement, AKT and IP3R phosphorylation control inter-organelle Ca^{2+}

**Figure 4**

Hypothetical relationship between PP2A activity and ER–mitochondria interactions in several metabolic diseases. Several diseases associated with disrupted metabolism, such as type 2 diabetes mellitus (T2DM), Alzheimer's diseases or viral infection show deregulated PP2A activity (upregulated at left or downregulated at right) and change in ER–mitochondria contact (reinforcement at top and disruption at bottom). Wolfram syndrome shows ER–mitochondria miscommunication, but no link with PP2A has been made. At opposite, cancers are associated with reduced PP2A activity, but MAM integrity is unknown.

transfer efficiency (Khan *et al.* 2006, Marchi *et al.* 2008, Giorgi *et al.* 2010). Other proteins of MAM are also phosphorylated at this subdomain, such as mTORC2, PACS2 and hexokinase-2 (Betz *et al.* 2013). Further studies are necessary to clarify the role of post-translational modifications in the regulation of MAM integrity and functionality.

Conclusions

Although the importance of ER–mitochondria communication in organelle homeostasis and cell fate is widely recognized, recent evidence indicates that MAM could also be an important hub for hormonal and nutrient signaling in hepatocytes, thus regulating metabolic homeostasis. Furthermore, recent studies highlight that ER–mitochondria miscommunication

in the liver could contribute to metabolic diseases. However, these observations are clearly still in the initial stages to determine whether or not ER–mitochondria miscommunication contributes to metabolic diseases. Therefore, it will be important in the future to determine whether MAM integrity is required for hormone and nutrient signaling in other cell types as well as in hepatocytes. Furthermore, the results described in this review are rather correlative and future studies should determine whether ER–mitochondria miscommunication is a cause or a consequence of metabolic diseases. Finally, the relevance of this interesting relationship between MAM and metabolic homeostasis needs to be investigated in humans. Although care should be taken to ensure proper analysis of MAM components, functions and subsequent regulated signaling pathways, further dynamic studies are now required to clarify the nutritional regulation of

MAM, the role of MAM in cellular metabolism and the role of MAM disruption in the development of metabolic diseases. Strategies that specifically dampen or reinforce ER–mitochondria interactions *in vivo*, preferably in an inducible-manner, should help in the future to validate the role of MAM in metabolic diseases. Similarly, the molecular determinants of MAM, as well as the dynamic regulation of ER–mitochondria interactions, should be investigated to identify the key regulators of MAM integrity and functionality that could reveal themselves as pharmacological targets to modulate MAM and improve metabolic diseases. Whether PP2A could be a good target for the modulation of ER–mitochondria interactions and Ca²⁺ transfer requires further investigations. Nevertheless, it is unlikely that PP2A is the pharmacological target for improving metabolic diseases as this phosphatase has pleiotropic effects and its inhibition is pro-cancerous. Therefore, the next challenge is either to specifically target PP2A at MAM or to identify targets of PP2A at MAM, to specifically modulate ER–mitochondria crosstalk and potentially improve metabolic diseases. As PP2A is at the crossroad of several cellular signaling pathways and of several pathologies, there is no doubt that future studies will soon answer these fascinating questions.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

This work was funded by INSERM. E T was supported by a post-doc fellowship from Lund University.

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Received in final form 7 December 2016

Accepted 13 December 2016

Accepted Preprint published online 13 December 2016