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REVIEW

The complex web of membrane contact sites in brain aging and neurodegeneration

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Abstract

To sustain the essential biological functions required for life, eukaryotic cells rely on complex interactions between different intracellular compartments. Membrane contact sites (MCS), regions where organelles come into close proximity, have recently emerged as major hubs for cellular communication, mediating a broad range of physiological processes, including calcium signalling, lipid synthesis and bioenergetics. MCS are particularly abundant and indispensable in polarized and long-lived cells, such as neurons, where they support both structural and functional integrity. In this review, we explore the functional diversity, molecular composition, and dynamic regulation of key mammalian MCS: endoplasmic reticulum (ER)-plasma membrane, ER-mitochondria and contact sites involving lipid droplets. We highlight their central role in neuronal health and discuss how MCS dysfunction has increasingly been recognized as a hallmark of brain aging and various neurodegenerative diseases, most notably Alzheimer's disease, where altered MCS dynamics contribute to pathogenesis. Finally, we emphasize the therapeutic potential of targeting MCS and outline key unanswered questions to guide future research.

Keywords Organelle contacts · Neuronal homeostasis · Synaptic dysfunction · Therapeutic targets · Inter-organelle crosstalk

Introduction

In all eukaryotic cells, membrane-bound organelles spatiotemporally compartmentalize biochemical reactions. Based on this concept, early studies in cell biology primarily viewed organelles as independent entities, with cellular processes believed to rely on vesicular trafficking or the diffusion of small molecules and proteins through the cytoplasm. This perspective established the basis of the "autonomous model", which assumed that specific proteins and phospholipids were synthesized entirely within their respective organelle. However, alternative models for mitochondria soon emerged, challenging this view. The "non-autonomous model" posited that all mitochondrial proteins and phospholipid building blocks are synthesized elsewhere in the cell and subsequently transported to mitochondria for assembly. In contrast, the "hybrid model" proposed that while some mitochondrial proteins and phospholipids are synthesized by mitochondrial enzymes, others originate from different cellular compartments and then imported [1]. In the 1950s, the hybrid model gained experimental support through the seminal work of Bernhard and Rouiller. Using electron microscopy (EM), they observed clear physical contacts between the endoplasmic reticulum (ER) and mitochondria, as well as between the ER and the plasma membrane (PM) [2]. Remarkably, over a decade before the term "autophagy" was coined, they reported that these contacts increased in liver cells of animals that were refed after a prolonged period of starvation [2]. Although such contacts were later observed in different biological systems [3], including early electron micrographs from Keith Porter and George Palade showing that the ER makes physical contacts

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with mitochondria and PM [4, 5], their physiological significance remained underappreciated for decades, largely due to the lack of tools to confirm whether organelle proximity facilitated functional crosstalk essential for cellular homeostasis. It was not until 40 years after Bernhard and Rouiller's observations that Jance E. Vance demonstrated that membrane contact sites (MCS) have distinct cellular functions [6]. In an elegant study, Vance combined biochemical fractionation with a Percoll density gradient and radiolabelling to show that the ER actively transfers phosphatidylserine (PS) into mitochondria through a "collision-based mechanism" occurring at mitochondria-associated membranes (MAMs) [6]. This PS transfer is now known to be the rate-limiting step in producing phosphatidylethanolamine (PE), which is synthesized through PS decarboxylation by mitochondrial PS decarboxylase [7]. Moreover, given that many MCS mediate lipid transfer, it is now widely accepted that these structures evolved in modern eukaryotes to maintain the unique lipid composition of cellular membranes in the face of extensive vesicular trafficking [8].

Since then, technical breakthroughs have significantly advanced our understanding of inter-organelle communication, revealing heterotypic MCS (interactions between two different organelles) across virtually all organelles and cell types, thereby creating an intricate, interconnected web of functional interactions [9] (Fig. 1). Over the past decade, the diversity of identified contact sites has grown substantially, unveiling novel molecular mechanisms that facilitate contact formation and revealing unexpected functional roles. Among organelles, the ER stands out as the largest and most dynamic organelle, making it unsurprising that the best-characterized MCS involve ER tubules [10, 11]. ER-mitochondria contact sites are implicated in calcium (Ca^{2+}) transfer, lipid exchange, mitochondrial fission, autophagy, inflammation and the integration of apoptotic stimuli [12–23]. ER-PM junctions regulate lipid metabolism, store-operated Ca^{2+} entry (SOCE) and autophagy [24–31], while ER-Golgi apparatus (GA) contacts play critical roles in vesicle trafficking and lipid exchange [32–34]. ER-lysosome (ER-Lys) interactions coordinate lipid metabolism and facilitate Ca^{2+} exchange [35, 36], while ER-lipid droplets (ER-LD) interfaces are essential for LD biogenesis [37–41]. Endosomal contacts with the ER regulate cholesterol trafficking and endosome fission [10, 42, 43], and ER-peroxisome (PO) connections are essential for PO division and lipid handling [44, 45]. In the mitochondria field, the last 15 years have seen an explosion in contact site studies. Interactions between mitochondria and Lys regulate mitophagy and Ca^{2+} homeostasis [46, 47], while mitochondria-LD contacts influence metabolic states [48–50]. Mitochondria-nucleus MCS enable retrograde signalling and phospholipid transfer [51, 52], and mitochondria-GA contacts are linked to apoptotic pathways [53]. Mitochondria-endosome interfaces regulate iron transfer and serve as mRNA translation platforms [54]. Mitochondria-PO MCS contribute to reactive oxygen species (ROS) metabolism and fatty acid oxidation [55], while mitochondria-PM contacts regulate mitochondrial shape [56]. Lys–GA interfaces mediate nutrient sensing [57], while Lys-PO contacts play a role in cholesterol trafficking [58]. Lys-LD MCS regulate LD degradation via stillunknown tether(s) [59]. Finally, PO-LD interfaces have been shown to facilitate lipid traffic

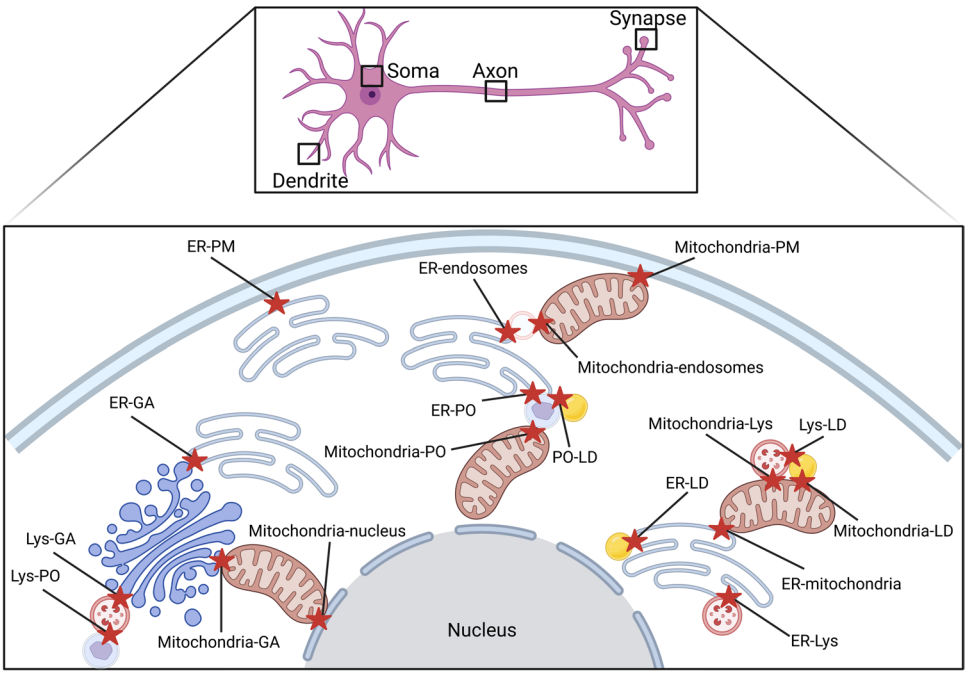

Fig. 1 The extensive landscape of membrane contact sites (MCS). Schematic overview illustrating the diversity of membrane contact sites (MCS) identified to date. The top panel shows the spatial architecture of a neuron, including the soma, dendrites, axon, and synaptic terminals. The lower panel depicts the intricate network of inter-organelle interactions, highlighting key MCS between the endoplasmic reticulum (ER), mitochondria, Golgi apparatus (GA), lysosomes (Lys), peroxisomes (PO), lipid droplets (LD), endosomes, the nucleus, and the plasma membrane (PM). Each red star indicates a heterotypic MCS observed in mammalian cells

[60]. Recently, homotypic contact sites have been identified between two PO [61] and two LD [62], as well as contact sites involving three organelles simultaneously, referred to as "three-way contacts".

