Cellular and Molecular Neurobiology (2022) 42:917–930
https://doi.org/10.1007/s10571-020-01008-9

REVIEW PAPER

Mitochondria-ER Tethering in Neurodegenerative Diseases

Reza Raeisossadati[1] · **Merari F. R. Ferrari**[1]

Received: 22 August 2020 / Accepted: 11 November 2020 / Published online: 16 November 2020

Abstract

Organelles juxtaposition has been detected for decades, although only recently gained importance due to a pivotal role in the regulation of cellular processes dependent on membrane contact sites. Endoplasmic reticulum (ER) and mitochondria interaction is a prime example of organelles contact sites. Mitochondria-associated membranes (MAM) are proposed to harbor ER-mitochondria tether complexes, mainly when these organelles are less than 30 nm apart. Dysfunctions of proteins located at the MAM are associated with neurodegenerative diseases such as Parkinson's, Alzheimer's and amyotrophic lateral sclerosis, as well as neurodevelopmental disorders; hence any malfunction in MAM can potentially trigger cell death. This review will focus on the role of ER-mitochondria contact sites, regarding calcium homeostasis, lipid metabolism, autophagy, morphology and dynamics of mitochondria, mainly in the context of neurodegenerative diseases. Approaches that have been employed so far to study organelles contact sites, as well as methods that were not used in neurosciences yet, but are promising and accurate ways to unveil the functions of MAM during neurodegeneration, is also discussed in the present review.

Keyword Mitochondria-associated membranes (MAM) · Contact sites methodologies · Autophagy · Lipid metabolism · Calcium · Neurodegeneration

Abbreviations

α-syn	Alpha-synuclein
Aβ	Amyloid-beta peptide
ACAT1	Cholesterol acyltransferase/sterol O-acyltransferase 1 (same as SOAT1)
AD	Alzheimer's disease
ALS	Amyotrophic lateral sclerosis
APEX	Ascorbate peroxidase
APOE4	Apolipoprotein E4
APP	Amyloid precursor protein
ATG2A	Autophagy-related protein 2 homolog A
ATG9A	Autophagy-related protein 9 homolog A
ATG14L	Autophagy-related 14-like
BAP31	B-cell receptor-associated protein 31
BECN1	Beclin-1
BiC	Bimolecular complementation
BioID	Proximity based biotin identification
BRET	Bioluminescence resonance energy transfer
CACNA1A	Calcium Voltage-Gated Channel Subunit Alpha1 A
CE	Cholesteryl-ester
CLEM	Correlative light and electron microscopy
CMT2A	Charcot–Marie–Tooth type 2A
Cryo-ET	Electron cryo-tomography
Cryo-FIB	Cryo-focused ion beam
ddFP	Dimerization-dependent fluorescent protein
DFCP1	Double FYVE domain-containing protein 1
DGAT2	Diacylglycerol O-acyltransferase 2
DRP1	Dynamin-Related Protein 1
EI24	Etoposide-induced protein 2.4
EM	Electron microscopy
ER	Endoplasmic reticulum
FACL4	Fatty acid CoA ligase 4 (same as ACS4)
FIB-SEM	Focused ion beam-scanning EM

✉ Reza Raeisossadati
raeisossadati@ib.usp.br

✉ Merari F. R. Ferrari
merari@usp.br

[1] Departamento de Genetica e Biologia Evolutiva, Instituto de Biociencias, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

FIP200	Focal adhesion kinase (FAK) family interacting protein of 200 kDa
FIS1	Mitochondrial fission 1 protein
FRET	Fluorescence resonance energy transfer
GRP75	Glucose regulated protein 75
GSK-3β	Glycogen synthase kinase-3 beta
hiPS	Human-induced pluripotent stem cell
IP3R	Inositol 1, 4, 5-trisphosphate receptor
KIF5C	Kinesin Family Member 5C
LB	Lewy bodies
LRRK2	Leucine-rich repeat kinase 2
MAM	Mitochondria-associated membranes
MEF	Mouse embryonic fibroblasts
MERCs	Mitochondria-ER contact sites
MFN1 and MFN2	Mitochondrial fusion GTPases mitofusins 1 and 2
MID49	Mitochondrial dynamics protein of 49 kDa
MIRO1 and 2	Mitochondrial Rho GTPase 1 and 2 (same as RHOT1 and 2)
MITOL	Mitochondrial ubiquitin ligase (same as MARCH5)
Mul1	Mitochondrial E3 ubiquitin protein ligase 1
ORP5/8	Oxysterol-binding protein related-protein 5 and 8
PC	Phosphatidylcholine
PD	Parkinson's disease
PDZD8	PDZ domain-containing protein 8
PE	Phosphatidylethanolamine
PEMT2	Phosphatidylethanolamine N-methyltransferase 2
PINK1	PTEN-induced kinase 1
PLA	Proximity ligation assay
PSEN 1 and 2	Presenilin 1 and 2
PSS 1 and 2	Phosphatidylserine synthases 1 and 2
PTPIP51	Protein tyrosine phosphatase interacting protein 51 (same as RMDN3)
ROS	Reactive oxygen species
SBF-SEM	Serial block-face scanning electron microscopy
SEM	Scanning electron microscopy
Sig-1R	Sigma-1 receptor
SNCA	Alpha-synuclein gene
TDP-43	TAR DNA binding protein 43
TEM	Transmission electron microscopy
TRAK	Trafficking kinesin protein (same as Milton)
ULK1	Unc-51 like autophagy activating kinase
VAPB	Vesicle-associated membrane protein (VAMP)-associated protein B
VDAC	Voltage-dependent anion-selective channel protein 1
VPS13A	Vacuolar Protein Sorting 13 Homolog A

Introduction

Membrane-bound organelles are a distinctive feature of eukaryotic cells; proper compartmentalization enables cells to process incompatible biological reactions simultaneously. Organelles can communicate through vesicles as well as cellular structures by which surfaces of two different organelles are closely apposed (10–30 nm). Organelles contact was first described between the endoplasmic reticulum (ER) and mitochondria (Bernhard et al. 1952; Bernhard and Rouiller 1956); but several other contact sites have been studied in the past decades (Cohen et al. 2018).

Interaction between the ER and mitochondria occurs through a specialized region known as mitochondria-associated membranes (MAM), which is also called mitochondria-ER contact sites (MERCs). It was thought that the physiological role of MAM was only limited to lipid, calcium, ions and proteins transfer between these two organelles; however, recent studies shed light on the importance of contact sites in many other different biological processes, such as inflammasome formation, autophagy, ER stress and mitochondria morphology (Friedman et al. 2011; Paillusson et al. 2016).

In mammalian cells, lipid and protein complexes control the machinery underlying the contact sites. Proteins located on the ER surface, such as mitochondrial fusion GTPase mitofusin 1 and 2 (MFN2), BAP31, IP3R, VAPB and ORP5/8, interact with their counterparts on the outer mitochondria membrane, like MFN1 and 2, FIS1, VDAC, and PTPIP51 (Lee and Min 2018).

Malfunction in the mitochondria-ER communication can cause metabolic and neurodegenerative diseases (Stoica et al. 2014). Several studies have documented the structural and functional role of MAM in neurodegeneration (Area-Gomez et al. 2018; Rodriguez-Arribas et al. 2017; Stoica et al. 2014; Tambini et al. 2016).

Mitochondria-ER Contact Sites

Mitochondria-ER contact sites have pivotal roles in a variety of cellular functions, for this reason it is reasonable to expect that its dysfunction is associated to several neuropathologies.

MAM and Ca^{2+} Storage: A Crosstalk Between ER and Mitochondria

ER and mitochondria have been considered the major Ca^{2+} storage sites within the cell. Efflux of Ca^{2+} from ER via inositol 1,4,5- triphosphate receptors (IP3R) stimulates its

uptake by mitochondria via VDAC channels (Rowland and Voeltz 2012). Fine-tuning of intracellular Ca^{2+} concentration is vital for proper mitochondrial trafficking and ATP generation. Nonetheless, uncontrolled uptake of Ca^{2+} leads to increased mitochondrial permeability and signaling for apoptosis (Paillusson et al. 2016; van Vliet et al. 2014).

Several studies have shown the importance of MAM in Ca^{2+} shuttling for neurodegenerative diseases as depicted in Fig. 1.

