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► **To cite this version:**

Nadine Camougrand, Stéphen Manon. Mitochondria-associated membranes in the maintenance of cell homeostasis. *Mitochondrial Physiology and Vegetal Molecules*, pp.151 - 169, 2021, 10.1016/b978-0-12-821562-3.00043-5 . hal-03395328

**HAL Id: hal-03395328**

**<https://hal.science/hal-03395328>**

Submitted on 25 Oct 2021

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# **Mitochondria-Associated Membranes in the Maintenance of Cell Homeostasis.**

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In most eukaryotic cells, mitochondria and the endoplasmic reticulum (ER) occupy a large proportion of the cytoplasm, and form intricate networks that are physically close to each other. Physical contacts between these organelles have been observed through microscopy methods since 1960 (Robertson, 1960; Franke and Kartenbeck, 1971; Morré et al., 1971). For example, up to 20% of mitochondrial membranes is in contact with ER in HeLa cells (Rizzuto et al., 1998). However, it remained unclear for long if the formation of these contacts was regulated or if they were randomly distributed. The second question is whether they have a function in exchanges between ER and mitochondria.

Since mitochondria have a much higher density than the ER, differential centrifugation allows to separate these two organelles. However, it is well-known that the mitochondrial fraction is always “contaminated” by components from the ER (and from other subcellular compartments). Further purification by density gradient methods allowed to separate this “Mitochondria-Associated Membranes” fraction (MAM) (Meier et al., 1980), that was further characterized by a high capacity to synthesize phospholipids (Vance, 1990; Rusiñol et al., 1994). The function of MAM has first been limited to the transfer/synthesis of lipids between ER and mitochondria (Vance, 2014, for review). However, the function of MAM has been further extended to other processes involving a dialog between ER and mitochondria, namely  $\text{Ca}^{2+}$  signaling. Both phospholipid transfer and  $\text{Ca}^{2+}$  transport play a major role in fundamental cellular processes such as autophagy/mitophagy and cell death. This short review will cover the current knowledge on the links between MAM formation and stability and these fundamental cellular processes.

## **MAM and phospholipid synthesis and transfer**

The definitive identification of MAM as a compartment distinct from the ER bulk was based on the presence of enzymes involved in phospholipid synthesis (Vance and Schiao, 1996; Stone and Vance, 2000). In mammalian cells, full-length forms of Phosphatidylserine synthases 1 and 2 (PSS-1 and PSS-2) are enriched in MAM, and largely excluded from the ER bulk (Stone and Vance, 2000). This might be related to an efficient transfer of PS to mitochondria, where PS decarboxylation is the main source of PE (Shiao et al., 1995). This did not exclude that other (truncated) forms of these enzymes were present in other parts of the ER, providing PS to this organelle. A similar process of localized PS synthesis in MAM has been observed in yeast (Achleitner et al., 1999). Other enzymes involved in phospholipid synthesis have a dual localization between MAM and the ER bulk, such as liver Phosphatidylethanolamine N-methyl Transferases, that convert PE to PC (Vance et al., 1997). In this case, two distinct isoenzymes (PEMT1 and PEMT2) are involved. Following these pioneering studies, a growing number of lipid syntheses have been found to take place in MAM, such as ceramide (Stiban et al., 2008) and triacylglycerol (Stone et al., 2009). In yeast, PE can be synthesized from Lyso-PE by the Acyltransferase Ale1, located in MAM (Riekhof et al., 2007), suggesting that this localization of lipid syntheses is conserved among different pathways and organisms. Indeed, the localization of lipid syntheses in MAM has been connected to the requirement for an efficient transfer of ER-synthesized lipid to mitochondria (Tatsuta et al., 2014). One can intuitively imagine that the transport of hydrophobic lipids from ER to mitochondria through the aqueous cytosolic phase is not energetically favorable. A vesicular transport of lipid may occur, but has not been observed so far between ER and mitochondria. Lipid transfer proteins exist, and are involved in the transport of ceramide between ER and Golgi (Hanada et al., 2003), but have not been found for mitochondria. The existence of MAM offers a support for the diffusion of lipid between the donor membrane (ER) and the acceptor membrane (Outer Mitochondrial Membrane, OMM) through close junctions. PS synthesis by PSS located in MAM would therefore be channeled to PE synthesis through PSD located in mitochondria. Conversely, mitochondrial PE could be “re-exported” and converted to PC through PEMT in MAM. Given the localization of PSD in the matrix (but probably close to the inner mitochondrial membrane, IMM), it is likely that a component of the OMM (not identified yet) or contacts sites between OMM and IMM are also involved. The possible connection between MAM and OMM/IMM contact sites is thus an exciting issue. Mitochondrial contact sites are enriched with negatively charged phospholipids, namely cardiolipin, that is specific to mitochondria (Hoch, 1992; Schlattner et al., 2014, for reviews).