As interest in contact sites grows and new concepts emerge, this review provides a comprehensive update on the current understanding of mammalian MCS biology. We focus on their functions and molecular components, including tethers and regulatory proteins, with a particular emphasis on their roles in neurons. This emphasis is motivated by a growing body of evidence suggesting that MCS are essential for neuronal development, supporting high metabolic demands, extensive signalling needs, and the elaborate architecture of these highly specialized cells, which must transmit information over long distances and integrate signals from thousands of synaptic inputs [63, 64]. Moreover, given the long lifespan of neurons, it is not surprising that MCS dysregulation is associated with neurodegenerative diseases [65, 66] and the progressive decline of cellular homeostasis during brain aging [67, 68]. Since these processes are multifactorial, and considering the current state of knowledge, it is impossible to address every aspect in this review. Therefore, we focus primarily on ER-PM, ER-mitochondria, and LD contact sites, describing the proteins that are directly linked to their formation and function, particularly those implicated in Alzheimer's disease (AD), and highlighting their emerging potential as targets for therapeutic intervention.

Definition and experimental approaches for studying membrane contact sites

MCS are defined as extended areas of close apposition between two membrane-bound compartments [69], typically maintained at a distance of 10–30 nm, although exceptions exist [70]. These sites are stabilized by molecular bridges known as tethers, specialized proteins that, by forming complexes with other proteins or directly interacting with membrane lipids, play a dual role: they establish and regulate MCS while preventing membrane fusion [69]. Indeed, unlike incidental associations or SNARE (soluble NSF attachment protein receptor)-mediated fusion events, MCS can be either stable or transient, yet consistently serve as biological "hotspots" that enable functional crosstalk between organelles [9]. This crosstalk fulfils specific biological functions essential for cellular homeostasis and depends on the enrichment of specific lipids, proteins, and enzymes at a certain MCS [9, 69, 71].

To elucidate MCS composition, researchers have significantly expanded the experimental toolkit, using a wide range of methods including EM, biochemical fractionation, co-immunoprecipitation, affinity chromatography, proximity enzymatic biotinylation and mass spectrometry [72]. Emerging high-resolution approaches, such as cryo-electron tomography (cryo-ET), which enables label-free imaging of macromolecular assemblies in their native cellular context with resolutions of 4–10 nm [73, 74], and focused ion-beam scanning electron microscopy (FIB-SEM), offering resolutions below 10 nm in all planes [75–77], are expected to become the techniques of choice for visualizing MCS in the near future. However, many of these methods require fixed and processed samples, making them unsuitable for capturing the transient nature of organelle contact sites in living cells. For this reason, fluorescence microscopy, combined with genetically encoded organelle-specific markers, has remained the gold standard for several years. While super-resolution imaging techniques and atomic force microscopy offer higher precision for visualizing sub-diffraction-limit MCS, confocal microscopy, with its resolution limit of approximately 250 nm, remains widely used due to its ability to analyse temporal dynamics and lower technical demands [72]. Using this approach, MCS have been identified in combination with a number of proximity-based assays, where the activation of a bipartite reporter depends on close membrane association. Examples of such techniques include bimolecular fluorescence complementation (BiFC), as split-GFP [78, 79], and Förster Resonance Energy Transfer (FRET) [33, 80, 81]. Although these techniques allow live-cell imaging, they may introduce artefacts depending on irreversible complementation, protein overexpression or the need to calibrate experiment by rapamycin-induced dimerization of the FRET couple. To overcome these limitations, proximity-ligation assays (PLAs), which use antibody-oligonucleotide conjugates to probe endogenous proteins, are now frequently used [82, 83], though the readout might be affected by the expression levels of the target proteins and the specificity of selected antibodies. Recently, a series of reversible chemogenetic reporters, tailored from the splitFAST (split Fluorescence-activating and Absorption Shifting Tag) system, has been developed to monitor MCS dynamics in living cells and organisms [84]. Thanks to the combination of these probes with Ca^{2+}-sensing modules, it was possible to dynamically study MCS while simultaneously measuring the associated Ca^{2+} signals occurring at these nanodomains [84]. Importantly, splitFAST-based probes can also be used to monitor the recruitment of specific proteins at certain MCS [84], helping the definition of the molecular apparatus involved in the assembly/modulation of a particular MCS. We anticipate that combining this approach with CRISPR-Cas9 technology will enable the tracking of dynamics and functions of endogenous proteins at MCS with unprecedented resolution. Nevertheless, we [85] and others [69] have emphasized that

achieving a comprehensive characterization of contact sites requires a combination of multiple methodologies, including biochemical, fluorescence, and EM-based techniques.

To date, technological advances have revealed that MCS possess a unique protein and lipid composition. Tethering and spacer proteins form the structural framework of these sites, maintaining the appropriate intermembrane distance by anchoring to organelles or binding to proteins or lipids in the opposing membranes [86]. Functional proteins, such as ion channels, lipid transfer proteins, and metabolite transporters, are enriched at MCS and mediate key processes, like Ca^{2+} signalling and lipid exchange [87]. Additionally, sorting proteins regulate the recruitment and organization of MCS components, shaping the unique proteome and lipidome of each contact site [64, 88]. Regulatory proteins further control both the extent of contact and the activity of other proteins within the site. These diverse protein classes collectively orchestrate MCS formation and function. Many exhibit overlapping roles (*e.g.*, tethers can also act as lipid transporters or regulators), while others contribute to function without being involved in the formation of the contact site, or *vice versa*. Moreover, numerous MCS proteins also perform roles outside the contact site, complicating their study. This structural complexity is underscored by the observation that deleting a single tether typically reduces MCS abundance, rather than eliminating them entirely [69].

Despite significant progress, many aspects of MCS remain poorly understood. Numerous tethers are yet to be identified, and the lipid composition of MCS has received far less attention than their protein counterparts. Addressing these gaps with advanced techniques and suitable probes will be essential to uncover the spatiotemporal organization and dynamic roles of lipids at MCS.

Ca^{2+} and lipid exchange at ER-PM contact sites in neurons

ER-PM contact sites are increasingly recognized as key hubs for cellular communication, coordinating Ca^{2+} signalling, lipid transport, and membrane dynamics [89–91] (Fig. 2). These contacts were first observed by Keith Porter and George Palade using EM [4, 5] and were later extensively studied in the context of excitation–contraction (E-C) coupling in muscle cells [92]. In cardiac and skeletal muscle, specialized ER-PM contacts form dyad and triad junctions, respectively, where the sarcoplasmic reticulum (a muscle-specific ER subtype) contacts the tubular invaginations of sarcolemma (the PM in muscle cells) [93]. These sites enable rapid Ca^{2+} signalling through direct coupling of the dihydropyridine receptor (DHPR) on the PM with the ryanodine receptor (RyR) on the ER, providing a spatial