In Alzheimer's disease (AD), which is defined by progressive neuronal loss in the hippocampus and cortex, with extracellular deposits of amyloid-beta (Aβ) plaques, produced by the unusual processing of amyloid precursor protein (APP); and intracellular accumulation of neurofibrillary

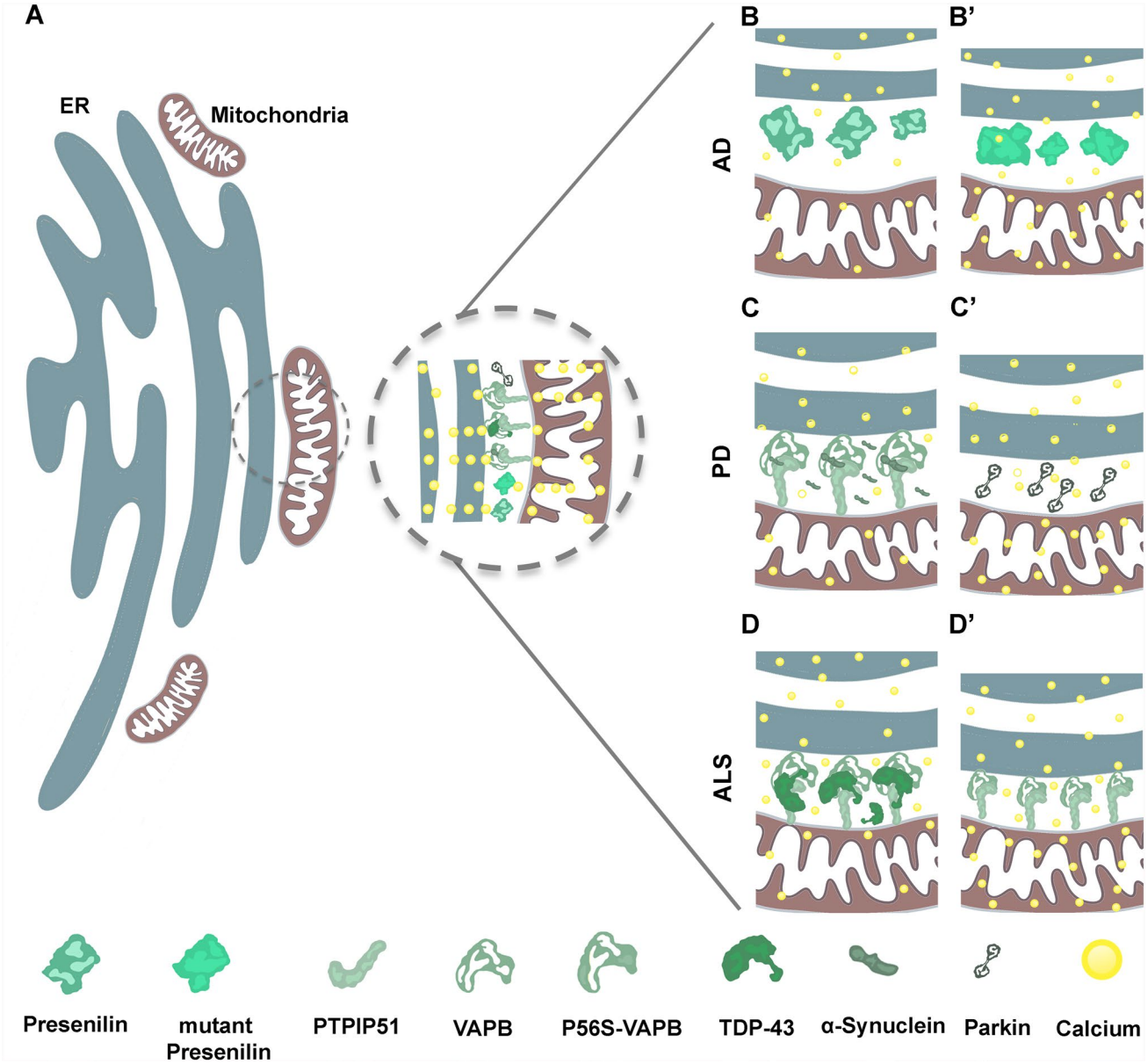

Fig. 1 **a** ER-Mitochondria contact sites illustration and mitochondria-associated membranes (MAM) influence on Ca^{2+} homeostasis in neurodegenerative disease. **b–b'** In Alzheimer's disease (AD) mutation in Presenilin (PSEN1) located at the MAMs tightens contact sites and increases Ca^{2+} flux into the mitochondria. **c–c'** In Parkinson's disease, α-syn interacts with VAPB-PTPIP51, disturbing this tethering complex and decreasing MAM and Ca^{2+} shuttling into the mitochondria. Parkin overexpression increases the contact sites and elevates the Ca^{2+} flux into the mitochondria. **d–d'** In ALS, TDP-43 reduces the binding of VAPB-PTPIP51; therefore, reducing Ca^{2+} shuttling into mitochondria. Mutation in VAPB (P56S) leads to a higher affinity to other tethering counterpart of PTPIP5 that triggers Ca^{2+} transfer to mitochondria

tangle mainly formed by hyperphosphorylated microtubule-associated protein tau (Goedert and Spillantini 2006), components of the γ-secretase Presenilin 1 (PSEN1) and Presenilin 2 (PSEN2) are present in the MAM (Zampese et al. 2011). Catalytic loss-of-function mutations in these genes have been linked to modulation of the ER-mitochondria contact sites and mitochondrial Ca^{2+} uptake (Area-Gomez et al. 2018; Area-Gomez et al. 2012; Sepulveda-Falla et al. 2014). Presenilins mutation change ER-mitochondria dynamics contributing to amyloidogenic processing of APP as well as Ca^{2+} imbalance between the two organelles (Area-Gomez et al. 2012; Sepulveda-Falla et al. 2014). It is noteworthy to remember that mutations in APP, *PSEN1 or PSEN2* are associated with familial AD, although other genes have been considered risk or protective factors for AD (Dorszewska et al. 2016), and the involvement of MAM for the AD must be taken into acount.

In mouse embryonic fibroblast (MEF) lacking PSEN1 and in fibroblasts from AD patients the number of ER-mitochondria contact sites is increased (Fig. 1b, b'); it was proposed that the Presenilin might affect the same pathway that MFN2 does. A point mutation in PSEN1 (E280A) resulted in decrease of ER-mitochondria tethering, down-regulation of the Ca^{2+} channels IP3Rs and CACNA1A, and reduction of the Ca^{2+}-dependent mitochondrial transport proteins MIRO1 and KIF5C (Sepulveda-Falla et al. 2014).

PSEN2 is also associated with ER-mitochondria contact sites. In SH-SY5Y cells models, overexpression and down-regulation of PSEN2 resulted in increase and decrease of the contact sites, respectively, which is directly related to the capacity of mitochondria to uptake Ca^{2+} (Zampese et al. 2011). It is proposed that the number and distance of contact sites are not associated with the enzymatic activity of PSEN2 itself, but the ability of maintaining together the two membranes (Zampese et al. 2011).

In Parkinson's disease (PD), the second most prevailing neurodegenerative disease after Alzheimer's disease, α-syn, Parkin, PINK1, *VPS35,* DJ-1, and LRRK2 proteins can alter MAM and influence Ca^{2+} balance, all these proteins are encoded by genes associated to dominant or recessive-inherited PD (Gomez-Suaga et al. 2018). PD symptoms appear as a result of the dopaminergic neurons loss in substantia nigra pars compacta, and intraneuronal proteinaceous inclusions called Lewy bodies (LB), which are composed mainly of α-syn (Kalia and Lang 2015).

It has been documented that α-syn interacts with vesicle-associated membrane protein (VAMP)—associated protein B (VAPB) in ER, thus disturbing the contact between VAPB and tyrosine phosphatase–interacting protein 51 (PTPIP51). Therefore an abnormal increase in α-syn levels can alter the number of contact sites and disrupt the Ca^{2+} transfer to mitochondria (Fig. 1c, c') (Guardia-Laguarta et al. 2014; Paillusson et al. 2017; Vicario et al. 2018).

In the SH-SY5Y cell model, Parkin overexpression resulted in the maintenance of ER-mitochondria tethering and Ca^{2+} homeostasis, while Parkin knockdown leads to mitochondrial fragmentation, dysfunction in mitochondrial Ca^{2+} handling, and reduced the ER–mitochondria contact sites (Cali et al. 2013).

Consistent with the previous study it has been shown that Parkin knockdown in the S2R + Drosophila cell line, MEF, and also human Parkin mutant fibroblasts, impaired tethering between ER and mitochondria (Basso et al. 2018). The absence of Parkin resulted in decreased ubiquitylation of MFN2 ending up with impaired tethering between these organelles (Basso et al. 2018).

In HeLa cells, DJ-1 down-regulation can alter the Ca^{2+} transferring from ER to mitochondria, which is associated to changes in mitochondria morphology that influence the contact sites (Ottolini et al. 2013). It has been shown that DJ-1 in the M17 cell lines physically interacts with the tethering complexes IP3R-GRP75-VDAC (Liu et al. 2019). *DJ-1* knockout resulted in reduced ER-mitochondria association, increased levels of IP3R type 3 and its aggregation that end up to MAM dysfunctions (Liu et al. 2019). In the brain of DJ-1 knockout mice, similar deficits were confirmed; furthermore, in the substantia nigra of sporadic PD, diminishing ER-mitochondria association is linked to DJ-1 level and IP3R3-DJ-1 interaction (Liu et al. 2019).

In Drosophila PD model, dopaminergic neurons harboring PINK1 mutation present strengthen ER-mitochondrial contact sites, and consequently raise in the levels of mitochondrial Ca^{2+}; alteration in the contact sites is probably due to impaired control of MIRO1 abundance, that resulted in increased Ca^{2+} transfer from the ER to mitochondria (Lee et al. 2018). The mitochondrial Rho GTPase MIRO1 (RHOT1) has been studied as an adaptor protein for mitochondrial transport; in fibroblast of PD patients, RHOT1 dominant mutation promotes decreases in MAM and causes calcium dyshomeostasis (Grossmann et al. 2019).