The architecture of the contact sites is stabilized by a complex called MICOS, both in yeast (Harner et al., 2011; Horvath and Daum, 2013) and human cells (Kozjak-Pavlovic, 2017, for review) that is involved in the exchange of cardiolipin, PA and probably other phospholipids between OMM and IMM. To date, there is no structural evidence that MAM and mitochondrial contact sites are physically connected but the transfer of neosynthesized PS in MAM to the IMM/matrix PSD makes it likely. Other lipids, such as sterols, also might be transported by a similar system from the ER to mitochondria. Indeed, sterols are synthesized in the ER and are transported to other membranes by specific transporters (Sullivan et al., 2006) and there is no direct evidence of enrichment of sterol synthesis enzymes in MAM compared to bulk ER. However, sterol transport to the IMM (mostly as a substrate for steroid hormones synthesis) occurs through a complex spanning the two mitochondrial membranes and including TSPO, VDAC1 and ANT1 (Gatliff and Capanella, 2012, for review) thus stabilizing a contact site between OMM and IMM. How this type of contact site might or not be connected to MAM is an interesting issue.

### **The yeast ERMES complex**

In yeast, a genetic screen allowed to identify the complex ERMES (ER-mitochondria encounter structure) as required for the tethering between ER and mitochondria in yeast (Kornmann et al., 2009). ERMES is a complex composed of at least four proteins: OMM proteins Mdm10 and Mdm34, ER membrane protein Mmm1, and cytosolic protein Mdm12. Mutations in ERMES components cause diverse mitochondrial phenotypes, including mitochondrial morphology defects and the inability of cells to grow on a non-fermentable carbon source. These phenotypes can be reversed by the expression of an artificial tether, suggesting that the main function of the complex is to act as a mechanical link between ER and mitochondria. Considering the pleiotropic effect of ERMES alterations, it was likely that a fundamental maintenance process, such as phospholipid homeostasis, was affected. Indeed, Mmm1, Mdm12 and Mdm34 contain a SMP domain that is closely related to the lipid-binding domain Tulip (Kopec et al., 2010; Michel and Kornmann, 2012). The structure of the Mmm1-Mdm12 sub-complex show that it forms a hydrophobic tunnel allowing the diffusion of phospholipids (Jeong et al., 2017). It is therefore likely that ERMES is directly involved in the transfer of lipids between ER and MOM. The fact that the deletion of Mmm1, Mdm12 or Mdm10 dramatically alter mitochondrial function and render yeast cells unable to grow on a non-fermentable carbon source, with a concomitant

loss of mitochondrial DNA (Tan et al., 2013), pointed at the importance of this ER-mitochondria lipid transfer process.

No obvious homologous structure of the ERMES complex has been identified in mammalian cells. However, the yeast GTPase Gem1 is also associated to ERMES and regulates MAM stability (Kornmann et al., 2011). Gem1 is the yeast homolog of the mammalian protein Miro that forms foci at the contacts between ER and mitochondria (Kornmann et al., 2011). Recently, Miro clusters have been found to regulate ER-mitochondria contacts, with further effects on mitochondrial transport and cristae organization (Modi et al., 2019). This suggests that the molecular organization of MAM involved in lipid transfer might be conserved between yeast and mammals, although other mammalian components remain to be identified.