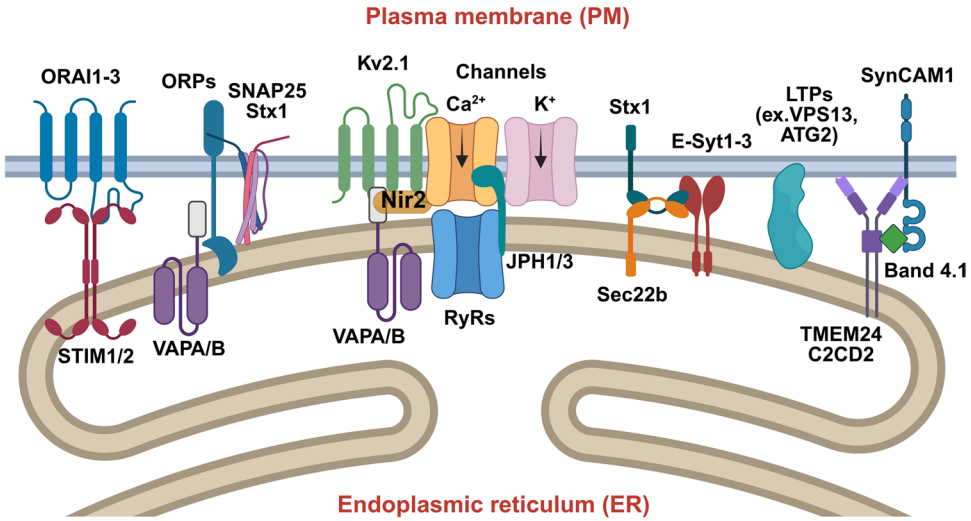

Fig. 2 Functional architecture of ER-PM contact sites in Ca^{2+} signaling and lipid transport. The cartoon illustrates the diversity of proteins enriched at endoplasmic reticulum-plasma membrane (ER-PM) contact sites, involved in Ca^{2+} homeostasis and lipid exchange. Store-operated Ca^{2+} entry (SOCE) is initiated upon ER Ca^{2+} depletion, leading to STIM1/2 oligomerization and their interaction with PM-localized Orai1-3 channels, facilitating Ca^{2+} influx. VAPA/B tethers mediate ER-PM contact formation via interactions with proteins carrying FFAT motifs, including oxysterol-binding protein-related proteins (ORPs), which coordinate lipid exchange. The lipid transfer protein Nir2 also associates with VAPs and contributes to phosphoinositide homeostasis. Kv2.1 channels function both as voltage-gated potassium channels and structural ER-PM tethers. They interact with ryanodine receptors (RyRs), forming a tripartite complex with junctophilins (JPH1/3) and supporting ER-PM Ca^{2+} crosstalk. Extended synaptotagmins (E-Syt1–3), via their Ca^{2+}-sensitive C2 domains and SMP domain, mediate lipid transfer at these MCS and form non-fusogenic SNARE complexes with syntaxin-1 (Stx1) and Sec22b. TMEM24 interacts with cytoskeletal adapters (Band 4.1) and synaptic adhesion proteins (SynCAM1), linking membrane lipid composition to neuronal excitability. Large lipid transfer proteins (LTPs), such as VPS13 and ATG2, mediate bulk lipid transport through extended hydrophobic bridges, addressing high membrane demand during neuronal growth and activity

platform for E-C coupling [92, 94–96]. Building on these pioneering studies, ER-PM contacts have since been identified in a variety of non-muscle cells, including neurons, where they are particularly abundant in cell bodies [97]. Here, they modulate neuronal excitability and lipid homeostasis during synaptic activity, axonal growth, and repair [98].

ER-PM contact sites as gatekeepers of neuronal Ca^{2+} signalling

A key example of Ca^{2+} signalling at ER-PM contacts is the SOCE, a Ca^{2+} entry pathway through PM channels triggered by ER Ca^{2+} depletion to replenish ER stores and prevent toxic cytoplasmic Ca^{2+} overload. The ER-resident stromal interaction molecule (STIM) proteins, STIM1 and STIM2, play central roles in SOCE by detecting ER Ca^{2+} depletion via their EF-hand domains (Fig. 2) [99, 100]. Upon Ca^{2+} depletion, STIM proteins undergo conformational changes, oligomerize, and translocate to ER-PM contact sites, where they interact with the PM Ca^{2+}-permeable Orai channels (Orai1-3), facilitating Ca^{2+} entry into the ER lumen [25, 26, 29, 30, 99–102]. Recent findings reveal that STIM1 organizes ER-PM contacts into specialized "ER ladder" structures in distal axons of developing neurons [103]. Additionally, acute ER Ca^{2+} depletion and ER stress increase presynaptic Ca^{2+} levels and glutamate release via a pathway involving STIM2 and the synaptic Ca^{2+} sensor synaptotagmin-7 [104]. Beyond its classical role in refilling ER Ca^{2+} stores, the SOCE machinery, which includes a broader network of soluble and membrane-bound proteins, is essential for neuronal functions at both pre- and post-synaptic sites, in both excitatory and inhibitory neurons, as well as in glial cells [105]. Indeed, SOCE plays a pivotal role in regulating basal synaptic transmission, dendritic spine morphology and synaptic plasticity, with inactivation of key SOCE components leading to learning deficits in animal models [105]. Furthermore, emerging evidence links SOCE dysfunction to aging and neurodegenerative diseases, including Alzheimer's disease (AD) and Huntington's disease (HD). For instance, selective STIM1 deletion in Purkinje cells reduces the levels of specific SOCE-associated proteins, such as Orai3 and the inositol 1,4,5-trisphosphate receptor (IP3R1), while downregulating ion transporters, leading to age-dependent changes in neuronal excitability [106]. In AD, STIM2 downregulation impairs SOCE and disrupts Ca^{2+} homeostasis, contributing to synaptic loss [107], while in HD, hyperactivation of SOCE leads to cytosolic Ca^{2+} overload and synaptic dysfunction [108]. Familial AD (FAD) cellular models expressing either presenilin 1 or 2 (PS1 or PS2) mutations show a blunted SOCE [109–113], likely due to a reduced expression level of STIM1 found in mutant PS-expressing cells, human FAD fibroblasts, and in the brain of sporadic AD patients [112–114]. Accordingly, modulating SOCE activity has been proposed as a therapeutic strategy to restore synaptic function and mitigate neurodegeneration [105].

Lipid homeostasis at neuronal ER-PM contact sites

In addition to Ca^{2+} signalling, ER-PM contact sites play a central role in lipid transport, particularly in neurons, where they meet the high PM synthesis demands during axonal growth and repair [63, 64, 90]. Among model systems where lipid exchange at ER–PM contact sites has been extensively characterized, *Drosophila* photoreceptors represent a prominent example [115]. These primary sensory neurons transduce light into electrical signals and are structurally specialized to maximize photon capture. To sustain their activity, they contain specialized smooth ER subdomains called sub-microvillar cisternae (SMC), which lie within ~ 10 nm from the PM rich in rhodopsin receptors [116]. Phototransduction here depends on the non-vesicular transfer of lipids, including phosphatidylinositol (PI) and phosphatidic acid (PA). The SMC are enriched in lipid transfer proteins, such as RDGBα (also known in mammals as PITPNM1/Nir2) [117], which shuttles PI and PA during G-protein-mediated $PI(4,5)P_2$ hydrolysis, and the diacylglycerol kinase DGK, which phosphorylates diacylglycerol (DAG) to generate PA [118]. Loss of RDGBα or DGK impairs lipid turnover and leads to light-dependent retinal degeneration [118, 119].

In mammalian cells, lipid identity at ER-PM contacts is maintained by tether proteins such as the vesicle-associated membrane protein (VAMP)-associated proteins A and B (VAPA and VAPB). Anchored to the ER by their transmembrane domains, these proteins contain a cytosolic Ig-like major sperm protein (MSP) domain and a coiled-coil region involved in dimerization [28]. The MSP domain interacts with a specific short peptide motif known as the FFAT motif, found in more than 100 proteins across various cell types and collectively referred to as the "VAPome" [28]. These interactions not only stabilize ER-PM contacts but also support other inter-organelle contacts, such as ER-GA and ER-mitochondria connections, highlighting the pleiotropic roles of VAPs in inter-organelle communication [28]. At ER-PM contacts, VAPA/B interact with oxysterol-binding protein (OSBP)-related proteins (ORPs), a family of lipid transfer proteins (Fig. 2) [120, 121]. ORPs facilitate the countertransport of specific lipids, such as newly synthesized cholesterol and phosphatidylserine (PS) from the ER, in exchange for phosphatidylinositol 4-phosphate (PI4P) at the PM [120–122]. While most of the 12 ORP homologues target the ER through FFAT-mediated