Missense mutations in the leucine-rich repeat kinase 2 (LRRK2) gene is one common cause of familial PD. It has been shown that MEF carrying G2019S and D1994A LRRK2 mutations have impaired mitochondrial Ca^{2+} transferring (Toyofuku et al. 2020). Interestingly the numbers of ER–mitochondrial contact sites are reduced in the $LRRK2^{-/-}$ and LRRK2 (G2019S). Since LRRK2 regulates the activities of E3 ubiquitin ligases such as MITOL, MULAN, and Parkin, which are enriched in the MAM, depletion or loss-of-function mutation of LRRK2 leads to missing of MAM integrity that results in calcium imbalance (Toyofuku et al. 2020). Contrary to MEF study, in astrocytes isolated form G2019S-LRRK2 mice the number of ER-mitochondria contact sites are increased, which resulted in increment of Ca^{2+} transfer from ER to mitochondria (Lee et al. 2019a). It is proposed that this may happen due to change

in the redistribution pattern of proteins such as MFN2, calnexin, IP3R that are located to MAM (Lee et al. 2019a).

In amyotrophic lateral sclerosis (ALS) that is distinguished by progressive degeneration of lower and upper motor neurons in the brainstem, spinal cord, and motor cortex, most cases of familial disease are linked to mutations of superoxide dismutase-1 (*SOD1*), VAPB, TAR DNA-binding protein 43 (*TDP-43*) and *C9ORF72* genes, that are responsible for about 50% of familial ALS patients (DeJesus-Hernandez et al. 2011; Rosen et al. 1993; Sreedharan et al. 2008; Turner et al. 2013).

Disruption in VAPB-PTPIP51 tethering plays an essential role in ALS autosomal-dominant form; the mutation P56S-VAPB has higher affinity for PTPIP51 that can lead to increase in Ca^{2+} transfer to mitochondria (De Vos et al. 2012; Moustaqim-Barrette et al. 2014). Also, TDP-43 can modulate the VAPB-PTPI51 interaction by activation of glycogen synthase kinase-3 beta (GSK-3β) (Fig. 1d, d'). Overexpression of TDP-43 resulted in GSK-3β activation that leads to decreased VAPB-PTPI51 interaction and reduced ER-mitochondria association (Stoica et al. 2014).

Sigma-1 receptor (Sig-1R) is an ER chaperone that resides mainly at MAM. Sig-1R interacts with and stabilizes IP3R3s, playing a significant role in the regulation of the Ca^{2+} signaling between the ER and mitochondria (Hayashi and Su 2007). Mutation in Sig-1R is associated with juvenile ALS (Al-Saif et al. 2011). Homozygous frameshift mutation (p.L95fs) of Sig-1R resulted in inability to bind to IP3R3; loss of this interaction leads to Ca^{2+} deregulation at MAM in Sig-1R linked ALS (Watanabe et al. 2016).

In cortical pyramidal neurons, the PDZ domain-containing protein 8 (PDZD8) is essential for the ER and mitochondrial membranes tethering and is required for Ca^{2+} transferring from ER to mitochondria (Hirabayashi et al. 2017). In the axon injury model, overexpression of the glucose-regulated protein 75 (GRP75) leads to increase in ER-mitochondria tethering, which consequently elevates the mitochondrial Ca^{2+} and enhances ATP generation (Lee et al. 2019b).

Many proteins are enumerated that are part of and can change MAM and Ca^{2+} homeostasis. However, it remains to be elucidated the role of other tethering proteins in the context of neurodegenerative disease. Isolation of MAM, followed by proteomic studies, can provide other potential targets, which modulate MAM in physiological and pathological conditions such as neurodegeneration.

MAMs in Lipid Metabolism

Lipid molecules are associated with multiple cellular processes, such as cell membrane formation, cell signaling, transduction and synaptic transmission (Mesa-Herrera et al. 2019). Organelles demonstrate a unique lipid profile, which directly relates to their function and physical properties. The substantial lipid classes elucidated in the organelles' membranes are phospholipids, sterols, and sphingolipids (van Meer et al. 2008). However, endoplasmic reticulum plays a paramount role in lipid synthesis, storage and distribution within the cells.

Synthesis of two most abundant phospholipids phosphatidylcholine (PC) and phosphatidylethanolamine (PE) is dependent on trafficking between ER and mitochondria, because of the location of the required synthesizing enzymes (Kojima et al. 2016; Tatsuta and Langer 2017). In fact, since most of the organelles have limited capacity to produce their lipids, lipid transport via specific proteins or tethering is mandatory for the correct lipid composition of each organelle (Flis and Daum 2013).

The MAM harbors diverse lipid trafficking proteins and synthesizing enzymes, such as diacylglycerol O-acyltransferase 2 (DGAT2), fatty acid CoA ligase 4 (FACL4/ACS4), phosphatidylserine synthases 1 and 2 (PSS1 and PSS2), phosphatidylethanolamine N-methyltransferase 2 (PEMT2), and cholesterol acyltransferase/sterol O-acyltransferase 1 (ACAT1/SOAT1) (Lewin et al. 2002; Stone et al. 2009; Stone and Vance 2000).

Level of cholesteryl-ester (CE) is directly associated with Aβ production in AD; the ACAT1, which is responsible for CE synthesis, modulates Aβ generation via a balance between free cholesterol and cholesteryl esters (Puglielli et al. 2001). The C99, the 99-aa C- terminal domain of the amyloid precursor protein (APP) that is produced by cleavage of APP with β-secretase, is associated with familial AD and is involved in the lipid homeostasis and regulation of MAM activity. In cell models of AD and cells from AD patients, the unprocessed C99 accumulates in the MAM, which resulted in alteration of lipid profile in MAMs and elevated sphingolipid turnover (Pera et al. 2017).

The presence of apolipoprotein E4 allele (APOE4) is one of the main genetic risk factors for AD. APOE4 can modulate MAM function, in human fibroblasts treated with astrocyte-conditioned media obtained from APOE4 and APOE3 transgenic mice. This alteration in MAM resulted in an increase of phospholipids and cholesteryl esters in cells treated with APOE4 (Tambini et al. 2016). As previously mentioned, Presenilin is enriched in the MAM in Presenilin-deficient cells and cells from AD patients; this accumulation resulted in increased phospholipid and cholesteryl ester synthesis (Area-Gomez et al. 2012).

In induced peptidergic neurons from Parkinson's disease patients and ventral lateral neurons from mutant flies, loss of Parkin resulted in a higher number of contact sites compared to control groups; this excessive interaction can cause dysfunction of lipid trafficking that depletes phosphatidylserine from ER (Valadas et al. 2018). Moreover, in PD, α-syn mutation or its triplication change MAM; α-syn

overexpression or mutation E46K can increase the level of oleic acid (OA, 18:1) diglycerides, and triglycerides level in the whole-cell lipidomic profiling (Fanning et al. 2019). Interestingly, overexpression or knockdown of α-syn is associated with alteration of lipid metabolism (Alza et al. 2019). In SNCA knockout mice, the docosahexaenoic acid (DHA), phosphatidylethanolamine and phosphatidylinositol are increased compared to control (Golovko et al. 2007). Furthermore, overexpression of α-syn resulted in lipid droplet accumulation and an increase in triacylglycerol (Alza et al. 2019). These modulations may be correlated with the enrichment of α-syn in MAM.

It has been shown in fibroblasts of ALS patients that 220 lipids isolated from MAM were changed compared to control, such as phosphatidylcholine PC (36:4p), phosphatidylcholines (42:2), ceramide (d18: 2/22:0), phosphatidylethanolamines (18:0p/22:6) and triglycerides (16:1/14:0/16:1) (Veyrat-Durebex et al. 2019). Further combined omic approaches are required to elucidate the interaction of enriched proteins in MAM and their association with the lipid composition of this multifunctional platform. Lipid alterations in the MAM probably change the distribution and contribute to aggregation of proteins that are associated to neuropathological conditions.

MAM in Autophagy

Autophagy is a conserved cellular process that may be classified as macroautophagy, chaperone-mediated autophagy and microautophagy, in which organelles and free components are delivered to lysosomes for degradation.

In macroautophagy, the transport of cargoes occurs via double-membrane vesicles called autophagosomes, in contrast to the other two autophagy pathways. The autophagic flux involves a series of steps, such as autophagosome formation, maturation, and closure; the process entails crosstalk among autophagosome biogenesis, lysosomal degradation and dynein machinery on microtubules (Bento et al. 2016; Kimura et al. 2008; Rubinsztein et al. 2012).

An emerging body of evidence demonstrates the pivotal role of MAMs in autophagy and a possible participation during the accumulation of oligomers and aggregates of misfolded proteins in neurodegenerative diseases (Menzies et al. 2017).

The ER-mitochondria contact sites participate of phagophore assembly, as it has been documented that the autophagosomes may form at the MAM in mammalian cells. Following autophagy triggering the pre-autophagosome and autophagosome markers autophagy-related 14-like (ATG14L), double FYVE domain-containing protein 1 (DFCP1), and autophagosome-formation marker ATG5 relocalizes to the MAM (Hamasaki et al. 2013). Furthermore, ATG2A can interact with ATG9A at the ER-mitochondrial contact sites to promote phagophore growth (Tang et al. 2019).

Recently it has been shown that the tethering complex VAPB/PTPIP51 regulates autophagy, since overexpression of either proteins tightens ER-mitochondria contacts and decrease autophagosome formation, on the other hand, depletion of VAPB or PTPIP51 stimulates autophagy flux (Gomez-Suaga et al. 2017).

The etoposide-induced protein 2.4 (EI24) is anchored at the MAM via its C-terminal domain. In pancreatic β cells, this protein is vital for MAM integrity and autophagy flux through regulation of IP3R–GRP75–VDAC1 complex (Yuan et al. 2019). In the neuronal context, EI24 is also a main component of the autophagy pathway; since the EI24 conditional knockout mice present massive axon degeneration and extensive neuron loss in the brain and spinal cord (Zhao et al. 2012), although the contribution of EI24 for MAMs integrity in neuronal cells is still an open question.