### **MAM and Ca<sup>2+</sup> signaling**

Ca<sup>2+</sup> movements between ER and mitochondria are crucial to cell fate. Indeed, Ca<sup>2+</sup> is required for the activity of several Krebs cycle enzymes, such as pyruvate dehydrogenase,  $\alpha$ -ketoglutarate dehydrogenase and isocitrate dehydrogenase (Denton and McCormack, 1986; Hansford, 1994). Conversely, high intramitochondrial concentrations of calcium are responsible for the opening of the mitochondrial permeability transition pore (mPTP) (Giorgio et al., 2018, for a recent review). mPTP opening initiates necrotic cell death following, namely, ischemia/reperfusion (Baines et al., 2005; Nakagawa et al., 2005).

Local intramitochondrial calcium concentration was shown to be higher in the regions close to ER-mitochondria contacts (Rizzuto et al., 1998). It has been postulated that the mitochondrial calcium uniporter (MCU) could be physically close to the ER calcium transporter IP3R and the OMM VDAC (Szabadkai et al., 2006), thus allowing a calcium channeling process that would increase the efficiency of ER-mitochondria Ca<sup>2+</sup>-signaling (Pizzo and Pozzan, 2007). Strikingly, the isoform III of IP3R (Mendes et al., 2005), The ER calcium sensor Sigma-1 receptor (Hayashi and Su, 2007), and Grp75 (Szabadkai et al., 2006) were found to be enriched in MAM and to contribute to the ER-mitochondria calcium transport. This does not imply that this localization and function are exclusive, but more likely suggests that close ER-mitochondria contacts dependent on these proteins might form in a transitory manner to efficiently channel calcium when needed (Shkryl and Shirokova, 2010; Csordas et al., 2010). Considering the role of Ca<sup>2+</sup> in necrotic cell death, the role of MAM in this type of death is related to the above-mentioned Ca<sup>2+</sup>-channeling between ER and mitochondria. Local elevated mitochondrial

Ca<sup>2+</sup> concentration at the vicinity of ER-mitochondria contact sites containing IP3R, VDAC and MCU would trigger the opening of the mPTP, massive release of mitochondrial molecules (including Ca<sup>2+</sup>, in the process called “calcium-induced calcium release”), loss of mitochondrial energetic processes, and ultimately cell death (Giorgio et al., 2018, for review). mPTP components have for long been identified as being VDAC on the OMM and ANT on the IMM. This is now largely regarded as wrong, and the exact identity of mPTP components is still discussed (Baines and Gutierrez-Aguilar, 2018, for review). Most recent experiments point at the involvement of ATP synthase dimers (Carraro et al., 2019, for review), but it is not known if the localization of these dimers has something to do with mPTP regulation by calcium.

### **MAM and apoptotic cell death**

Until the demonstration that the loss of cyclophilin D, a major regulator of mPTP, did not impair apoptotic cell death (Nakagawa et al., 2005), it was believed that mPTP opening was also directly involved in apoptosis (Jacotot et al., 1999). This mistake was largely due to the fact that mammalian cells in culture engaged into a secondary necrosis (mPTP-dependent) following the initial apoptotic signal (mPTP-independent), because of the absence of elimination of apoptotic cells by the immune system.

It is now largely accepted that the permeabilisation of the OMM during apoptosis is due to the formation of large channels formed of oligomers of Bax (Pavlov et al., 2001) and Bak (Bleicken et al., 2013), the pro-apoptotic members of the Bcl-2 family. These large channels allow the release of apoptogenic factors from the intermembrane space to the cytosol (Renault and Manon, 2011; Birkinshaw and Czabotar, 2017, for reviews). Bax and Bak are regulated by the other members of the Bcl-2 family at different levels. The anti-apoptotic proteins Bcl-2, Bcl-xL and Mcl-1 prevent their activation. For long, the “rheostat” model considered that the formation of *e.g.* Bax/Bcl-2 dimers allowed to sequester Bax away from mitochondria and that the rupture of the interaction (by a BH3-only protein such as Bad) was sufficient to promote Bax mitochondrial relocation and activation (Korsmeyer et al., 1993). Although this view is still partly valid, it was largely incomplete (Volkman et al., 2014). First it did not explain the regulation of Bak, that is constitutively mitochondrial, where it is maintained under an inactive state by the interaction with VDAC2 (Cheng et al., 2003). Also, the overexpression of Bcl-2 (Teijido and Dejean, 2010) or Bcl-xL (Renault et al., 2015) tends to increase the localization of inactive Bax at the mitochondria in non-apoptotic cells. The process of translocation/