VAPA/B interaction, a subset contains PH domains that directly bind phosphoinositides [120, 121]. In neurons, ORP6 and ORP3 facilitate the exchange of PI4P and PS, while ORP2 regulates PM cholesterol and neurotransmitter release through interactions with the PM-localized SNARE proteins syntaxin 1A and SNAP-25 (Fig. 2) [123]. VAPs also interact with the lipid transporter Nir2 [124], promoting the clustering of phosphorylated voltage-gated potassium (Kv) channels and enhancing neuronal excitability via a voltage-independent mechanism (Fig. 2) [27, 125, 126]. Despite lacking a typical FFAT motif, Kv2.1's C-terminal VAP-binding domain is essential for enabling ER Ca^{2+} uptake during electrical activity [125]. Additionally, Kv2.1 channels also facilitate the coupling between PM L-type Ca^{2+} channels and ER RyRs, as well as the recruitment of Nir2 to regulate phosphoinositide homeostasis [127, 128]. Increased levels of nitrosylation and oxidation of RyRs, accompanied by a decrease in the channel-stabilizing subunit calstabin1, have been observed in the skeletal muscle of aged mice, resulting in elevated ROS production and ER/PM Ca^{2+} leakage [129]. Given the critical role of RyRs in Ca^{2+} signalling, it would be informative to explore whether similar mechanisms contribute to aging-related changes in the brain. Kv2.1 channels also associate with the type I protein kinase A (PKA) signalosome [130], linking membrane depolarization to PKA activity. Similarly, neuronal ER-localized Juctophilin isoforms (JPH1, 2, 3) stabilize a tripartite complex of Cav1 Ca^{2+} channels, RyR2, and KCa3.1 potassium channels at ER-PM contacts (Fig. 2) [131, 132]. These junctions, periodically arranged along dendrites and stabilized by JPH3, serve as microdomains for Ca^{2+} influx and release, supporting long-distance neuronal signalling and plasticity [133]. Interestingly, a recent study showed that JPHss, through interactions with a family of curvature-sensing proteins containing the Eps15 homology domain, display a preference for curved regions of the plasma membrane. The relevance of this mechanism in neurons remains to be fully investigated [134]. Nevertheless, consistent with their essential functions in neurons, both VAPA/B and JPH3 have been linked to progressive neurodegenerative diseases, such as Amyotrophic Lateral Sclerosis (ALS) and Parkinson's disease (PD) [98].

The interplay between neuronal lipid transfer and Ca^{2+} handling at ER-PM contacts is further illustrated by proteins such as extended-synaptotagmin 2 (E-Syt2) and transmembrane protein 24 (TMEM24) (Fig. 2). E-Syt2, a member of the evolutionarily conserved extended synaptotagmin (E-Syt1/2/3) family, contains Ca^{2+}-binding C2 domains and a synaptotagmin-like mitochondrial lipid-binding protein (SMP) domain, which dimerizes to form hydrophobic tunnels that transfer lipids like diacylglycerol (DAG) between the ER and PM under elevated cytosolic Ca^{2+} levels [24]. E-Syt2 also forms a tripartite complex with the ER-resident SNARE protein Sec22b and syntaxin-1 (Stx1), tethering the axonal ER to the PM and supporting lipid transport for PM expansion [135]. Unlike traditional SNARE complexes that mediate membrane fusion, the Sec22b-Stx1 assembly at ER-PM contact sites is non-fusogenic. Instead, it acts as a short-range tether, maintaining a precise distance (~10 nm) between the ER and PM. In line with this, overexpression of E-Syt2 promotes axonal branching and filopodia formation, effects dependent on both Sec22b and Stx1 [135]. Interestingly, E-Syt1 silencing in hippocampal neurons impairs synaptic plasticity [136], and E-Syt2- and E-Syt3-mediated ER-PM contacts have also been implicated in autophagy [31]. Despite their significant roles, E-Syt triple knockout (E-Syt1/2/3 KO) mice exhibit no changes in ER morphology or neuronal survival, reflecting the functional redundancy of tether proteins [137]. In contrast, loss of *Drosophila* extended synaptotagmin (dEsyt) leads to a reduction in ER-PM contacts and mislocalization of the phosphatidylinositol transfer protein RDGB, ultimately resulting in progressive retinal degeneration [138]. In *Drosophila* photoreceptors, Ca^{2+} binding is a prerequisite for dEsyt function [139]. Instead, TMEM24 and its paralogue C2CD2, by interacting with band 4.1 family members and cell adhesion molecules, such as SynCAM1, regulate phosphatidylinositol (PI) transport from the ER to the PM in response to changes in cytoplasmic Ca^{2+} levels (Fig. 2), potentially linking lipid dynamics with neuronal excitability [140, 141].

Emerging evidence highlights the roles of large lipid transfer proteins (LTPs) in mediating bulk lipid transport by a shuttle mechanism between MCS involving the ER, PM, and other organelles, addressing the extensive membrane demands of growing neurons [64, 142]. These proteins include: VPS13, ATG2, the Hob proteins, Tweek/Csf1/KIAA1109 and SHIP164 [64]. Most LTPs function as lipid bridges, enabling continuous lipid flow between organelles without vesicular transport (Fig. 2). This process involves a hydrophobic groove along the protein's rod-like structure, allowing lipids to slide directly from one bilayer to another, bypassing the cytosol thereby enhancing efficiency [143]. Beyond their bridge-like transport capabilities, many LTPs operate in conjunction with other proteins to maintain membrane asymmetry and homeostasis. For example, SHIP164 has been implicated in endosomal lipid dynamics and may contribute to maintaining lipid balance across organelles [144]. Mutations in these proteins are linked to several neurological disorders, including PD (VPS13C), chorea-acanthocytosis (VPS13A), ataxia (VPS13D), Cohen syndrome (VPS13B) and Alkuraya-Kučinskas syndrome (KIA1109), further emphasizing the clinical relevance of ER-PM contact sites [64].

Despite being the most well-characterized MCS, ER-PM contacts remain an active focus of investigation. Key unanswered questions include the extent of functional redundancy among tethering proteins, their tissue-specific roles, and how these structures adapt under stress conditions.

The multifaced roles of ER-mitochondria contact sites: functions and molecules

Contact sites between both smooth and rough ER and the outer mitochondrial membrane (OMM), known as mitochondria-ER contact sites (MERCS), define specialized subcellular regions called MAMs (Fig. 3). Highly responsive to the cellular metabolic state, these structures play a crucial role in preserving the distinct architecture and function of both organelles while supporting their communication during key cellular processes, including Ca^{2+} homeostasis [145], lipid metabolism [7], mitochondrial dynamics [146], ER stress response [147], autophagy regulation [148], apoptosis induction [149, 150] and the inflammatory response [151]. In neurons, MAMs are particularly abundant in the soma, neurites and synaptic regions [97], where they are indispensable for proper synaptic transmission, axonal growth, and energy supply [63, 65]. Given that MAM alterations have been implicated in several neurodegenerative disorders, including AD, PD and ALS, and that mitochondrial dysfunction is considered one of the nine hallmarks of aging [67], there is increasing interest in therapeutic strategies aimed at modulating MAM levels and functions. For example, a high-throughput screening of 1,200 FDA-approved compounds identified luteolin as a promising candidate that enhances ER-mitochondria contacts, promoting mitochondrial Ca^{2+} uptake and improving locomotor activity in an HD model by boosting Ca^{2+}-dependent pyruvate dehydrogenase activity [152].

