Molecules and Cells

Minireview

The Interface Between ER and Mitochondria: Molecular Compositions and Functions

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Mitochondria and endoplasmic reticulum (ER) are essential organelles in eukaryotic cells, which play key roles in various biological pathways. Mitochondria are responsible for ATP production, maintenance of Ca^{2+} homeostasis and regulation of apoptosis, while ER is involved in protein folding, lipid metabolism as well as Ca^{2+} homeostasis. These organelles have their own functions, but they also communicate via mitochondrial-associated ER membrane (MAM) to provide another level of regulations in energy production, lipid process, Ca^{2+} buffering, and apoptosis. Hence, defects in MAM alter cell survival and death. Here, we review components forming the molecular junctions of MAM and how MAM regulates cellular functions. Furthermore, we discuss the effects of impaired ER-mitochondrial communication in various neurodegenerative diseases.

Keywords: ER-mitochondria tethering, mitochondrial-associated ER membrane (MAM), neurodegenerative disease

In contrast to prokaryotes, eukaryotic cells contain small organelles that have distinct biological roles. Furthermore, compartmentalization of each organelle allows spatiotemporal regulation of various functions within a cell, and these functions are highly inter-dependent among different organelles. Communications among the organelles occur through direct contacts employing membranes and membrane proteins of different organelles. Membrane contacts between organelles were first discovered by using electron microscopy (Bernhard, 1956; Copeland, 1959). Since then, various specific protein complexes providing platforms for the contacts have been discovered by using biochemical and microscopic methods (Csordas et al., 2010; Harmon et al., 2017). Membrane contacts have been reported to occur between ER and plasma membrane (PM), ER and endosomes, ER and Golgi, as well as ER and mitochondria (Eisenberg-Bord et al., 2016; Prinz, 2014; Rowland et al., 2014; Wu et al., 2018). ER-PM modulates Ca^{2+} homeostasis and lipid exchange, and is mediated by proteins called Orai, stromal interaction molecule 1 (STIM1), and oxysterol-binding protein (ORP) (Chung et al., 2015; Liou et al., 2007). ER-endosome tethering regulates endosomal fission and transport through VAPB, Rab and ORP (De Vos et al., 2012; Raiborg et al., 2015; Rowland et al., 2014). ER-Golgi contact coordinates post-translational modification and protein transport. Also, lipid transfer between ER and Golgi is carried out by VAPB, ORP, ceramide transfer protein (CERT) and NIR2 (Mesmin et al., 2013; Murphy and Levine, 2016; Peretti et al., 2008). ER-mitochondria tethering is important in Ca^{2+} buffering and lipid traffic that regulates lipid processing (de Brito and Scorrano, 2010; Hayashi et al., 2009; Rowland and Voeltz, 2012; Vance, 2014). ER-mitochondria tethering is formed by numerous proteins such as mitofusin (MFN), inositol triphosphate receptor (IP_3R), voltage-dependent anion channel (VDAC), glucose-regulated protein 75 (Grp75), mitochondrial fission 1 protein (Fis1), B cell receptor associated protein 31 (BAP31), protein tyrosine phosphatase interacting

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protein 51 (PTPIP51) and vesicle-associated membrane protein-associated protein B (VAPB) (Iwasawa et al., 2011; Merkwirth and Langer, 2008; Szabadkai et al., 2006).

Here, we focus our discussion on the molecular compositions of ER-mitochondria tethering, and its role in Ca^{2+} maintenance and cell survival. Additionally, we will assess how defects in ER-mitochondria tethering affect neurodegenerative disease.

MOLECULAR COMPOSITIONS OF ER-MITOCHONDRIA TETHERING

The interface between ER and mitochondria is called mitochondrial-associated ER membrane (MAM) (Fig. 1 and Table 1). Numerous studies found different molecules in MAM and have attempted to investigate biological roles of MAM, but it still is not yet fully understood. In yeast cells, a structure of protein complexes that connects ER and mitochondria called the ER-mitochondria encounter structure (ERMES), has been reported to contain Mdm12, Mdm34, Mdm10 and Mmm1 proteins (Kornmann et al., 2009). Mdm12 is a linker protein connecting ER membrane protein Mmm1 to mitochondrial outer membrane proteins, Mdm34 or Mdm10. This physical tethering establishes ERMES, which then allow efficient lipid transport by soluble lipid-carrier proteins such as CERT and OSBP (D'Angelo et al., 2008). Mutant proteins disrupting ERMES cause defects in phospholipid exchange between ER and mitochondria, resulting in impaired cellular growth or organelle recycling (Kornmann et al., 2009).