In the SH-SY5Y cells, proteins related to mitophagy are located at MAM such as PINK1 and BECN1; and promote the enhancement of contact sites and autophagosome formation followed by mitophagy (Gelmetti et al. 2017). In neuronal cells, Mul1 (Mitochondrial E3 ubiquitin protein ligase 1) is anchored in the outer mitochondrial membrane; knockdown of MUL1 in cortical neurons resulted in reduction of ER-Mito contact sites, mitochondrial fragmentation and Parkin-mediated mitophagy (Puri et al. 2019). MUL1 deficiency resulted in increase of MFN2 activity, which can perturb the ER-Mito contact site (Puri et al. 2019).

As detailed earlier, mutation in VAPB (P56S) resulted in an autosomal-dominant form of ALS; this mutation increases the binding to PTPIP51, which causes inhibition of autophagy by reducing the ULK1/FIP200 interaction (Zhao et al. 2018).

Due to the importance of MAM in autophagy, it seems possible to postulate that malfunction in the MAM or any tethering protein may trigger cell death to the majority of organs, including the nervous system.

MAM and Mitochondria Dynamics

Although to date little is known about the reciprocal relationships between MAM and organelles morphology and motility, emerging data shed light on a new aspect of organelles contact site. Motor proteins are tightly regulated by Ca^{2+} and control mitochondrial movement along microtubules. Reduced mitochondrial movement is related to increase in ER-mitochondrial contact sites (Pizzo and Pozzan 2007).

MIRO1 and MIRO2 form complexes with TRAK adaptors and molecular motors kinesin/dynein to orchestrate mitochondria transport. It has been shown in mouse embryonic fibroblasts that MIRO proteins are required for mitochondria and endoplasmic reticulum contact sites,

and also for mitochondrial cristae architecture (Modi et al. 2019). Besides, in fibroblast of PD patients, MIRO1 mutations T351A or T610A disturb the calcium homeostasis and reduce the amount of ER-mitochondrial contact sites (Berenguer-Escuder et al. 2019). Autosomal dominant mutation in MIRO1 (R272Q, R450C) in PD patient fibroblasts reduced mitochondrial mass and ER-mitochondrial contact sites (Grossmann et al. 2019). Interestingly number of ER-mitochondrial contact sites is increased, but the mass of the mitochondria did not change in iPSC-derived neurons harboring MIRO1 (R272Q); the alteration in the contact site is associated to the localization of MIRO1 (R272Q) to the MAM, which collaborates to reduced mitochondrial movement and calcium dyshomeostasis in these cells (Berenguer-Escuder et al. 2020).

Disruption in calcium homeostasis has a direct influence on mitochondrial morphology. Mitochondrial ubiquitin ligase (MITOL) is an integral mitochondrial outer membrane protein that regulates mitochondrial dynamic via fission proteins such as Drp1 and Mid49 (Xu et al. 2016). Furthermore, it is documented that MITOL is partially located to MAM and intervene mitofusin 2 (MFN2) ubiquitylation. Therefore, MITOL can mediate MFN2 activation enabling MAM formation (Sugiura et al. 2013).

Depletion of MITOL in neurons leads to reduction of ER-mitochondria contact sites and absence of branched mitochondria, which is probably associated to accumulation of mitochondrial fission proteins (Nagashima et al. 2019). Moreover, in the absence of MITOL, the level of cardiolipin (major phospholipid in the mitochondrial inner membrane) becomes lower; this may happen due to MAM malfunction that resulted in disruption of phospholipids transfer, which is necessary for cardiolipin biogenesis (Nagashima et al. 2019).

MFN2 is one of the well-studied proteins involved in mitochondrial fusion, it is located on outer mitochondrial membranes (OMM) and the ER membranes in MAM, providing stability to MAM structure. In murine fibroblasts, depletion of MFN2 promotes an increase in distance between two organelles, which leads to morphology change of ER and mitochondria (de Brito and Scorrano 2008).

Mutation in MFN2 has been linked to Charcot–Marie–Tooth type 2A (CMT2A) disease, an axonal sensorimotor neuropathy. In patient fibroblasts harboring the mutation MFN2 (R94C), a significant decrease in contact between mitochondria and ER has been reported. The CMT2A mouse model manifests impairment of several MAM linked processes such as calcium handling defects, mitochondria morphology, transport changes and ER stress (Bernard-Marissal et al. 2019).

Recently, it has been shown that mutation in VPS13A, a peripheral membrane protein localized at membrane contact sites, is associated with the neurodegenerative disorder Chorea Acanthocytosis; in VPS13A-depleted cells, not only the number of MAM but also the mitochondria size and mitophagy are decreased (Yeshaw et al. 2019). Both mitochondrial motility and morphology are directly involved in MAM formation and function. Although, molecular events underlying mitochondrial movement and morphology are well studied, further investigation is necessary to understand how they control MAM structure and function in different neuropathological conditions.

MAM Detection Methodologies

Several methodologies have been developed since early as the 1950s to quantify the nanometric distance between mitochondria and ER in different experimental, physiological, and pathological conditions. Most of the findings related to MAM visualization in healthy neurons and during neurodegenerative diseases are included in Table 1 and discussed in the following subtopics.

Confocal Microscopy

The most readily available approach to measure organelles contact sites is confocal microscopy, often coupled to super-resolution devices. Confocal microscopy was first applied to detect the ER-mitochondria contacts in living HeLa cells (Rizzuto et al. 1998).

The methodology is based on fluorescent detection in live and fixed cells, in immunostained structures or cells expressing fluorescent tag proteins, and different barcoding strategies (Hu et al. 2018; Scorrano et al. 2019). Like all methodologies, confocal microscopy approach suffers some limitations, the axial resolution is approximately 500–700 nm, and the lateral resolution is around 200 nm (Jing et al. 2019), which is not enough to detect the organelle's contact sites accurately.

In the case of immunostaining for different tethering proteins, the output relies on antibodies specificity. Also, chemical fixation can disrupt the contact site. To overcome this issue, Cryo-Confocal microscopy can be a great alternative. In the Cryo approach the materials are frozen under high-pressure freezing or plunge freezing, then samples are imaged in a frozen state (Elgass et al. 2015).

Confocal or fluorescent microscopy can be combined with several biochemical techniques to improve the limitations. The majority of these approaches are based on proximity-driven fluorescent signal generations such as fluorescence resonance energy transfer (FRET), bioluminescence resonance energy transfer (BRET), bimolecular complementation (BiC), dimerization-dependent fluorescent protein (ddFP) and proximity ligation assay (PLA). The descriptions of these techniques are illustrated in Fig. 2.

Table 1 Methodologies available for detection of in situ ER-mitochondria contact sites in neurosciences

Approaches	Organelles	Cell types	References
Confocal	Mito-ER	Neurons from animal models of neurodegeneration, AD patient fibroblasts, Postmortem AD human brain, mice brain, M17 cells, MEF cells, PD hiPS, Fly dopaminergic neurons, SH-SY5Y cells	Area-Gomez et al. 2012; Guardia-Laguarta et al. 2014; Guardia-Laguarta et al. 2015; Hedskog et al. 2013; Lee et al. 2019b; Cali et al. 2013; Grossmann et al. 2019; Lee et al. 2018; Moustaqim-Barrette et al. 2014; Ottolini et al. 2013; Paillusson et al. 2017
PLA	Mito-ER	SH-SY5Y, PD hiPS, HT22 cells, neuron, NSC-34 cells,	Bernard-Marissal et al. 2019; Gomez-Suaga et al. 2019; Honrath et al. 2017; Paillusson et al. 2017; Stoica et al. 2016
TEM	Mito-ER	AD patient fibroblasts, Postmortem AD human brain, mice brain, SH-SY5Y, PD hiPS, Fly neurons, NSC-34 cells	Area-Gomez et al. 2012; Hedskog et al. 2013; Moustaqim-Barrette et al. 2014; Paillusson et al. 2017; Stoica et al. 2016
SBF-SEM	Mito-ER	Mice hippocampal neurons	Nagashima et al. 2019
FIB-SEM	Mito-ER	Cortical neurons, Cerebral cortex	Hirabayashi et al. 2017; Wu et al. 2017
**ET	Mito-ER	COS-7 cells	Elgass et al. 2015
*CLEM	_	Mice brain	Hirabayashi et al. 2018
*Cryo-ET	_	Hippocampal neurons, Neocortex	Fischer et al. 2018; Guo et al. 2018; Tao et al. 2018
*Cryo-EM	_	AD tissue from frontal and temporal cortex	Arakhamia et al. 2020; Fitzpatrick et al. 2017
*BioID	_	Hippocampal neurons	Spence et al. 2019
*APEX	Mito-ER	HEK239T cells, mice brain	Hung et al. 2017; Hirabayashi et al. 2018

Detailed discussion of these methods is found in Sect. 3. Recently developed approaches (*) still need to be optimized for visualization and mapping contact sites

**ET was not applied to neurosciences studies yet. Abbreviations were defined in abbreviation list

Electron Microscopy

Electron microscopy (EM) is a gold standard technique that provides nano-scale resolution to visualize cell structures such as MAM.