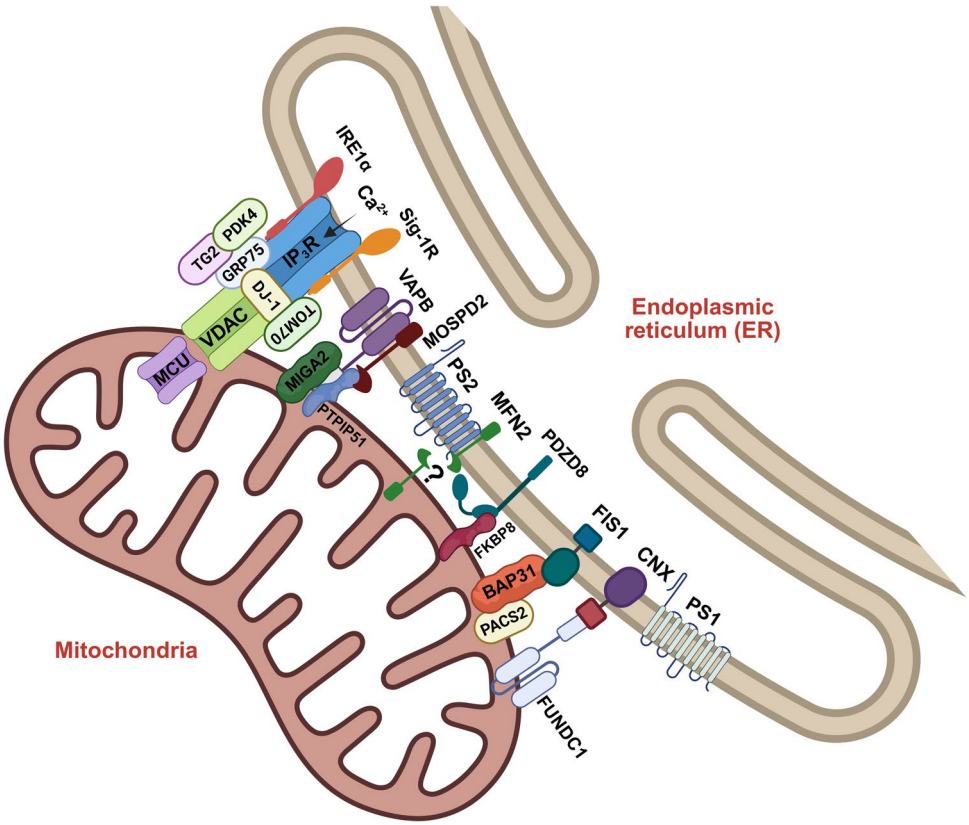

Fig. 3 Structural and regulatory proteins of ER-mitochondria contact sites. Overview of key proteins and complexes enriched at mitochondria-associated membranes (MAMs), which coordinate Ca^{2+} transfer, lipid exchange, mitochondrial dynamics, and stress responses. Central to Ca^{2+} signaling, the ER-localized inositol 1,4,5-trisphosphate receptors (IP3Rs) release Ca^{2+} upon stimulation, transferring it to mitochondria through the IP3R-GRP75-VDAC1 complex, allowing organelle Ca^{2+} uptake mediated by the mitochondrial Ca^{2+} uniporter (MCU) complex. This process is fine-tuned by additional components, including DJ-1, TG2, PDK4, TOM70, IRE1α, and Sig-1R. Close apposition between the ER and mitochondria is also modulated by homotypic or heterotypic interactions between MFN2 or MFN1, as well as by PDZD8 and VAPB. Through interactions with PTPIP51, MOSPD2 and MIGA2, these proteins facilitate not only tethering but also phosphatidylserine (PS) and IP3R-mediated Ca^{2+} transfer. PDZD8 additionally mediates Ca^{2+} shuttling, while FIS1, BAP31, PACS-2, FUNDC1 and CNX link MAMs to apoptotic and autophagic signaling

Molecular mechanisms of Ca^{2+} exchange at ER-mitochondria contact sites

One of the primary roles of MERCS is the regulation of Ca^{2+} flux between the ER, the main intracellular Ca^{2+} store, and mitochondria, which rely on Ca^{2+} for metabolic bioenergetics. This transfer is primarily regulated by two key ER-resident proteins: the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), which actively pumps Ca^{2+} from the cytosol into the ER lumen to maintain steady-state ER Ca^{2+} concentration, and the inositol 1,4,5-trisphosphate receptors (IP3Rs), which release Ca^{2+} from the ER upon IP3-generating stimuli [19, 22, 23]. Notably, IP3Rs are also required to maintain ER-mitochondrial contacts, independently of Ca^{2+} fluxes [153]. IP3Rs form a tethering complex with the voltage-dependent anion channels (VDAC1) on the OMM, an interaction facilitated by the cytosolic molecular chaperone glucose-regulated protein 75 (GRP75) [12]. Upon IP3R-mediated ER Ca^{2+} release, this molecular complex and other ER-mitochondria tethering complexes enriched at MAMs allow the formation of nanodomains with high Ca^{2+} concentrations (defined as "hot spots") at the ER-mitochondria interface [80, 154]. These regions enable efficient Ca^{2+} uptake by the low-affinity mitochondrial Ca^{2+} uniporter complex (MCUC) located in the inner mitochondrial membrane (IMM) [22], thereby stimulating mitochondrial ATP production through activation of Krebs cycle enzymes and metabolite transporters [155]. Additional proteins, such as translocase of the outer membrane (TOM) protein 70 (TOM70), the ER stress sensor inositol-requiring enzyme 1 (IRE1α), transglutaminase type 2 (TG2), the ER sigma-1 receptor (Sig-1R), and pyruvate dehydrogenase kinase 4 (PDK4), interact with components of the IP3R-GRP75-VDAC complex, further refining its regulatory functions (Fig. 3) [13, 156–159]. In neurons, this ER-to-mitochondria Ca^{2+} transfer supports retrograde mitochondrial movement, neurite elongation, and axon regeneration following injury [160, 161]. Accordingly, loss of any of these proteins impairs essential processes, such as cellular bioenergetics, autophagy and cell proliferation [13, 156–159]. The IP3R-GRP75-VDAC complex also interacts with the PD-associated deglycase DJ-1 and α-synuclein. DJ-1 depletion results in reduced ER-mitochondria MCS, decreasing Ca^{2+} transfer between these organelles [14, 162, 163].

While the IP3R-GRP75-VDAC complex is the best-characterized pathway for Ca^{2+} release at MERCS, especially in non-excitable cells [164], the potential role of RyRs (previously discussed in the ER-PM contact sites section), should also be considered in neurons. Given their high expression in many neuronal subtypes, RyRs may contribute to ER-mitochondria Ca^{2+} exchange, particularly at synapses, although direct evidence for this mechanism remains limited.

In addition to its well-accepted role in mitochondrial fusion, Mitofusin 2 (MFN2) also regulates ER-mitochondria tethering (Fig. 3). Unlike its paralog MFN1, a fraction of MFN2 localizes to the ER [165], where it is proposed to tether the ER and mitochondria via homotypic or heterotypic interactions with MFN2 or MFN1, respectively, on the OMM [17]. Early studies supported this tethering role, linking MFN2 to MAM-regulated processes such as autophagosome formation [166]. However, more recent studies have challenged this view, showing that loss of MFN2 leads to increased ER-mitochondria contacts, as revealed by EM and functional assays measuring Ca^{2+} transfer at MAMs [78, 167–171]. Despite this, MFN2 is widely considered a modulator, though not essential, of ER-mitochondria tethering, and its precise function remains an active area of investigation. In neurons, MFN2 overexpression or conditional knockout impairs Ca^{2+} uptake and disrupts IP3R-GRP75 interactions [172] and it is downregulated in post-mortem brains from AD patients [173]. Mutations in MFN2, linked to Charcot-Marie-Tooth disease type 2A (CMT2A), a disorder characterized by progressive axonal degeneration, disrupt ER-mitochondria contacts resulting in mitochondrial fragmentation, defective axonal transport, and progressive neuropathy [174]. Similarly, PD-associated mutations in the mitochondrial adaptor protein Miro1 (RHOT1) reduce MAM formation in fibroblasts derived from PD patients [175], while PINK1/Parkin-mediated phosphorylation and ubiquitination of MFN2 destabilizes MAMs [176]. Conversely, Parkin overexpression strengthens ER-mitochondria coupling and Ca^{2+} exchange [177]. Although contrasting evidence exists regarding the precise role of MFN2 in ER-mitochondria apposition, a promising approach has emerged from the use of a cell-permeant minipeptide that, by altering MFN2 conformation, enhances organelle tethering and mitochondrial fusion without interfering with MFN2 GTPase activity [178]. Although it has been demonstrated that this strategy can reverse mitochondrial fragmentation and depolarization in both MFN2-deficient fibroblasts and neurons carrying a pathogenic CMT2A-linked MFN2 mutation [178], its effect on ER-mitochondria contacts remains to be investigated.