Mammalian cells have more complicated protein complexes in the ER-mitochondria interface. Proteins in MAM either play a direct role in physical connection between ER and mitochondria or modulates the tethering complexes in MAM. MFN1 and 2 (MFN1/2), a mitochondrial fusion GTPase, localized to the outer membrane of mitochondria is found in the MAM complex. MFN1/2 plays a role in mitochondrial fusion together with OPA1, another mitochondrial fusion GTPase, located on the inner membrane of mitochondria (Cipolat et al., 2004). During mitochondrial fusion process, mitochondrial MFN1/2 assembles homo- or heterodimer complexes with MFN2 presented in ER membrane (de Brito and Scorrano, 2008; Detmer and Chan, 2007). Fis1 and BAP31 interaction is also found in MAM (Iwasawa et al., 2011). Fis1 located on the mitochondrial outer membrane recruits dynamin related protein 1 (DRP1) to mitochondrial fission sites (Stojanovski et al., 2004). BAP31 is a chaperone located on the ER membrane, which regulates degradation of misfolded protein and apoptotic pathway (Nguyen et al., 2000; Wakana et al., 2008). When Fis1 binds to BAP31 in MAM, apoptotic signals is conveyed to ER, initiating apoptotic pathway (Iwasawa et al., 2011). Another interaction at the interface of ER and mitochondria occurs between PTPIP51 and VAPB (De Vos et al., 2012). While PTPIP51 is a mitochondrial outer membrane protein that modulates cellular development and tumorigenesis (Yu et al., 2008); VAPB is an ER membrane protein involved in vesicle trafficking and unfolded protein response (Kanekura et al., 2006; Nishimura et al., 2004). However, VABP and PTPIP51 complex in MAM acts on different pathways such as Ca^{2+} regulation and autophagy (De Vos et al., 2012; Gomez-Suaga et al., 2017). PTPIP51 also connects to other mitochondrial proteins, ORP5 and ORP8 found in MAM. It is reported that mutations in the *orp* genes induce defective mitochondrial morphology and impaired respiratory chain system (Galmes et al., 2016), however, roles of PTPIP51-ORP in MAM are not clear. Another important molecular complex in MAM is IP_3R-Grp75-VDAC interaction. IP_3R is an inositol triphosphate-dependent calcium channel located on ER membrane, controlling Ca^{2+} efflux from ER to cytosol. IP_3R plays a role in cellular differentiation, survival, and apoptosis (Joseph and Hajnoczky, 2007; Mikoshiba, 2007). VDAC is a mitochondrial outer membrane protein, which regulates Ca^{2+} influx to mitochondria together with mitochondrial calcium uniporter (MCU) (Hoppe, 2010). VDAC also modulates ATP release to cytosol and controls an apoptotic pathway (Colombini, 2012; Rostovtseva et al., 2005). Interestingly, IP_3R and VDAC in MAM does not directly interact but require a linker protein, cytoplasmic Grp75 (Szabadkai et al., 2006). Grp75 is known as a chaperone protein and mostly located in the mitochondrial matrix, but a low level of Grp75 is found in the cytoplasm