retrotranslocation, that is controlled not only by anti-apoptotic proteins (Edlich et al., 2011), but also by protein kinases such as AKT (Schellenberg et al., 2013), adds to the complexity of the regulation. Ultimately, the distribution of Bax in different compartments seems to be a more crucial determining factor in the capacity of a cell to engage into apoptosis than its capacity to oligomerize (Kuwana et al., 2020).

Conversely, BH3-only proteins tBid, Bim and Puma activate Bax and Bak, either directly, or indirectly through the inhibition of anti-apoptotic proteins (Shamas-Din et al., 2011, for review). It is now largely accepted that there is a dynamic equilibrium of the repartition of all different Bcl-2 family members (pro-apoptotic, anti-apoptotic, BH3-only) between the cytosol and the OMM (Edlich, 2018). However, these proteins have also been found in the ER, such as Bax (Zong et al., 2003; Gajkowska et al., 2004; Stout et al., 2007; Zhang et al., 2013), Bcl-2 (Gotow et al., 2000; Annis et al., 2001; Thomenius et al., 2003) and Bcl-xL (Eno et al., 2012) (Popgeorgiev et al., 2018, for review). ER-localized Bcl-2 regulates  $Ca^{2+}$  transport (Lam et al., 1994; He et al., 1997; Foyouzi-Youssefi et al., 2000): actually, this connection between its ER-localization and calcium-regulated cell death was described even before its inhibitory function on Bax activation. This effect might also happen with Bcl-xL (Williams et al., 2016). Conversely, ER-localized Bax was found to stimulate  $Ca^{2+}$  fluxes that trigger cell death (Oakes et al., 2003). It was also found that the zebrafish pro-apoptotic protein Bcl-w regulated both VDAC and MCU to control  $Ca^{2+}$  flow into mitochondria (Prudent et al., 2013). Consequently, both pro- and anti-apoptotic proteins modulate calcium-induced death (*i.e.* necrosis), indirectly, through their capacity to modulate ER calcium. Considering the importance of MAM in calcium-induced death, the question of a MAM localization of Bcl-2 family members is fully relevant.

It has been observed that the cytomegalovirus anti-apoptotic protein vMIA induced a re-targeting of Bax to MAM, instead of OMM (Zhang et al., 2013). This re-targeting prevents mitochondria permeabilization and Bax is further degraded by the proteasome. Proteins of the Bcl-2 family can be ectopically expressed in yeast, where they keep their ability to localize and permeabilize the OMM (Renault and Manon, 2011, for review). When human Bax was expressed in yeast cells carrying a deletion in a ERMES protein (Mdm34), the protein was less capable to reach the OMM (Légitot et al., 2019). These data suggest that MAM might be a transitory location for Bax, where it might be blocked if an anti-apoptotic signal (such as vMIA) is present. According to the Bax translocation/retrotranslocation processes (Edlich et al., 2011; Schellenberg et al., 2013, Renault et al., 2015), one can speculate that MAM stability might have a crucial role in the cycling of Bax between

mitochondria (where it can be activated to promote apoptosis) and the ER (where it cannot be activated), providing an additional level of regulation of Bax function and apoptosis.

Ceramide, like PS, are synthesized in MAM, and can be transferred to the OMM following an apoptotic signal (Stiban et al., 2008). They are able to permeabilize OMM to apoptogenic factors, alone or in combination with Bax, in a way sensitive to Bcl-2/Bcl-xL (Colombini, 2017, for review).

Interestingly, ERMES also regulates acetic acid-induced death in yeast where calcium, Bcl-2 family members, or ceramides are not involved (Martins et al., 2019), suggesting that ER-mitochondria contacts are involved in many other death signaling processes.