Transmission electron microscopy (TEM) and its combination with immunogold labeling using different gold particle sizes, is an acceptable approach to detect the tethering proteins (Scorrano et al. 2019). On the other hand, the TEM has limitations such as the impossibility of handling live cells, chemical fixation procedures that may interfere with the native state of MAM, and the possibility of single plane only (Scorrano et al. 2019; Stacchiotti et al. 2018).

Scanning electron microscopy (SEM) provides the capability of high-resolution 3D imaging of large volume specimens (Scorrano et al. 2019). Different SEM approaches, such as serial block-face scanning electron microscopy (SBF-SEM) has now been initially used in neuroscience (Lippens et al. 2019). This technique is based on collecting hundreds of sequential serial sections producing three-dimensional views of the specimen (Lippens et al. 2019).

In focused ion beam-scanning EM (FIB-SEM) an ion beam is used to remove a thin layer of the sample (15–50 nm), followed by electron beam SEM image capture. With this approach, the series of images are reconstructed ion of in three-dimension (3D) for better visualization of the specimen (Hirabayashi et al. 2017).

Electron Tomography

Electron tomography (ET) can generate 3D reconstructions of cellular structures. ET is based on multiple imaging, while the sample is tilting along the axis, followed by 3D reconstruction of the images (Scorrano et al. 2019).

Like other EM-based approaches, samples preparation procedure with chemical fixation may disrupt the intact architecture of the cells. Thus, electron cryo-tomography (Cryo-ET) offers the advantage of immobilizing samples in non-crystalline ice and imaging under cryogenic conditions (Tocheva et al. 2010). The combination of cryo-focused ion beam (Cryo-FIB) milling and Cryo-ET can overcome the sample thickness and so far make this approach as a unique methodology to visualize cell structures (Collado and Fernandez-Busnadiego 2017; Schaffer et al. 2019).

Correlative Light and Electron Microscopy

Correlative light and electron microscopy (CLEM) is a combination of fluorescence microscopy with different high-resolution electron microscopy, which provides a powerful technique to answer both structural and functional questions in different fields of neuroscience (Begemann and Galic 2016; Hirabayashi et al. 2018).

Taking advantage of utilizing specific genetic tags such as MiniSOG (mini Singlet Oxygen Generator) makes CLEM a more robust approach (Shu et al. 2011). MiniSOG

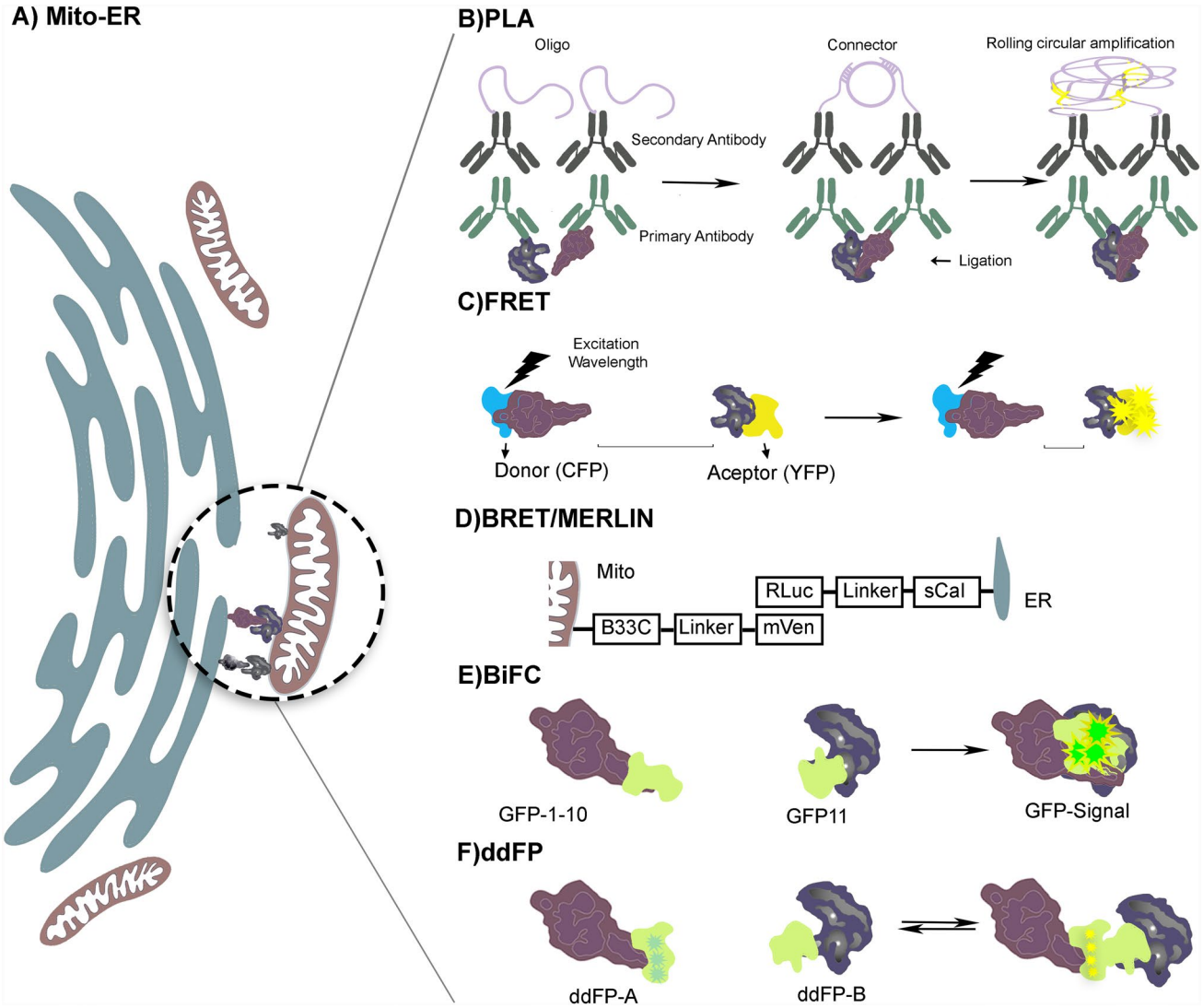

Fig. 2 **a** Representation of endoplasmic reticulum (ER)-Mitochondria contact sites. Magnified area (dashed circle) illustrates tethering complexes and the methods using fluorescent approaches employed to study interaction between mitochondria and ER are exemplified in B to F. **b** Proximity ligation assay (PLA) is a broadly used method to detect the endogenous protein–protein interactions in fixed samples. It is based on the labeling of the target proteins with primary antibodies, followed by secondary antibodies conjugated to specific oligonucleotides; in close proximity targeted proteins can hybridize with connector oligonucleotides that serve as templates for rolling circular amplification by using fluorophore-labeled nucleotides (Soderberg et al. 2006). This technique has potential to detect MAM via tethering proteins in the presence of specific antibodies. **c** Fluorescence resonance energy transfer (FRET) is based on the transfer of energy between two fluorophores (donor/acceptor) fused to membrane proteins that has been used to visualize organelles' contact site, FRET provides high-resolution detection (approximately 10 nm) and is compatible to live cells analysis (Csordas et al. 2010; Scorrano et al. 2019). **d** Bioluminescence resonance energy transfer (BRET) is a new methodology that has been recently developed to quantify the contact site between mitochondria and ER using the so-called MERLIN (Mitochondria–ER Length Indicator Nanosensor). The Renilla Luciferase 8 (RLuc) acts as a donor linked to a truncated non-functional variant of calnexin (sCal) and mVenus as an acceptor fused to alpha-helical C-terminal domain of Bcl-xL (Hertlein et al. 2020). BRET provides higher resolution than fluorescence microscopy, it is applicable to live cell imaging and has limited phototoxicity as compared with FRET. **e** Biomolecular fluorescence complementation (BiFC) is based on split-fluorescent proteins such as split Venus or GFP composes of two complementary non-fluorescent residues fused to N or C terminal fragment of membranes bounded proteins, while targeted membranes locate in proximity the split fragments bind to each other and allowing the fluorescence to bright (Cieri et al. 2018; Kakimoto et al. 2018; Miller et al. 2015). The complementation is stable in this approach; therefore, it is not suitable to study the dynamic of the contact sites (Scorrano et al. 2019). The split probes irreversibly bind, which may alter the function of contact sites; on the other hand, it is applicable to live imaging. **f** Dimerization-dependent fluorescent protein (ddFP) works similar to BiFC, this approach is based on a reversible binding of weak or non-fluorescent protein monomers (Alford et al. 2012; Scorrano et al. 2019). Due to reversibility, the dynamic of contact sites can be monitored when using this approach

is a 106 amino acid flavin-binding protein that becomes fluorescent and produces reactive oxygen species (ROS) when excited at the correct wavelength (Shu et al. 2011). When tissue slices or fixed cells are exposed to diaminobenzidine (DAB), it reacts with the ROS and polymerizes at the site of MiniSOG, the polymerized DAB is stained with osmium tetroxide and visualized with EM (Shu et al. 2011). Recently, split-MiniSOG was developed to detect in vitro protein–protein interactions allowing the study of α-syn aggregation (Boassa et al. 2019). It seems that split-MiniSOG will be an invaluable strategy to detect the ER-mitochondria contact sites, which was not applied yet.