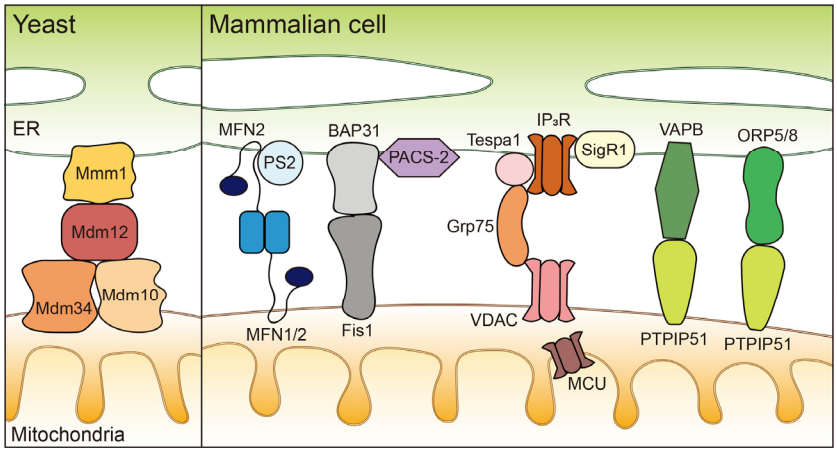

Fig 1. Composition of ER-mitochondria interface. Yeast-specific ERMES is composed by a multiprotein complex including ER protein Mmm1, cytosolic protein Mdm12, as well as mitochondrial protein Mdm34 and Mdm10. In mammalian cells, the interface between ER and mitochondria contains MFN2-MFN1/2, BAP31-Fis1, IP3R-Grp75-VDAC, and PTPIP51-VAPB or -ORP5/8 tethering complexes, which makes the two organelles close juxtaposition. Other single proteins, such as PS2, PACS-2, Tespa1, SigR1 and MCU, are also associated in the ER-mitochondria tethering complexes.

Table 1. List of protein components involved in MAM

Species	Protein components	Biological roles	Possible related diseases	Reference
Yeast	Mmm1-Mdm12-Mdm34-Mdm10	Efficient phospholipid exchange		Kornmann et al., 2009
Mammalian	IP_3R-Grp75-VDAC1	Ca^{2+} regulation	PD	Szabadkai et al., 2006; Davison et al., 2009
	BAP31-Fis1	Initiation of apoptosis		Iwasawa et al., 2011
	VAPB-PTPIP51	Ca^{2+} regulation and autophagy	ALS	De Vos et al., 2012; Gomez-Suaga et al., 2017; Nishimura et al., 2004; De Vos et al., 2012
	ORP5/8-PTPIP51	Lipid transfer		Galmes et al., 2016
	MFN2-MFN1/2	Physical tethering	PD	de Brito and Scorrano, 2008; McLelland et al., 2018
	PACS-2	Regulation of apoptosis	AD	Simmen et al., 2005; Hedskog et al., 2013
	SigR1	Ca^{2+} regulation by interacting with Ankyrin and BiP	AD, ALS	Su et al., 2016; Wu and Bowen, 2008; Hedskog et al., 2013; Al-Saif et al., 2011; Bernard-Marissal et al., 2015
	Tespa1	Ca^{2+} regulation by interacting with IP_3R and Grp75		Matsuzaki et al., 2013
	FUNDC1	Regulation of mitochondrial fission and mitophagy in hypoxia condition		Wu et al., 2016
	PS	Ca^{2+} regulation by interacting with MFN2	AD	Filadi et al., 2016; Zampese et al., 2011; Area-Gomez et al., 2012; Cheung et al., 2008

plasm and nucleus (Wadhwa et al., 2002). Cytoplasmic Grp75 linking IP_3R and VDAC enables close juxtaposition between ER and mitochondria to regulate Ca^{2+} transfer from ER to mitochondria (Szabadkai et al., 2006). Several studies report this Ca^{2+} delivery to mitochondria requires MCU, and this complex is called IP_3R-Grp75-VDAC-MCU calcium regulation axis (Rizzuto et al., 2009; Xu et al., 2018). Since Ca^{2+} is a key regulator involved in various biological functions, modulation of the IP_3R-Grp75-VDAC-MCU complex likely plays important roles in diverse cellular functions.