### **MAM and Autophagy/Mitophagy**

Autophagy is a degradation process, highly conserved among eukaryotes, by which cytosolic components such as organelles or proteins are degraded within specific compartments: the vacuole in yeast and plants, and lysosomes in mammalian cells. It involves *de novo* formation of double phospholipid membranes called phagophore, that elongate, close and form autophagosomes containing sequestered portions of cytosol and organelles. Mature autophagosomes merge with vacuole or lysosomes and the cargo is degraded by the hydrolytic enzymes. Degradation products are re-exported to the cytosol and reused by the cell (Xie and Klionsky, 2007).

The molecular mechanisms involved in autophagy have been widely studied, and this process has been increasingly understood both in yeast and mammalian cells. Autophagy requires more than 40 autophagy-related genes (denoted 'Atg') proteins. Among them, LC3/Atg8 plays a central role, being localized from the beginning until the end of the process in the membranes of the autophagosomes, through the conjugation of a PE at the C-ter end of the protein (Chimera et al., 2000).

Mitophagy allows selective degradation of mitochondria by autophagy, and is involved in quality control of mitochondria (Lemasters, 2005). This came from a work on *S.cerevisiae*, grown on a non-fermentable carbon source (lactate) and subjected to nitrogen starvation (Kiššová et al., 2004).

Multiple mitophagy programs either operate independently or undergo crosstalk and require modulated receptor activities in the mitochondrial membranes. In mammalian cells, two main mechanisms of mitophagy signaling have been identified: ubiquitin-mediated mitophagy and receptor-mediated mitophagy. In these pathways, ubiquitin or proteins, receptors or adaptors, containing a LC3-interacting domain (LIR), act to recruit LC3 family members to mitochondria and thus induce mitophagy. The mechanisms and factors involved in these mitophagy pathways are discussed thereafter.

### ***PINK1/Parkin-dependent mitophagy***

Ubiquitin-mediated mitophagy relies on ubiquitin chains made by E3 ligases to operate as the ‘destroy me’ signal. The best-characterized ubiquitin-mediated mitophagy pathway involves PINK1 and Parkin. PINK1 (PTEN-induced putative kinase 1) (park6) and PARKIN (park2) are two mutated genes in Parkinson’s disease (Gasser, 2005). Under basal conditions, PINK1 goes through the TOM complex due to its N-terminal mitochondrial-targeting sequence (MTS), and is processed by PARL in the IMM and rapidly degraded by the proteasome (N-end rule pathway). PINK1 is barely detectable due to its quick turnover (Matsuda et al., 2010; Narendra et al., 2008; Narendra et al., 2010). At the same time, the E3 ubiquitin-ligase Parkin is kept in the cytosol in an auto-inhibited state, through multiple intramolecular interactions (Chaugule et al., 2011). When mitochondria undergo damage such as loss of membrane potential, PINK1 stays anchored and accumulates in the OMM. Once PINK1 is stabilized to mitochondria, it phosphorylates Parkin on serine 65 (pS65-Parkin). pS65-Parkin is recruited to mitochondria, changes conformation and gets activated, resulting in an increase of its ubiquitin ligase activity. At the same time, PINK1 phosphorylates ubiquitin resulting in more Parkin recruitment and activation, amplifying the process. Polyubiquitinated mitochondrial proteins are recognized by adaptor proteins such as p62 (also known as Sequestosome-1/SQSTM1), OPTN (Optineurin), TAX1BP1 (Tax1 binding Protein 1), NDP52 (Nuclear dot protein 52kDa) and NBR1 (Pankiv et al., 2007; Kirkin et al., 2009; Wild et al., 2011). Phagophore initiation and elongation machineries are recruited to these highly ubiquitinated mitochondrial areas, resulting in mitochondria engulfment into autophagosomes and further lysosomal degradation.