Another well-characterized ER-mitochondria tethering complex involves VAPB and the tyrosine phosphatase interacting protein-51 (PTPIP51), which facilitates IP3R-mediated Ca^{2+} transfer from the ER to mitochondria, supporting mitochondrial ATP production and regulating autophagy (Fig. 3) [16, 179]. Similar to VAPB, the ER-resident motile sperm domain-containing protein 2 (MOSPD2), which also binds PTPIP51 via its MSP domain, functions as a master regulator of multiple contact sites across organelles [180]. In neurons, VAPB and PTPIP51 co-localize in synaptic regions, where their interaction is enhanced by

neuronal activity and is crucial for synaptic transmission [181]. Accordingly, loss of this complex reduces dendritic spine density and impairs synaptic functions [181]. Notably, their expression is decreased in the temporal cortex of AD patients [182], and PD-linked α-synuclein mutations disrupt this tethering, impairing Ca^{2+} homeostasis and ATP production [183]. While the VAPB-PTPIP51 axis is also implicated in ALS and frontotemporal dementia (FTD) [16], further studies are needed to determine whether MAM dysfunction is a common feature across different cohorts. However, both wild-type and ALS/FTD-associated mutant TDP-43 have been reported to disrupt VAPB-PTPIP51 interaction [184], and overexpression of either protein restores synaptic function in TDP-43 disease models [185]. From a therapeutic perspective, a small high-throughput screen identified LCD3 (also known as Dynarrestin) as a compound with high affinity for PTPIP51, which enhances the VAPB-PTPIP51 interaction [186]. Although the impact of LCD3 on MERCS ultrastructure and function has not been investigated, future studies will be important to clarify this and to evaluate its potential effects on neuropathies, particularly ALS, where the VAPB-PTPIP51 interaction is disrupted. Alongside these major players, the PDZ domain-containing protein 8 (PDZD8), located at the ER, and VPS (discussed earlier) have been identified as important regulators at MAMs in neurons [187, 188], where PDZD8 directs ER Ca^{2+} released by IP3Rs or RYRs toward mitochondria (Fig. 3). This process is essential for dendritic excitability and synaptic plasticity [187]. Although its mitochondrial binding partner remained unknown for some time, the OMM protein FKBP8 has recently been identified as a PDZD8 interactor [189]. Consistently, PDZD8 deletion disrupts ER-mitochondria communication, leading to excessive cytosolic Ca^{2+} accumulation and neuronal dysfunction [187], whereas FKBP8 overexpression is sufficient to narrow ER-mitochondria distance [189].

ER-mitochondria coupling in Alzheimer's disease and aging

Alterations in the structure and/or functions of MAMs have emerged as a common feature in different neurodegenerative diseases and during brain aging [190], suggesting that disruptions of these MCS may actively contribute to neurodegeneration rather than being mere secondary consequence of disease progression. In the context of AD, three genes frequently mutated in FAD encoding for amyloid precursor protein (APP), PS1, and PS2, are involved in Aβ peptide generation [191]. Aβ peptides are produced through proteolytic cleavage of APP by the γ-secretase complex, an intramembrane aspartyl protease in which PSs (PS1 or PS2) serve as the catalytic core [191]. Despite the central role of Aβ accumulation in AD pathogenesis, pharmacological strategies targeting amyloid plaques have largely failed in clinical trials [192]. As a result, the discovery that PSs are enriched at MAMs (Fig. 3) [193] and that FAD-linked PS2 mutants increase ER-mitochondria physical coupling and enhance Ca^{2+} transfer efficiency in different cellular models [194], have shifted focus toward MAM dysfunction as a potential driver of AD pathology. Several studies reported that, post-mortem human AD brains, APP transgenic mice, primary hippocampal neurons treated with $Aβ_{42}$, and fibroblasts from both familial and sporadic AD patients display upregulated ER-mitochondria contacts and elevated levels of VDAC1 and other MAM-associated proteins [195, 196]. Similarly, decreased expression of mitochondrial Ca^{2+} influx transporter genes and upregulation of efflux pathway components have been observed in post-mortem AD human brains, likely reflecting a compensatory response to limit mitochondrial Ca^{2+} overload, thereby potentially linked to altered ER-mitochondria contact sites [197]. Instead, reduced Sig-1R levels, observed in primary hippocampal neurons from 3xTG-AD exposed to $Aβ_{25-35}$, increase ER-mitochondria distance and impair IP3-dependent Ca^{2+} transfer [198]. Interestingly, Aβ exposure increases MAM formation and Ca^{2+} transfer in young rat hippocampal neurons, but reduces them in aged neurons, mirroring the age-related decline in mitochondrial Ca^{2+} handling seen in AD [199]. Moreover, in two animal models mimicking HD, the expression levels of GRP75, IP3R and MFN2 significantly decline with age, suggesting a possible disruption of ER-mitochondria contacts [200]. Additionally, FAD-associated PS1 and PS2 mutations enhance IP3R channel gating kinetics [201]. PS2 also strengthens ER-mitochondria contacts [194, 202, 203] via its cytosolic loop [204] and interaction with MFN2 (Fig. 3) [203], thereby antagonizing MFN2's inhibitory effect on organelle tethering [167]. These effects are exacerbated by FAD-linked PS2 mutations, resulting in further MAM upregulation [203]. In addition, the same mutations in PS2 impair mitochondrial functions by disrupting Ca^{2+} signalling and displacing hexokinase 1 (HK1) from mitochondria in a GSK-3β-dependent manner [205, 206]. Of note, the flavonoid luteolin, recently identified through a high-throughput screen for mitochondrial modulators, enhances mitochondrial respiration and ATP production in human neuroblastoma cells by increasing ER-mitochondria Ca^{2+} transfer via IP3Rs at MAMs [152], highlighting the critical role of MCS, and the associated Ca^{2+} signal, for mitochondrial function and neuronal health. Finally, FAD-PS2 mutations also interfere with autophagosome-Lys fusion, thus blocking the autophagy flux by reducing ER Ca^{2+} content and dampening cytosolic Ca^{2+} rises upon IP3-generating stimulations [207].

Compelling evidence also suggests that MAMs serve as platforms for Aβ production. Although APP cleavage by γ-secretase has traditionally been linked to the GA and PM, emerging studies show that multiple components of this pathway, including APP, PS1, PS2, and γ-secretase activating proteins (*e.g.*, GSAP), are enriched at MAMs [196, 208–211], supporting the hypothesis that a significant portion of Aβ production may occur directly at ER-mitochondria interfaces. The biochemical and structural properties of MAMs also appear to influence γ-secretase activity and Aβ generation. For instance, deletion of the γ-secretase activating protein GSAP has been shown to affect the cellular lipid landscape, thereby restoring cognitive behavior in an AD animal model [211]. Moreover, MAM upregulation, such as by MFN2 knock-down, reduces Aβ production in HEK293 cells expressing FAD-linked APP mutations [169]. Interestingly, elevated glucocorticoid levels, a known stress-related AD risk factor, upregulate MAMs and increase PS1 recruitment to these sites, thereby enhancing γ-secretase activity and Aβ production, ultimately contributing to mitochondrial dysfunction [212]. Further supporting the role of MAMs in Aβ metabolism, a 3D neural cell culture AD model showed that palmitoylation of APP promotes its localization to MAMs, influencing APP trafficking to the PM and modulating β-secretase activity, particularly in axons and neurites [213]. Together, these findings reveal a bidirectional relationship: on the one hand, MAMs serve as central hubs for Aβ production, while on the other, Aβ itself can modulate ER-mitochondria contacts.

In conclusion, given their pivotal roles in Ca^{2+} signalling, mitochondria dynamics, and proteostasis, it is not surprising that MAM dysfunctions have been implicated in neurodegenerative diseases and brain aging, emphasizing the urgent need for further research to uncover potential therapeutic targets. For example, in a therapy-induced senescence model, increased ER-mitochondria contacts were observed alongside reduced Ca^{2+} flux, attributed to a diminished interaction between IP3R and VDAC1 [214]. Supporting the therapeutic potential of modulating MAM function, pharmacological activation of Sig-1R using selective agonists has shown promise in models of neurodegeneration. Notably, pridopidine, a high-affinity Sig-1R agonist is capable of rescuing IP3R-dependent Ca^{2+} release in HD models, including patient-derived induced pluripotent stem cells (iPSCs) [215]. Increased mitochondrial respiration, reduced ROS production, and improved antioxidant responses have also been reported following pridopidine treatment in striatal neurons from HD mouse models expressing the human mutant form of huntingtin [216]. These mice also showed improved motor coordination upon treatment, suggesting a delay in symptom onset [216]. In AD, pridopidine exhibits strong positive effects, notably preventing the loss of mushroom spines which correlates with cognitive impairment, and rescuing long-term potentiation deficits induced by Aβ42 oligomers in hippocampal slices [217]. These effects depend on Sig-1R activity and involve the restoration of ER Ca^{2+} content and enhanced SOCE in dendritic spines [217]. Nevertheless, although pridopidine targets the MERCS-resident protein Sig-1R, future studies are needed to unravel the modulation of MAM function upon agonist treatment. In contrast, while the role of the antihyperglycemic drug metformin in normalizing the number of upregulated MERCS in a model of Duchenne muscular dystrophy [218], and that of sulforaphane in improving disrupted ER-mitochondria interactions in hepatocytes isolated from obese mouse models are well established [219], similar studies in neurodegenerative diseases are lacking. Nevertheless, whether MAM alterations act as primary drivers, secondary consequences, or compensatory mechanisms in disease progression remains unclear. Clarifying these dynamics is critical for identifying new therapeutic targets.