Proximity Biotinylation Labeling

The proximity-based biotin identification (BioID) and ascorbate peroxidase (APEX) methods are based on enzymes that turn a substrate into a reactive radical that tags neighboring proteins with biotin. BioID has been used to map protein–protein interaction based on the mutant form of the biotin ligase BirA (Scorrano et al. 2019). APEX is an engineered ascorbate peroxidase, which can catalyze the oxidation of biotin-phenol in the presence of H_2O_2. APEX catalyzes the oxidation of biotin phenol into short-lived radicals that can attach to electron-rich amino acids (Tyr, Trp, His, and Cys) in nearby endogenous proteins. Following the H_2O_2 removal, the reaction will stop and biotinylated proteins can be purified using streptavidin beads and identified through mass spectrometry (Hung et al. 2017; Scorrano et al. 2019).

Isolation of MAM

Organelle interface fraction is another widely used biochemical approach. Mitochondrial-associated ER membranes fraction was first separated and characterized in 1990 (Vance 1990). This methodology is based on sequential gradient (Percoll) ultracentrifugation that is applicable for both tissues and cells, which results in subcellular fractionation of microsomes (endoplasmic reticulum), crude mitochondria, pure mitochondria, and MAM (Lewis et al. 2016; Wieckowski et al. 2009). A combination of fractionation with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) for particular protein detection or multi-omic approaches is commonly used (Liu et al. 2019; Ma et al. 2017; Veyrat-Durebex et al. 2019). The purity of the subcellular fractions is the main concern. For this reason, normalization with several subcellular markers such as Sig-1R, calnexin, VDAC, GRP75 is mandatory.

Conclusion

The interplay between ER and mitochondria is an emerging field that undeniably plays a crucial role in maintaining and regulating several cellular essential pathways in neuronal cells. Although evidence suggests that disruption of MAM function can perturb calcium homeostasis, autophagy, lipid metabolism, and organelles motility, many questions remain unclear. Therefore, a meticulous investigation is necessary to gain better insights into the mechanisms of tethering in brain cells. Hence, the composition of all tethering complexes still needs to be elucidated in different neurodegenerative diseases by implementing new approaches that were not done in neurosciences yet. Moreover, a clear understanding of MAM by means of development of new methodologies to manipulate the tethering and in vivo barcoding of tethering complexes may contribute to early detection, prevention and treatment of neurodegenerative diseases in the future.

Acknowledgements Authors were awarded with research grants from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (FAPESP #13/08028-1, #18/07592-4 and #19/01290-9).

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no competing interests.

References

AlSaif A, AlMohanna F, Bohlega S (2011) A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. Ann Neurol 70:913–919. https://doi.org/10.1002/ana.22534

Alford SC, Ding Y, Simmen T, Campbell RE (2012) Dimerization-dependent green and yellow fluorescent proteins. ACS Synth Biol 1:569–575. https://doi.org/10.1021/sb300050j

Alza NP, Iglesias Gonzalez PA, Conde MA, Uranga RM, Salvador GA (2019) Lipids at the crossroad of alpha-synuclein function and dysfunction: biological and pathological implications. Front Cell Neurosci 13:175. https://doi.org/10.3389/fncel.2019.00175

Arakhamia T et al (2020) Posttranslational modifications mediate the structural diversity of tauopathy strains. Cell 180:633–644. https://doi.org/10.1016/j.cell.2020.01.027

Area-Gomez E et al (2018) A key role for MAM in mediating mitochondrial dysfunction in alzheimer disease. Cell Death Dis 9:335. https://doi.org/10.1038/s41419-017-0215-0

Area-Gomez E et al (2012) Upregulated function of mitochondria-associated ER membranes in alzheimer disease. EMBO J 31:4106–4123. https://doi.org/10.1038/emboj.2012.202

Basso V et al (2018) Regulation of ER-mitochondria contacts by Parkin via Mfn2. Pharmacol Res 138:43–56. https://doi.org/10.1016/j.phrs.2018.09.006

Begemann I, Galic M (2016) Correlative light electron microscopy: connecting synaptic structure and function. Front Synaptic Neurosci 8:28. https://doi.org/10.3389/fnsyn.2016.00028

Bento CF et al (2016) Mammalian autophagy: how does it work? Annu Rev Biochem 85:685–713. https://doi.org/10.1146/annurev-biochem-060815-014556

Berenguer-Escuder C et al (2020) Impaired mitochondrial-endoplasmic reticulum interaction and mitophagy in miro1-mutant neurons in parkinson's disease. Hum Mol Genet 29:1353–1364. https://doi.org/10.1093/hmg/ddaa066

Berenguer-Escuder C et al (2019) Variants in miro1 cause alterations of ER-mitochondria contact sites in fibroblasts from parkinson's disease patients. J Clin Med. https://doi.org/10.3390/jcm8122226

Bernard-Marissal N et al (2019) Altered interplay between endoplasmic reticulum and mitochondria in charcot-marie-tooth type 2A neuropathy. Proc Natl Acad Sci U S A 116:2328–2337. https://doi.org/10.1073/pnas.1810932116

Bernhard W, Haguenau F, Gautier A, Oberling C (1952) Submicroscopical structure of cytoplasmic basophils in the liver, pancreas and salivary gland; study of ultrafine slices by electron microscope. Z Zellforsch Mikrosk Anat 37:281–300

Bernhard W, Rouiller C (1956) Close topographical relationship between mitochondria and ergastoplasm of liver cells in a definite phase of cellular activity. J Biophys Biochem Cytol 2:73–78. https://doi.org/10.1083/jcb.2.4.73

Boassa D et al (2019) Split-miniSOG for spatially detecting intracellular protein-protein interactions by correlated light and electron microscopy. Cell Chem Biol 26:1407–1416. https://doi.org/10.1016/j.chembiol.2019.07.007

Cali T, Ottolini D, Negro A, Brini M (2013) Enhanced parkin levels favor ER-mitochondria crosstalk and guarantee Ca(2+) transfer to sustain cell bioenergetics. Biochim Biophys Acta 1832:495–508. https://doi.org/10.1016/j.bbadis.2013.01.004

Cieri D et al (2018) SPLICS: a split green fluorescent protein-based contact site sensor for narrow and wide heterotypic organelle juxtaposition. Cell Death Differ 25:1131–1145. https://doi.org/10.1038/s41418-017-0033-z

Cohen S, Valm AM, Lippincott-Schwartz J (2018) Interacting organelles. Curr Opin Cell Biol 53:84–91. https://doi.org/10.1016/j.ceb.2018.06.003

Collado J, Fernandez-Busnadiego R (2017) Deciphering the molecular architecture of membrane contact sites by cryo-electron tomography. Biochim Biophys Acta Mol Cell Res 1864:1507–1512. https://doi.org/10.1016/j.bbamcr.2017.03.009

Csordas G et al (2010) Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. Mol Cell 39:121–132. https://doi.org/10.1016/j.molcel.2010.06.029

de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456:605–610. https://doi.org/10.1038/nature07534

De Vos KJ et al (2012) VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. Hum Mol Genet 21:1299–1311. https://doi.org/10.1093/hmg/ddr559

DeJesus-Hernandez M et al (2011) Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72:245–256. https://doi.org/10.1016/j.neuron.2011.09.011

Dorszewska J, Prendecki M, Oczkowska A, Dezor M, Kozubski W (2016) Molecular basis of familial and sporadic alzheimer's disease. Curr Alzheimer Res 13:952–963. https://doi.org/10.2174/1567205013666160314150501

Elgass KD, Smith EA, LeGros MA, Larabell CA, Ryan MT (2015) Analysis of ER-mitochondria contacts using correlative fluorescence microscopy and soft X-ray tomography of mammalian cells. J Cell Sci 128:2795–2804. https://doi.org/10.1242/jcs.169136

Fanning S et al (2019) Lipidomic analysis of alpha-synuclein neurotoxicity identifies stearoyl CoA desaturase as a target for parkinson treatment. Mol Cell 73:1001–1014. https://doi.org/10.1016/j.molcel.2018.11.028

Fischer TD, Dash PK, Liu J, Waxham MN (2018) Morphology of mitochondria in spatially restricted axons revealed by cryo-electron tomography. PLoS Biol 16:e2006169. https://doi.org/10.1371/journal.pbio.2006169

Fitzpatrick AWP et al (2017) Cryo-EM structures of tau filaments from alzheimer's disease. Nature 547:185–190. https://doi.org/10.1038/nature23002

Flis VV, Daum G (2013) Lipid transport between the endoplasmic reticulum and mitochondria. Cold Spring Harb Perspect Biol. https://doi.org/10.1101/cshperspect.a013235

Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK (2011) ER tubules mark sites of mitochondrial division. Science 334:358–362. https://doi.org/10.1126/science.1207385

Gelmetti V et al (2017) PINK1 and BECN1 relocalize at mitochondria-associated membranes during mitophagy and promote ER-mitochondria tethering and autophagosome formation. Autophagy 13:654–669. https://doi.org/10.1080/15548627.2016.1277309

Goedert M, Spillantini MG (2006) A century of alzheimer's disease. Science 314:777–781. https://doi.org/10.1126/science.1132814

Golovko MY, Rosenberger TA, Feddersen S, Faergeman NJ, Murphy EJ (2007) Alpha-synuclein gene ablation increases docosahexaenoic acid incorporation and turnover in brain phospholipids. J Neurochem 101:201–211. https://doi.org/10.1111/j.1471-4159.2006.04357.x

Gomez-Suaga P, Bravo-San Pedro JM, Gonzalez-Polo RA, Fuentes JM, Niso-Santano M (2018) ER-mitochondria signaling in parkinson's disease. Cell Death Dis 9:337. https://doi.org/10.1038/s41419-017-0079-3