### ***PINK1/Parkin-independent mitophagy, specific receptor characterization***

Mitophagy events have also been reported when PINK1/Parkin proteins were dispensable. PINK1/Parkin-independent mitophagy relies on receptors located in the OMM. Several receptors have been characterized so far: FUNDC1 (Liu et al., 2012), NIX/BNIP3L (Novak et al., 2010; Zhu et al., 2013), BCL2L13 (Murakawa et al., 2015), and prohibitin 2 (PBH2) (Wei et al., 2017). All these receptors contain a LIR domain and can bind LC3 proteins. FUNDC1 and BNIP3 receptors are dephosphorylated and phosphorylated upon membrane potential loss and hypoxia respectively, resulting in an increase of LC3 affinity. In *S.cerevisiae*, the mitochondrial receptor Atg32p is phosphorylated when mitophagy is induced. The human BCL2L13 protein may be homolog to Atg32p.

Indeed, ectopic expression of BCL2L13 in the *atg32Δ* yeast mutant can rescue mitophagy, thereby reinforcing its role as a mitophagy receptor (Murakawa et al., 2015). NIX and BNIP3 are involved in both mitophagy and apoptosis (Novak et al., 2010; Zhu et al., 2013). NIX is implicated in erythrocyte maturation by promoting mitochondrial elimination and acts as a mitophagy receptor recognized by autophagy machinery (Schweers et al., 2007; Sandoval et al., 2008). It is involved in autophagy induction and Parkin translocation to mitochondria (Ding et al., 2010). BNIP3L could also be ubiquitinated by Parkin and induce mitophagy (Gao et al., 2015). The pro-autophagic protein AMBRA1 can interact with LC3 protein and promote mitophagy in Parkin knock-down cells (Strappazzon et al., 2015). These results show the high level of complexity in mitochondrial degradation, and that mitophagy is far from being a linear event.

Recently, prohibitin 2 (PHB2), an IMM protein, was identified as a crucial mitophagy receptor involved in mitochondrial targeting for autophagic degradation (Wei et al., 2017). PHB2 binds LC3 through the LIR domain upon mitochondrial depolarization and proteasome-dependent OMM rupture. PHB2 is required for Parkin-induced mitophagy in mammalian cells and for the clearance of paternal mitochondria after embryonic fertilization in *C.elegans* (Wei et al., 2017).

In mammalian cells, mitophagy can also be triggered by hypoxia (Liu et al., 2012). In this situation, FUNDC1, a mitochondrial outer-membrane protein, is a receptor of selective mitophagy through its interaction with LC3.

The role of cardiolipin in mitophagy induction was characterized in neuronal cells (Chu et al., 2013). After treatments with mitophagy inducers (rotenone, staurosporine, 6-hydroxydopamine,...), cardiolipin is translocated from the IMM to the OMM and can interact with LC3. This translocation could be one of the signals of mitophagy induction.

### ***Role of MAM in providing membranes and PE for autophagy/mitophagy processes***

Many studies in yeast and mammalian cells have shown that different cellular compartments, such as mitochondria (Hailey et al., 2010), the Golgi (Van der Vaart et al., 2010), ER (Axe et al., 2008) and the plasma membrane (Ravikumar et al., 2010) may supply autophagosome membranes. However, the interplay between ER and mitochondria in autophagy has been highlighted in recent studies. Indeed, in starved cells, autophagosomes are derived from OMM (Hailey et al., 2010). The transient localization of the early autophagosomal marker, Atg5, in puncta formed on the surface of mitochondria was observed, followed by LC3-positive autophagosomes transiently associated with mitochondria. This