Lipid regulation at ER-mitochondria contact sites

MAMs also represent highly specialized subdomains that play a critical role in regulating intracellular lipid metabolism (Fig. 3) [220]. Characterized by a unique lipid composition enriched in cholesterol, sphingolipids, and saturated phospholipids, MAMs act not only as structural platforms for ER-mitochondria tethering but also as functional hubs for non-vesicular lipid exchange and lipid production. Among the key proteins that regulate lipid transport at MAMs, the OMM protein Mitoguardin-2 (MIGA2) has emerged as a central player. By binding to VAPB, MIGA2 localizes to ER-mitochondria and mitochondria-LD contact sites and facilitates the transfer of PS, thereby maintaining mitochondrial membrane integrity and dynamics [221–224]. Lipid transport at MAMs is also mediated by VPS13D, which interacts with VAPs on the ER and Miro proteins on mitochondria and PO [188]. Importantly, their unique lipid composition, resembling lipid rafts, provides an optimal environment for the recruitment and activity of lipid-processing enzymes. These include acyl-CoA:cholesterol acyltransferase 1 (ACAT1/SOAT1), which esterifies cholesterol into cholesteryl esters (CE) [225], followed by their deposition in LDs; DGAT2, which catalyzes the final step of triacylglycerol (TAG) synthesis and LD formation [226]; the fatty acid CoA ligase 4 (FACL4/ACSL4), which activates long-chain fatty acids (FAs) for incorporation into complex lipids or protein acylation [227]. Additionally, phosphatidylserine synthases 1 and 2 (PSS1 and PSS2) convert PC and phosphatidylethanolamine (PE) into PS in the ER. This PS is subsequently transferred to mitochondria, decarboxylated into PE, and then shuttled back to the ER, where it is methylated by

phosphatidylethanolamine N-methyltransferase 2 (PEMT2) to regenerate PC [228, 229]. In addition to phospholipids, cholesterol metabolism at MAMs has gained significant attention, particularly in the context of neurodegenerative diseases [230]. While the bulk ER membrane is low in cholesterol, it accumulates at MAMs, where ACAT1 catalyzes free cholesterol conversion into CE [231]. Under stress conditions, this esterification serves as a buffering mechanism to prevent cholesterol toxicity and preserve cellular homeostasis [231, 232]. The regulation of MAM lipid composition, particularly of cholesterol, sphingolipids, and saturated phospholipids, is orchestrated by sphingomyelinases (SMases) and various phospholipase enzymes [233]. Moreover, ER lipid raft-associated proteins 1 and 2 (ERLIN1/2), which localize at MAM raft-like microdomains, contribute to cholesterol homeostasis by modulating the activity of sterol regulatory element-binding proteins (SREBPs) and associated factors, including SREBP cleavage-activating protein (Scap) and insulin-induced gene 1 (Insig1) [234, 235]. Under cholesterol-depleted conditions, the SREBP-Scap complex associates with COPII-coated vesicles and is transported to the GA, activating FA biosynthesis. Conversely, when cholesterol levels are high, ERLINs stabilize the SREBP-Scap-Insig1 complex at the ER, thereby suppressing cholesterol synthesis [236]. In agreement with the importance of cholesterol in brain physiology, ε4 variant of apolipoprotein E (APOE), a cholesterol/lipid transporter in the central nervous system, is an important risk factor for AD development [237] and alterations in lipid composition have been observed in different AD cell models, as well as in plasma and frontal cortex of AD patients (for an extensive review see [196]). Moreover, in different AD models, cholesterol accumulation, increased ACAT1 activity, and elevated CE deposition have been observed, potentially contributing to tau hyperphosphorylation, impaired Aβ processing, and LD accumulation (reviewed in [196]). Intriguingly, emerging evidence suggests that cholesterol dysregulation in AD may underlie both ER-mitochondria tethering defects and local Aβ generation. For instance, in fibroblasts from an AD patient carrying a PS2 mutation, which enhances ER-mitochondria contacts, increased LD formation was normalized by genetic correction of organelle juxtaposition, reinforcing the role of MAMs in cholesterol handling and ACAT1 regulation [204]. Moreover, cholesterol itself appears capable of modulating ER-mitochondria contacts, with high levels promoting membrane contact site formation [231]. Interestingly, APP, and particularly its C99 fragment, has been proposed to function as cholesterol sensor. The C99 domain contains a cholesterol-binding motif and may influence the subcellular distribution of cholesterol, stabilizing MAMs [209, 238, 239]. Indeed, increased levels of C99 fragments, arising from FAD-APP mutations or cholesterol overload, may enhance MAM stabilization and disturb local lipid composition, thereby promoting Aβ generation at these sites [196]. This aligns with findings that γ-secretase activity is enriched in lipid rafts and highly sensitive to membrane lipid composition [240, 241], potentially contributing to the altered APP cleavage and the formation of aggregation-prone Aβ species. Furthermore in neurons, lipid rafts facilitate β-secretase-mediated cleavage of palmitoylated APP, and MAM upregulation enhances APP trafficking to the PM, where β-secretase cleavage and Aβ production are increased [213].

Given its critical involvement in AD pathogenesis, regulating MAM lipid composition, particularly cholesterol metabolism, is emerging as a promising therapeutic strategy. Statin, which inhibit cholesterol biosynthesis, and ACAT1 inhibitors have been shown to attenuate AD phenotypes in various in vitro and in vivo models [242, 243]. More recently, a study has identified that the ATPase family AAA-domain containing protein 3 A (ATAD3A), which oligomerizes and accumulates at MAMs in AD, drives cholesterol buildup at these sites. This, in turn, exacerbates AD-related alterations in APP processing and synaptic dysfunction. Inhibiting ATAD3A oligomerization, either pharmacologically or genetically, restores brain cholesterol homeostasis and alleviates AD pathology in 5xFAD transgenic mice [244], further emphasizing the central role of MAM lipid remodeling in AD progression.

The role of MAMs in mitochondrial dynamics and quality control, ROS production and cell death

MAMs also regulate the balance between mitochondrial fusion and fission, processes essential for proper mitochondrial distribution and function within cells [245]. At fission sites, ER tubules wrap around mitochondria, marking division points and facilitating the scission process [20, 246]. At these sites, ER-associated proteins, such as inverted formin-2 (INF2), polymerize actin to stabilize ER-mitochondria contacts [247, 248]. Actin polymerization also initiates IMM constriction, a process supported by Ca^{2+} influx through VDAC1 channels [247, 248]. The OMM protein Spire1c, working in tandem with INF2, nucleates actin filaments, further stabilizing these contacts [247]. Dynamin-related protein 1 (DRP1) is another key player in mitochondrial fission, ensuring the proper inheritance of mitochondrial DNA (mtDNA) at specific subpopulations of these division sites [21, 249]. Mitochondrial fusion, which counteracts fission, also relies on ER-mitochondria contact sites. The ER marks future fusion points where the dynamin-associated GTPase mitofusins, MFN1 and MFN2, mediate OMM fusion, while OPA1 orchestrates IMM fusion [245]. Although both homotypic and heterotypic interactions between MFN1 and

MFN2 occur, homotypic interactions are required for successful fusion [17, 250]. These fusion events are spatially and temporally coupled with fission, as MFN1 and DRP1 puncta colocalize at ER-mitochondria contacts to regulate these dynamic events [245]. In addition, the ER membrane-localized lipid hydrolase ABHD16A, the p97-UBXD8 complex and the sphingolipid desaturase DEGS1 maintain lipid composition at MAMs, thereby controlling the assembly and function of both fission and fusion machineries [251–253].

MAMs also play a crucial role in linking mitochondrial fission to mitophagy, particularly under hypoxic conditions, in both neuronal and non-neuronal cell lines. Under hypoxia, the OMM protein FUN14 domain-containing protein 1 (FUNDC1) interacts with the ER membrane protein calnexin at MAMs to promote mitophagy (Fig. 3). Initially, FUNDC1 accumulates at MAMs, stabilizing its association with calnexin. As mitophagy progresses, this interaction weakens, exposing a cytosolic loop of FUNDC1 that recruits DRP1 at MAMs, thus facilitating autophagosome formation and the clearance of damaged mitochondria [254, 255].