Several proteins are reported to mediate ER-mitochondria communications by interacting with the ER-mitochondria tethering protein complexes. Phosphofurin acidic cluster sorting 2 protein (PACS-2) is a multifunctional cytoplasmic protein, which controls ER quality and induces apoptosis (Myhill et al., 2008; Simmen et al., 2005). Whether PACS-2 directly attaches to MAM is not clear, however, depletion of PACS-2 causes reduction of ER-mitochondria contact and generation of mitochondrial fragmentation (Simmen et al., 2005), suggesting that PACS-2 modulates ER-mitochondria contacts. Sigma non-opioid intracellular receptor 1 (SigR1) and Tespa1 associate with the IP_3R-Grp75-VDAC-MCU calcium axis in MAM. Overexpression of SigR1 increases Ca^{2+} flux from the ER by interacting with Ankyrin and ER chaperone protein, BiP (Su et al., 2016; Wu and Bowen, 2008). Tespa1 binds to both IP_3R and Grp75, and Tespa1 knockdown decreases the levels of mitochondrial and cytoplasmic Ca^{2+} (Matsuzaki et al., 2013), however its mechanism is not known. FUN14 domain containing 1 (FUNDC1) is another protein that modulates MAM dynamics. FUNDC1 interacts with Calnexin, a ER chaperone protein, and this binding competes with FUNDC1's binding to Drp1 during early hypoxia. In later hypoxia condition FUNDC1 dissociates from Calnexin and instead interacts with Drp1, which then induces mitochondrial fission and mitophagy (Wu et al., 2016). Presenilin (PS) is a multifunctional protein involved in amyloid beta (Aβ) production pathway, and it is known that PS mutants cause familial Alzheimer's disease (AD) (De Strooper, 2007). Interestingly, PS affects Ca^{2+} dynamics in MAM by interacting with MFN2. Thus, mutations in the *ps2* gene interferes with Ca^{2+} delivery to mitochondria (Filadi et al., 2016; Zampese et al., 2011).

Physical interactions linking ER and mitochondria play roles not only in Ca^{2+} homeostasis and apoptosis, but in lipid transferring between the two organelles. Lipid synthesis is performed mostly in the ER but still requires cooperation of enzymes on the mitochondrial membrane, because ER and mitochondria have distinct lipid processing enzymes. For example, newly synthesized phosphatidylserine (PS) in ER is transferred to the mitochondrial inner membrane, where it converts to phosphatidylethanolamine (PE) by PS decarboxylase (PSD). PE then transports back to the ER membrane through MAM contact (Vance, 2014). While various studies have discovered molecules involved in the MAM structure and functions, a comprehensive understanding of the complex MAM system is still lacking.

MAM REGULATES Ca^{2+} HOMEOSTASIS

ER is the major site of Ca^{2+} storage within a cell, and IP_3R on

ER is highly accumulated in MAM (Marchi and Pinton, 2014; Patergnani et al., 2011). Numerous forms of interactions between Ca^{2+} channels and regulators are found in MAM, which regulate Ca^{2+}-dependent cellular functions as well as maintain Ca^{2+} homeostasis. Furthermore, elevated Ca^{2+} level in MAM activates Ca^{2+} influx to mitochondria through the IP_3R-Grp75-VDAC-MCU complex. When the linker protein Grp75 is reduced, mitochondrial Ca^{2+} level is decreased, suggesting that Grp75 connects ER and mitochondria indirectly by interacting with both IP_3R and VDAC. The resulting apposition of ER and mitochondria facilitates Ca^{2+} transfer from ER to mitochondria. Indeed, Grp75 knockdown prevents cell death due to excess Ca^{2+} in mitochondria (Honrath et al., 2017).

Proteins associated with the IP_3R-Grp75-VDAC-MCU complex can modulate Ca^{2+} transfer between ER and mitochondria. SigR1 interacts with BiP in normal condition. However, when ER is under stress or when ER Ca^{2+} is depleted, SigR1 switches its interacting partner from BiP to IP_3R. This process protects IP_3R from degradation, resulting in restoration of Ca^{2+} transfer from the ER to mitochondria (Hayashi and Su, 2007). Tespa-1 binds to both Grp75 and IP_3R in T-cells. Knockout of Tespa-1 impairs Ca^{2+} flux to both cytosol and mitochondria, which causes decreased Ca^{2+} signaling and ERK activation (Liang et al., 2017; Matsuzaki et al., 2012), suggesting that the Tespa-1-Grp75-IP_3R complex regulates Ca^{2+} efflux from ER to cytosol or mitochondria. Other physical tethering complexes in MAM that facilitate efficient Ca^{2+} transfer between ER and mitochondria are the VAPB-PTPIP51 complex and the MFN complex. For example, genetic modification of the *vapb* gene disturbs Ca^{2+} transfer from from ER to mitochondrial in neuronal cells (De Vos et al., 2012). Mitochondria without MFN2 also decrease Ca^{2+} uptake upon IP_3R activation (de Brito and Scorrano, 2008).