Gomez-Suaga P, Paillusson S, Stoica R, Noble W, Hanger DP, Miller CCJ (2017) The ER-mitochondria tethering complex VAPB-PTPIP51 regulates autophagy. Curr Biol 27:371–385. https://doi.org/10.1016/j.cub.2016.12.038

Gomez-Suaga P et al (2019) The VAPB-PTPIP51 endoplasmic reticulum-mitochondria tethering proteins are present in neuronal synapses and regulate synaptic activity. Acta Neuropathol Commun 7:35. https://doi.org/10.1186/s40478-019-0688-4

Grossmann D et al (2019) Mutations in RHOT1 disrupt endoplasmic reticulum-mitochondria contact sites interfering with calcium homeostasis and mitochondrial dynamics in parkinson's disease. Antioxid Redox Signal 31:1213–1234. https://doi.org/10.1089/ars.2018.7718

Guardia-Laguarta C et al (2014) alpha-Synuclein is localized to mitochondria-associated ER membranes. J Neurosci 34:249–259. https://doi.org/10.1523/JNEUROSCI.2507-13.2014

Guardia-Laguarta C, Area-Gomez E, Schon EA, Przedborski S (2015) A new role for alpha-synuclein in parkinson's disease: alteration of ER-mitochondrial communication. Mov Disord 30:1026–1033. https://doi.org/10.1002/mds.26239

Guo Q et al (2018) In situ structure of neuronal C9orf72 poly-GA aggregates reveals proteasome recruitment. Cell 172:696–705. https://doi.org/10.1016/j.cell.2017.12.030

Hamasaki M et al (2013) Autophagosomes form at ER-mitochondria contact sites. Nature 495:389–393. https://doi.org/10.1038/nature11910

Hayashi T, Su TP (2007) Sigma-1 receptor chaperones at the ER-mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell 131:596–610. https://doi.org/10.1016/j.cell.2007.08.036

Hedskog L et al (2013) Modulation of the endoplasmic reticulum-mitochondria interface in alzheimer's disease and related models. Proc Natl Acad Sci U S A 110:7916–7921. https://doi.org/10.1073/pnas.1300677110

Hertlein V et al (2020) MERLIN: a novel BRET-based proximity biosensor for studying mitochondria-ER contact sites. Life Sci Alliance. https://doi.org/10.26508/lsa.201900600

Hirabayashi Y et al (2017) ER-mitochondria tethering by PDZD8 regulates Ca(2+) dynamics in mammalian neurons. Science 358:623–630. https://doi.org/10.1126/science.aan6009

Hirabayashi Y, Tapia JC, Polleux F (2018) Correlated light-serial scanning electron microscopy (CoLSSEM) for ultrastructural visualization of single neurons in vivo. Sci Rep 8:14491. https://doi.org/10.1038/s41598-018-32820-5

Honrath B, Metz I, Bendridi N, Rieusset J, Culmsee C, Dolga AM (2017) Glucose-regulated protein 75 determines ER-mitochondrial coupling and sensitivity to oxidative stress in neuronal cells. Cell Death Discov 3:17076. https://doi.org/10.1038/cddiscovery.2017.76

Hu F, Zeng C, Long R, Miao Y, Wei L, Xu Q, Min W (2018) Supermultiplexed optical imaging and barcoding with engineered polyynes. Nat Methods 15:194–200. https://doi.org/10.1038/nmeth.4578

Hung V et al (2017) Proteomic mapping of cytosol-facing outer mitochondrial and ER membranes in living human cells by proximity biotinylation. Elife 6:e24463. https://doi.org/10.7554/eLife.24463

Jing J, Liu G, Huang Y, Zhou Y (2019) A molecular toolbox for interrogation of membrane contact sites. J Physiol 9:1725–1739. https://doi.org/10.1113/JP277761

Kakimoto Y, Tashiro S, Kojima R, Morozumi Y, Endo T, Tamura Y (2018) Visualizing multiple inter-organelle contact sites using the organelle-targeted split-GFP system. Sci Rep 8:6175. https://doi.org/10.1038/s41598-018-24466-0

Kalia LV, Lang AE (2015) Parkinson's disease. Lancet 386:896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Kimura S, Noda T, Yoshimori T (2008) Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. Cell Struct Funct 33:109–122. https://doi.org/10.1247/csf.08005

Kojima R, Endo T, Tamura Y (2016) A phospholipid transfer function of ER-mitochondria encounter structure revealed in vitro. Sci Rep 6:30777. https://doi.org/10.1038/srep30777

Lee JH, Han JH, Kim H, Park SM, Joe EH, Jou I (2019) Parkinson's disease-associated LRRK2-G2019S mutant acts through regulation of SERCA activity to control ER stress in astrocytes. Acta Neuropathol Commun 7:68. https://doi.org/10.1186/s40478-019-0716-4

Lee KS, Huh S, Lee S, Wu Z, Kim AK, Kang HY, Lu B (2018) Altered ER-mitochondria contact impacts mitochondria calcium homeostasis and contributes to neurodegeneration in vivo in disease models. Proc Natl Acad Sci U S A 115:E8844–E8853. https://doi.org/10.1073/pnas.1721136115

Lee S, Min KT (2018) The interface between ER and mitochondria: molecular compositions and functions. Mol Cells 41:1000–1007. https://doi.org/10.14348/molcells.2018.0438

Lee S, Wang W, Hwang J, Namgung U, Min KT (2019) Increased ER-mitochondria tethering promotes axon regeneration. Proc Natl Acad Sci U S A 116:16074–16079. https://doi.org/10.1073/pnas.1818830116

Lewin TM, Van Horn CG, Krisans SK, Coleman RA (2002) Rat liver acyl-CoA synthetase 4 is a peripheral-membrane protein located in two distinct subcellular organelles, peroxisomes, and mitochondrial-associated membrane. Arch Biochem Biophys 404:263–270. https://doi.org/10.1016/s0003-9861(02)00247-3

Lewis A, Tsai SY, Su TP (2016) Detection of isolated mitochondria-associated ER membranes using the sigma-1 receptor methods. Mol Biol 1376:133–140. https://doi.org/10.1007/978-1-4939-3170-5_11

Lippens S, Kremer A, Borghgraef P, Guerin CJ (2019) Serial block face-scanning electron microscopy for volume electron microscopy Methods. Cell Biol 152:69–85. https://doi.org/10.1016/bs.mcb.2019.04.002

Liu Y, Ma X, Fujioka H, Liu J, Chen S, Zhu X (2019) DJ-1 regulates the integrity and function of ER-mitochondria association through interaction with IP3R3-Grp75-VDAC1. Proc Natl Acad Sci U S A 116:25322–25328. https://doi.org/10.1073/pnas.1906565116

Ma JH et al (2017) Comparative proteomic analysis of the mitochondria-associated ER membrane (MAM) in a long-term type 2 diabetic rodent model. Sci Rep 7:2062. https://doi.org/10.1038/s41598-017-02213-1

Menzies FM et al (2017) Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron 93:1015–1034. https://doi.org/10.1016/j.neuron.2017.01.022

Mesa-Herrera F, Taoro-Gonzalez L, Valdes-Baizabal C, Diaz M, Marin R (2019) Lipid and lipid raft alteration in aging and neurodegenerative diseases: a window for the development of new biomarkers. Int J Mol Sci 20:3810. https://doi.org/10.3390/ijms20153810

Miller KE, Kim Y, Huh WK, Park HO (2015) Bimolecular fluorescence complementation (BiFC) analysis: advances and recent applications for genome-wide interaction studies. J Mol Biol 427:2039–2055. https://doi.org/10.1016/j.jmb.2015.03.005

Modi S et al (2019) Miro clusters regulate ER-mitochondria contact sites and link cristae organization to the mitochondrial transport machinery. Nat Commun 10:4399. https://doi.org/10.1038/s41467-019-12382-4

Moustaqim-Barrette A, Lin YQ, Pradhan S, Neely GG, Bellen HJ, Tsuda H (2014) The amyotrophic lateral sclerosis 8 protein, VAP, is required for ER protein quality control. Hum Mol Genet 23:1975–1989. https://doi.org/10.1093/hmg/ddt594

Nagashima S et al (2019) MITOL deletion in the brain impairs mitochondrial structure and ER tethering leading to oxidative stress. Life Sci Alliance 2:e201900308. https://doi.org/10.26508/lsa.201900308

Ottolini D, Cali T, Negro A, Brini M (2013) The Parkinson disease-related protein DJ-1 counteracts mitochondrial impairment induced by the tumour suppressor protein p53 by enhancing endoplasmic reticulum-mitochondria tethering. Hum Mol Genet 22:2152–2168. https://doi.org/10.1093/hmg/ddt068

Paillusson S et al (2017) Alpha-synuclein binds to the ER-mitochondria tethering protein VAPB to disrupt Ca(2+) homeostasis and mitochondrial ATP production. Acta Neuropathol 134:129–149. https://doi.org/10.1007/s00401-017-1704-z

Paillusson S, Stoica R, Gomez-Suaga P, Lau DHW, Mueller S, Miller T, Miller CCJ (2016) There's something wrong with my MAM; the ER-mitochondria axis and neurodegenerative diseases. Trends Neurosci 39:146–157. https://doi.org/10.1016/j.tins.2016.01.008

Pera M et al (2017) Increased localization of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in alzheimer disease. EMBO J 36:3356–3371. https://doi.org/10.15252/embj.201796797