was confirmed by the use of the fluorescent lipid, NBD-PS (which converts to PE in mitochondria) that transfers from mitochondria to autophagosomes in starved cells. In addition, when mitochondria/ER connections are perturbed by loss of mitofusin2, starvation-induced autophagosomes do not form. (Hailey et al., 2010). These observations were subsequently confirmed by showing that autophagosomes form at ER-mitochondria contact site in mammalian cells. Imaging data reveal that both the pre-autophagosome/autophagosome marker ATG14 (or ATG14L) and the autophagosome-formation marker ATG5 relocalize to ER-mitochondria contact sites after starvation, until formation is complete. Subcellular fractionation showed that ATG14 co-fractionates in MAM under starvation conditions. Disruption of the ER-mitochondria contact site prevents the formation of ATG14 puncta. The ER-resident SNARE protein syntaxin 17 (STX17) binds to ATG14 and recruits it to the ER-mitochondria contact site (Hamasaki et al., 2013). STX17 also regulates the localization of the fission protein DRP1. All these data are confirmed by a series of biochemical and molecular analyses carried out in human fibroblasts in order to characterize lipid rafts in MAM and to explore their possible implication in autophagosomes formation. In this study, the presence of lipid microdomains containing ganglioside GD3 has been detected in MAM, and an interaction of this lipid with core-initiator proteins of autophagy, such as AMBRA1 and WIPI1, was observed. This association seems thus to take place in the early phases of autophagy in which MAM have been hypothesized to play a key role (Garofalo et al., 2016).

In nitrogen starved yeast, the mitochondrial phosphatidylserine decarboxylase Psd1, leading to the synthesis of PE from PS, is required for the mitophagy process (Vigié et al., 2018). As highlighted above, the role of MAM acting as bridges between mitochondria and ER is crucial for this transfer.

### ***MAM components and autophagy/mitophagy***

In yeast, mutants lacking ERMES subunits,  $\Delta mdm10$ ,  $\Delta mdm12$ ,  $\Delta mdm34$ , and  $\Delta mmm1$ , showed severe mitophagy defects but are not affected in bulk autophagy. ERMES contributes to mitophagy at the step of isolation membrane formation (Böcker and Westermann, 2014). Moreover, Mdm12 and Mdm34 co-localize with two actors of mitophagy, Atg11 and Atg32, during its induction (Mao et al., 2013). Recently, it was shown that Mdm34 and Mdm12, are ubiquitinated by the E3 ligase Rsp5. This ubiquitination did not affect mitochondrial dynamics nor ERMES turnover, but was required for efficient mitophagy (Belgareh-Touze et al., 2017).

IP3R blockade triggered the autophagy of both ER and mitochondria, as exactly observed in starvation-induced autophagy. The IP3R is known to reside in the membranes of the endoplasmic reticulum (ER) as well as within ER-mitochondrial contact sites. The autophagic pathway triggered by IP3R inhibition follows a canonical pathway because it requires the obligate contribution of Beclin-1, Atg5, Atg10, Atg12 and hVps34 (Criollo et al., 2007; Cardenas et al., 2010).

The relocalization of the tumor suppressor promyelocytic leukemia protein (PML) at MAM negatively controlled autophagosome formation and, thus, autophagy induction (Missiroli et al., 2016).

It has also been shown that the integral ER protein vesicle-associated membrane protein-associated protein B (VAPB) binds to the OMM protein, protein tyrosine phosphatase interacting protein 51 (PTPIP51) to participate to MAM formation (Stoica et al., 2014). Modulating expression of VAPB or PTPIP51 affects  $Ca^{2+}$  exchange between the two organelles. Then, the manipulation of VAPB and PTPIP51 expression (that modulated ER-mitochondria association) impacted both basal and induced autophagy (Gomez-Suaga et al., 2017). Moreover, this study showed that the impact of modulation of ER-mitochondria tethering is dependent on how autophagy is induced by rapamycin or by starvation. There are, however, some differences between these various studies. Notably, loosening ER-mitochondria contacts induces autophagosome formation, whereas some others report that such loosening is inhibitory to their formation (Hailey et al., 2010 ; Hamasaki et al., 2013 ; Garofalo et al., 2016). The reasons for these dissimilar results may involve different experimental approaches.