Since energy production and cell death can be triggered by different levels of Ca^{2+}, MAM plays a key role in delicate refinement of Ca^{2+} level in mitochondria. Upregulation of mitochondrial Ca^{2+} in physiological condition activates mitochondrial enzymes, which facilitates TCA cycle and oxidative phosphorylation. Activities of α-ketoglutarate dehydrogenase, isocitrate dehydrogenase and pyruvate dehydrogenase are Ca^{2+}- dependent enzymes (McCormack and Denton, 1993). ATP synthase is also Ca^{2+}-dependent enzyme (Das and Harris, 1990). Thus, increased Ca^{2+} level in mitochondria enhances electron activity, resulting in elevated generation of ATP (Hansford and Zorov, 1998). In contrast, prolonged or excessive mitochondrial Ca^{2+} level activates apoptotic pathway. Increased Ca^{2+} flux from ER to mitochondria initiates oligomerization of Bcl-2-associated X protein (BAX), which translocates to mitochondrial membrane and increases permeability of mitochondrial membrane (Rostovtseva et al., 2005). Furthermore, mitochondrial permeability transition pore (PTP) is induced by high level of Ca^{2+} (Haworth and Hunter, 1979). PTP also increases mitochondrial membrane permeability, leading to apoptosis by releasing cytochrome c, apoptosis-inducing factor (AIF), and Smac/DIABLO (Petronilli et al., 2001). Cytochrome c and AIF initiates apoptosis through caspase cascade pathway, but Smac/DIABLO triggers cell death independently from the caspase cascade (Kroemer et al., 2007). Furthermore, MAM proteins such as PACS-2, Bid, Fis1, and Bap31 are involved in apoptosis. PACS-2 initiates apoptosis by recruiting Bid into mitochondrial membrane upon activation of cell death signals. To activate Bid, Fis1 cleaves Bap31 into p20Bap31, pro-apoptotic molecule, followed by p20Bap31 converting pro-caspase-8 to caspase-8 (Iwasawa et al., 2011). Caspase-8 then activates Bid, allowing releasing cytochrome c from mitochondria, which then forms apoptosome with caspase-3, 7 and 9 (Simmen et al., 2005).

MISCOMMUNICATION OF ER-MITOCHONDRIA IN NEURODEGENERATIVE DISEASE

Impaired ER-mitochondrial communications may lead to metabolic diseases, cancers, and neurodegenerative diseases. Numerous studies have observed structural or functional changes in MAM in neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS). AD is a neurodegenerative disease characterized by progressive loss of cognitive functions. AD patient brains contain accumulation of amyloid plaque composed of primarily of Aβ and neurofibrillary tangles containing mostly hyperphosphorylated Tau. PS is a subunit protein of γ-secretase, involved in the processing of amyloid precursor protein (APP). Several mutations in *ps1*, *ps2* or *app* genes are observed in early onset familiar AD, and the mutated proteins cause mis-processing of APP, leading to Aβ plaque formation (O'Brien and Wong, 2011). It is interesting to note that PS and APP are highly localized in MAM, and γ-secretase activity is increased in the MAM fraction (Area-Gomez et al., 2009). Furthermore, PS and APP mutant display enhanced ER-mitochondria contact, thereby accelerating Ca^{2+} and phospholipids/cholesteryl esters transfer from the ER to mitochondria (Area-Gomez et al., 2012; Cheung et al., 2008). Other MAM proteins such as PS synthase, PACS-2, and SigR1 are also highly upregulated in AD patients and animal AD models (Hedskog et al., 2013; Stone and Vance, 2000). These findings suggest that MAM may play a key role in generating AD pathogenesis. It is plausible that increased mitochondrial Ca^{2+} may cause cell death in AD neurons, however exact functions of MAM in generating AD pathology remains unclear.