Pizzo P, Pozzan T (2007) Mitochondria-endoplasmic reticulum choreography: structure and signaling dynamics. Trends Cell Biol 17:511–517. https://doi.org/10.1016/j.tcb.2007.07.011

Puglielli L et al (2001) Acyl-coenzyme A: cholesterol acyltransferase modulates the generation of the amyloid beta-peptide. Nat Cell Biol 3:905–912. https://doi.org/10.1038/ncb1001-905

Puri R, Cheng XT, Lin MY, Huang N, Sheng ZH (2019) Mul1 restrains parkin-mediated mitophagy in mature neurons by maintaining ER-mitochondrial contacts. Nat Commun 10:3645. https://doi.org/10.1038/s41467-019-11636-5

Rizzuto R et al (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science 280:1763–1766. https://doi.org/10.1126/science.280.5370.1763

Rodriguez-Arribas M et al (2017) Mitochondria-associated membranes (MAMs): overview and its role in parkinson's disease. Mol Neurobiol 54:6287–6303. https://doi.org/10.1007/s12035-016-0140-8

Rosen DR et al (1993) Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 362:59–62. https://doi.org/10.1038/362059a0

Rowland AA, Voeltz GK (2012) Endoplasmic reticulum-mitochondria contacts: function of the junction. Nat Rev Mol Cell Biol 13:607–625. https://doi.org/10.1038/nrm3440

Rubinsztein DC, Shpilka T, Elazar Z (2012) Mechanisms of autophagosome biogenesis. Curr Biol 22:R29-34. https://doi.org/10.1016/j.cub.2011.11.034

Schaffer M et al (2019) A cryo-FIB lift-out technique enables molecular-resolution cryo-ET within native caenorhabditis elegans tissue. Nat Methods 16:757–762. https://doi.org/10.1038/s41592-019-0497-5

Scorrano L et al (2019) Coming together to define membrane contact sites. Nat Commun 10:1287. https://doi.org/10.1038/s41467-019-09253-3

Sepulveda-Falla D et al (2014) Familial alzheimer's disease-associated presenilin-1 alters cerebellar activity and calcium homeostasis. J Clin Invest 124:1552–1567. https://doi.org/10.1172/JCI66407

Shu X et al (2011) A genetically encoded tag for correlated light and electron microscopy of intact cells, tissues, and organisms. PLoS Biol 9:e1001041. https://doi.org/10.1371/journal.pbio.1001041

Soderberg O et al (2006) Direct observation of individual endogenous protein complexes in situ by proximity ligation. Nat Methods 3:995–1000. https://doi.org/10.1038/nmeth947

Spence EF, Dube S, Uezu A, Locke M, Soderblom EJ, Soderling SH (2019) In vivo proximity proteomics of nascent synapses reveals a novel regulator of cytoskeleton-mediated synaptic maturation. Nat Commun 10:386. https://doi.org/10.1038/s41467-019-08288-w

Sreedharan J et al (2008) TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319:1668–1672. https://doi.org/10.1126/science.1154584

Stacchiotti A, Favero G, Lavazza A, Garcia-Gomez R, Monsalve M, Rezzani R (2018) Perspective: mitochondria-ER contacts in metabolic cellular stress assessed by microscopy. Cells 8:5. https://doi.org/10.3390/cells8010005

Stoica R et al (2014) ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. Nat Commun 5:3996. https://doi.org/10.1038/ncomms4996

Stoica R et al (2016) ALS/FTD-associated FUS activates GSK-3beta to disrupt the VAPB-PTPIP51 interaction and ER-mitochondria associations. EMBO Rep 17:1326–1342. https://doi.org/10.15252/embr.201541726

Stone SJ, Levin MC, Zhou P, Han J, Walther TC, Farese RV Jr (2009) The endoplasmic reticulum enzyme DGAT2 is found in mitochondria-associated membranes and has a mitochondrial targeting signal that promotes its association with mitochondria. J Biol Chem 284:5352–5361. https://doi.org/10.1074/jbc.M805768200

Stone SJ, Vance JE (2000) Phosphatidylserine synthase-1 and -2 are localized to mitochondria-associated. J Biol Chem 275:34534–34540. https://doi.org/10.1074/jbc.M002865200

Sugiura A et al (2013) MITOL regulates endoplasmic reticulum-mitochondria contacts via mitofusin2. Mol Cell 51:20–34. https://doi.org/10.1016/j.molcel.2013.04.023

Tambini MD et al (2016) ApoE4 upregulates the activity of mitochondria-associated ER membranes. EMBO Rep 17:27–36. https://doi.org/10.15252/embr.201540614

Tang Z et al (2019) TOM40 targets Atg2 to mitochondria-associated ER membranes for phagophore expansion. Cell Rep 28(1744–1757):e1745. https://doi.org/10.1016/j.celrep.2019.07.036

Tao CL et al (2018) Differentiation and characterization of excitatory and inhibitory synapses by cryo-electron tomography and correlative microscopy. J Neurosci 38:1493–1510. https://doi.org/10.1523/JNEUROSCI.1548-17.2017

Tatsuta T, Langer T (2017) Intramitochondrial phospholipid trafficking. Biochim Biophys Acta Mol Cell Biol Lipids 1862:81–89. https://doi.org/10.1016/j.bbalip.2016.08.006

Tocheva EI, Li Z, Jensen GJ (2010) Electron cryotomography. Cold Spring Harb Perspect Biol 2:a003442. https://doi.org/10.1101/cshperspect.a003442

Toyofuku T, Okamoto Y, Ishikawa T, Sasawatari S, Kumanogoh A (2020) LRRK2 regulates endoplasmic reticulum-mitochondrial tethering through the PERK-mediated ubiquitination pathway. EMBO J 39:e100875. https://doi.org/10.15252/embj.2018100875

Turner MR et al (2013) Controversies and priorities in amyotrophic lateral sclerosis. Lancet Neurol 12:310–322. https://doi.org/10.1016/S1474-4422(13)70036-X

Valadas JS et al (2018) ER lipid defects in neuropeptidergic neurons impair sleep patterns in parkinson's disease. Neuron 98(1155–1169):e1156. https://doi.org/10.1016/j.neuron.2018.05.022

van Meer G, Voelker DR, Feigenson GW (2008) Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol 9:112–124. https://doi.org/10.1038/nrm2330

van Vliet AR, Verfaillie T, Agostinis P (2014) New functions of mitochondria associated membranes in cellular signaling. Biochim Biophys Acta 1843:2253–2262. https://doi.org/10.1016/j.bbamcr.2014.03.009

Vance JE (1990) Phospholipid synthesis in a membrane fraction associated with mitochondria. J Biol Chem 265:7248–7256

Veyrat-Durebex C et al (2019) Metabo-lipidomics of fibroblasts and mitochondrial-endoplasmic reticulum extracts from ALS patients shows alterations in purine, pyrimidine, energetic, and phospholipid metabolisms. Mol Neurobiol 56:5780–5791. https://doi.org/10.1007/s12035-019-1484-7

Vicario M, Cieri D, Brini M, Cali T (2018) The close encounter between alpha-synuclein and mitochondria. Front Neurosci 12:388. https://doi.org/10.3389/fnins.2018.00388

Watanabe S et al (2016) Mitochondria-associated membrane collapse is a common pathomechanism in SIGMAR1- and SOD1-linked ALS. EMBO Mol Med 8:1421–1437. https://doi.org/10.15252/emmm.201606403

Wieckowski MR, Giorgi C, Lebiedzinska M, Duszynski J, Pinton P (2009) Isolation of mitochondria-associated membranes and mitochondria from animal tissues and cells. Nat Protoc 4:1582–1590. https://doi.org/10.1038/nprot.2009.151

Wu Y, Whiteus C, Xu CS, Hayworth KJ, Weinberg RJ, Hess HF, De Camilli P (2017) Contacts between the endoplasmic reticulum and other membranes in neurons. Proc Natl Acad Sci U S A 114:E4859–E4867. https://doi.org/10.1073/pnas.1701078114

Xu S et al (2016) Mitochondrial E3 ubiquitin ligase MARCH5 controls mitochondrial fission and cell sensitivity to stress-induced apoptosis through regulation of MiD49 protein. Mol Biol Cell 27:349–359. https://doi.org/10.1091/mbc.E15-09-0678

Yeshaw WM et al (2019) Human VPS13A is associated with multiple organelles and influences mitochondrial morphology and lipid droplet motility. Elife 8:e43561. https://doi.org/10.7554/eLife.43561

Yuan L, Liu Q, Wang Z, Hou J, Xu P (2019) EI24 tethers endoplasmic reticulum and mitochondria to regulate autophagy flux. Cell Mol Life Sci 8:1591–1606. https://doi.org/10.1007/s00018-019-03236-9

Zampese E, Fasolato C, Kipanyula MJ, Bortolozzi M, Pozzan T, Pizzo P (2011) Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca2+ cross-talk. Proc

Natl Acad Sci U S A 108:2777–2782. https://doi.org/10.1073/pnas.1100735108

Zhao YG, Liu N, Miao G, Chen Y, Zhao H, Zhang H (2018) The ER contact proteins VAPA/B interact with multiple autophagy proteins to modulate autophagosome biogenesis. Curr Biol 28(1234–1245):e1234. https://doi.org/10.1016/j.cub.2018.03.002

Zhao YG, Zhao H, Miao L, Wang L, Sun F, Zhang H (2012) The p53-induced gene Ei24 is an essential component of the basal autophagy pathway. J Biol Chem 287:42053–42063. https://doi.org/10.1074/jbc.M112.415968