Phosphofurin acidic cluster sorting 2 (PACS2) regulates ER-Mitochondria communications, ER apoptosis and homeostasis. It was demonstrated that the successful autophagy of dysfunctional mitochondria stimulates VSMCs (Vascular smooth muscle cells) survival against pro-apoptotic oxidized lipoproteins (Swiader et al., 2016) whereas defective autophagy promotes VSMC apoptosis and unstable atherosclerotic plaque phenotype . Then, by using high-resolution fluorescence methods (STED confocal microscopy and proximity ligation assay), Moulis et al. (2019) showed an increase in mitochondria-ER contacts in stressed VSMCs, and the involvement of PACS-2 to establish those organelle contacts. Indeed, the disruption of ER-mitochondrial contacts by knocking down PACS-2 impairs mitophagosome formation and favors oxidized lipoproteins-induced cell apoptosis. These data support the hypothesis that MAM integrity is a prerequisite for mitochondria autophagy in mammalian cells.

### ***Localization/Relocalization of autophagy/mitophagy actors on MAM***

Some actors of the core autophagic machinery are found in MAMs, such as Atg14, Atg8, Atg5 or WIPI. In addition, some mitochondrial proteins involved in mitophagy, located in mitochondria, have also been found in MAM. It has been shown that PARKIN could be recruited by Beclin1 in the cytosol and translocated to damaged mitochondria (Choubey *et al.*, 2014). Beclin1 is a key component of the class III phosphatidylinositol 3-kinase (PtdIns3K) complex (including Beclin, ATG14, PIK3C3/Vps34 and PIK3R4/Vps15 (Funderburk *et al.*, 2010)), that is required for the initiation of autophagosome formation and relocates to MAM upon starvation-induced autophagy (Hamasaki *et al.*, 2013). Also, after mitophagy induction, both Beclin1 and PINK1 relocate at ER-mitochondria contact sites, promoting ER-mitochondria tethering and autophagosome formation (Gelmetti *et al.*, 2017). CCCP treatment also increased ER-mitochondria tethering, that was abolished in either Beclin1 or PINK1 is silenced. PARKIN further translocated to mitochondria in a PINK1-dependent fashion, and was particularly enriched in MAM (Gelmetti *et al.*, 2017). This confirmed previous observations that PARK2 accumulates both at mitochondria and ER-mitochondria junctions in conditions of excitotoxicity (Van Laar *et al.*, 2015). The finding that PINK1, PARK2 and BECN1 all localize at MAM after CCCP treatment had never been observed previously. However, while PARK2 and BECN1 are dispensable for each other translocation at MAM, both proteins require PINK1.

FUNDC1 is a mitochondrial receptor that is involved in hypoxia induced mitophagy. In hypoxic cells, it is also accumulated at ER-mitochondria contact sites by interacting with the ER membrane protein calnexin in hypoxic cells (Wu *et al.*, 2016). FUNDC1 is specifically required for mitochondrial fragmentation by recruiting DRP1 to drive mitochondrial fission in response to hypoxic stress (Wu *et al.*, 2016). Moreover, MFN2, a protein required for mitochondria fusion and also participating in tethering between ER and mitochondria, could be a substrate for PINK1 (de Brito and Scorrano, 2008). All these data indicate that a close link exists between MAM and autophagy/mitophagy and that MAM play a role of platform for the formation of autophagosomes and for the course of autophagy and mitophagy in cell survival.

### **Concluding remarks**

This short overview highlights the dual nature of MAM, that fuels mitochondria with lipid and  $\text{Ca}^{2+}$ , but also initiates autophagy/mitophagy (that degrade mitochondria to sustain cell survival) and death pathways (that degrade cells to sustain organism survival). It is likely that these different pathways do not involve the same type of MAM. MAM containing lipid synthesis enzymes are probably not the

same MAM that contain IP3R or the Bcl-2 family members. However, some of the processes that depend on MAM are likely connected. For example, PS synthesis leading to PE generation and PE-dependent autophagosome formation most probably occur in the same membranes. Another example could be the synergic effect of ceramide and Bcl-2 family members leading to OMM permeabilization during the early steps of apoptosis. Consequently, each process involving a communication between ER and mitochondria may rely on a specific type of MAM, that is specific in terms of biochemical composition, but might also be specific in terms of morphology of contacts (Giacomello and Pellegrini, 2016). A recent original study allowed to identify new MAM components that had not been found before (Kwak et al., 2020). The actual function of these components in ER-mitochondria contacts and communication remains a vast domain to explore.

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