PD is a neurodegenerative disease that causes tremor and a progressive loss of movement, which are associated with degeneration of dopaminergic neurons in substantia nigra in the brain. Dopaminergic neurons in PD contain Lewy bodies, composed of mostly aggregation of α-synuclein (α-syn) (Maries et al., 2003). The α-syn protein is also enriched in MAM (Guardia-Laguarta et al., 2014). Mutations are found in the *α-syn* genes among familial PD patients, and these mutant α-syn decrease ER-mitochondria communication and induce mitochondrial fragmentation (Cali et al., 2012). Mutations found in *parkin*, *dj-1*, and *pink1* genes are also related to PD. Neuronal excitotoxicity increases Parkin, which translocates to MAM (Van Laar et al., 2015), where Parkin can ubiquitinate MFN2 and disrupt ER-mitochondria tethering (McLelland et al., 2018). Furthermore, under the stress conditions, PINK1 recruits Parkin to the outer mitochondria

membrane, which activates mitophagy (Matsuda et al., 2010). Another example is DJ-1 that is highly concentrated in MAM. Overexpression of DJ-1 increases ER-mitochondria tethering (Ottolini et al., 2013). Interestingly, these three PD related proteins, Parkin, Pink, and DJ-1, can interact with Grp75 (Davison et al., 2009). Together, these results suggest that ER-mitochondria tethering can play key roles in generating PD pathology. However, how and why these mutant proteins underlying PD pathology show opposite effects on the strength of the ER-mitochondria communication is not clear. Additional studies are required to provide a comprehensive view of how ER-mitochondria tethering affects PD.

ALS is a neurodegenerative disease caused by loss of motor neurons, resulting in gradual deterioration of muscles. Although SOD1 and other candidate genes are reported to associate with familial ALS, the exact cause of ALS is still not clear. However, a mutation in *sigr1* is discovered in a juvenile form of ALS (Al-Saif et al., 2011). Moreover, SigR1 knockout mouse exhibits ALS phenotypes such as muscle weakness and motor neuron loss. Loss of SigR1 reduces ER-mitochondria tethering, which disrupts Ca^{2+} homeostasis in mitochondria and alters mitochondrial dynamics (Bernard-Marissal et al., 2015). Another MAM protein, VAPB is also mutated in familiar ALS (Nishimura et al., 2004). A mutant VAPB increases its affinity to PTPIP51 and strengthens VAPB-PTPIP51 tethering, which alters Ca^{2+} shuttling between ER and mitochondria (De Vos et al., 2012). Mutations in the *tdp-43* genes are also found in familial ALS, and mutated TDP-43s have higher affinity for FUS (Stoica et al., 2014). Interestingly, TDP-43 and FUS interaction activates GSK3β, which disrupts VAPB-PTPIP51 and weakens ER-mitochondria tethering. Altogether, these results indicate that miscommunications between ER-mitochondria regardless of weakened or enhanced ER-mitochondria tethering plays a key role in various neurodegenerative diseases. It is tempting to speculate that strong ER-mitochondria tethering generates excessive Ca^{2+} influx into mitochondria, thereby activating apoptotic pathways. On the other hand, weak tethering fails to deliver the optimum amount of Ca^{2+} required to activate mitochondrial enzymes for ATP production. Thus, both strong or weak ER-tethering consequently affect cell survival, which is manifested in neuronal cell death in the disease. However, whether dysfunctional MAM is a cause or consequence of these neurodegenerative diseases remains to be elucidated.

CONCLUSION

In this review, we discuss the molecular compositions and functions of ER-mitochondria interface. It is now clear that various molecules in the ER-mitochondria tethering complex are important for Ca^{2+} or lipid homeostasis, and for cell survival and apoptotic regulation. Moreover, several proteins disrupting MAM structure or functions have been identified in neurodegenerative diseases such as AD, PD, and ALS. While numerous molecules have been found in MAM, new molecules that affect MAM are still being identified. This implies that new MAM functions are waiting to be discovered in different cellular environments, cell types, and disease conditions. Furthermore, the underlying mechanism of how MAM is associated with neurological disorders is not fully understood. Thus, future studies will require considerable efforts to precisely delineate the structure and function of MAM. A better understanding of MAM may contribute to new strategies to treat and prevent neurodegenerative diseases in the future.

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