Several studies revealed that Ca^{2+} signalling at MAMs also controls ROS production, a key event contributing to aging and several age-associated disorders [256, 257]. In this context, the 66-kDa isoform of the growth factor adapter protein Shc (p66Shc) translocates into the mitochondrial intermembrane space through the TIM/TOM complex, where it interacts with cytochrome c, inducing ROS production and triggering apoptotic pathways [258]. Interestingly, elevated levels of p66Shc have been observed in the MAM fraction of older animals [259] and in primary fibroblasts derived from centenarians [260]. In line with this, p66Shc KO mice exhibited extended lifespan [261], highlighting the role of MAM-associated ROS regulation in longevity.

Additionally, MAMs serve as platforms for apoptotic signalling (Fig. 3). In this context, the ER-resident chaperone B cell receptor-associated protein 31 (BAP31) interacts with mitochondrial fission protein 1 (FIS1) to trigger apoptosis [15]. This interaction facilitates Ca^{2+} transfer from the ER to mitochondria, which leads to the opening of the permeability transition pore (PTP), loss of the mitochondrial membrane potential, and release of cytochrome c into the cytosol. Cytochrome c then binds to the apoptotic protease-activating factor 1 (APAF1) to form the apoptosome, thereby activating caspases responsible for programmed cell death [15, 262]. Interestingly, BAP31 also binds the mitochondrial translocase TOM40 to recruit NDUSF4, a component of the mitochondrial electron transport chain complex I, suggesting an additional role in stress sensing. Dysregulation of BAP31 has been implicated in neurodegenerative diseases: its deletion in AD mice leads to increased reticulon-3 (RTN3) levels and subsequent Aβ accumulation [263]. Moreover, a significant increase in FIS1-DRP1 interaction has been observed in Aβ-treated neurons and fibroblasts from AD patients [264]. Like BAP31, the multifunctional sorting protein PACS-2 is required for ER-mitochondria contacts. Loss of PACS-2 results in BAP31-dependent mitochondria fragmentation and detachment from the ER [18].

Although MAM functions have been extensively studied, it is still unclear whether their excessive or prolonged formation may have context-dependent consequences, especially during aging. Experimental strategies that artificially enhance ER-mitochondria proximity using synthetic linkers have shown mixed outcomes. In *D. melanogaster*, for example, enforced tethering between the two organelles led to lifespan extension, with a concurrent rise in mitochondrial ROS levels [265]. Similarly, increased ER-mitochondria coupling in high-fat diet-fed mice resulted in higher ROS accumulation [266]. In senescent endothelial cells, tighter ER-mitochondria contacts elevated mitochondrial Ca^{2+} uptake and respiration, but also heightened susceptibility to Ca^{2+}-induced cell death [267]. A comparable scenario was observed in aging hippocampal neurons *in vitro*, where enhanced ER-mitochondria communication was associated with increased mitochondrial activity but decreased SOCE efficiency [268]. These findings collectively underscore the dual nature of MAMs: while beneficial in promoting bioenergetic output and stress adaptation, excessive tethering may contribute to oxidative damage and neuronal vulnerability in aging. Future studies are needed to clarify the context-dependent roles of MAMs in aging and age-associated diseases, and to determine whether modulating ER-mitochondria contact sites could serve as a therapeutic strategy for improving neuronal health in the elderly.

Lipid droplet contact sites in lipid storage and redistribution

LD are highly dynamic organelles that play a pivotal role in cellular energy by synthesizing, storing and breaking down neutral lipids, such as triacylglycerols (TAGs) and sterol esters (SEs) [269, 270]. Although initially viewed as inert fat deposits, LD form when neutral lipids accumulate between the cytoplasmic leaflets of the ER bilayer, eventually budding into the cytoplasm [269, 270]. Even after detachment, a significant portion of LD (approximately 85% in mammalian cells) remains in close physical contact with the ER, forming ER-LD MCS (Fig. 4). These contacts play critical roles in LD biogenesis, expansion, and function [269, 270]. A key player at ER-LD MCS is Seipin, an ER-resident protein enriched at ER-LD junctions [271]. Seipin facilitates the formation of nascent LD, ensuring the proper incorporation of neutral lipids and structural proteins [271], by forming

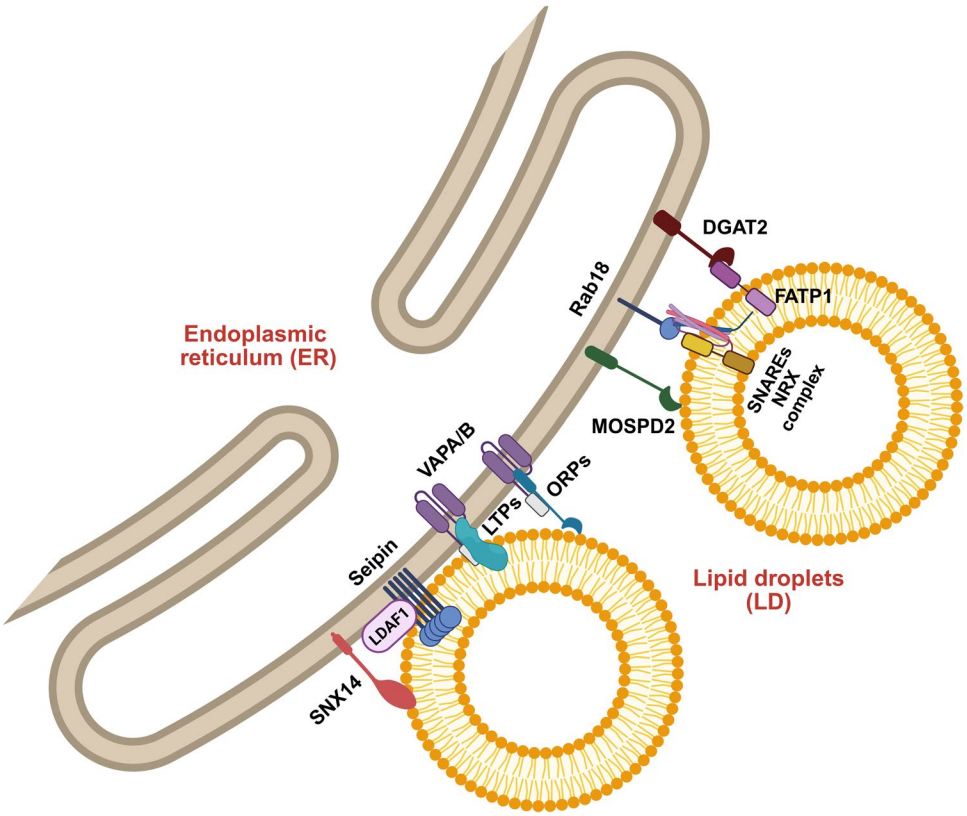

Fig. 4 Molecular machineries involved in ER-LD contact sites. Tethering proteins that regulate the biogenesis, growth, and function of lipid droplets (LD) at MCS with the endoplasmic reticulum (ER). A central component at ER-LD contacts is Seipin, an ER-resident protein that forms oligomeric ring structures and interacts with LDAF1 to promote LD biogenesis. SNX14, another ER-resident factor, also localizes to ER-LD contacts and promotes LD maturation independently of Seipin. VAP proteins (VAPA/B) support contact site formation by interacting with FFAT motif-containing lipid transfer proteins (LTPs), including oxysterol-binding protein-related proteins (ORPs). These LTPs mediate non-vesicular phospholipid and sterol transport at ER-LD interfaces. Other critical regulators include the fatty acid transport protein FATP1 and diacylglycerol acyltransferase 2 (DGAT2), which interact across the ER-LD contacts to coordinate triglyceride biosynthesis. Rab18, a small GTPase associated with the NRZ-SNARE tethering complex, promotes LD expansion and lipid storage. MOSPD2, an ER-anchored VAP-related protein, has both tethering and lipid transfer functions

symmetric ring-shaped oligomers and by interacting with LDAF1/promethin, which is also required for LD formation [272]. Therefore, Seipin loss causes aberrant ER-LD contacts, leading to defective LD assembly and lipid storage, while its toxic gain-of-function mutations can disrupt ER architecture [40, 273]. Indeed, EM analysis of Seipin-deficient cells revealed a local expansion of ER membranes at ER-LD contact sites, which is further exacerbated, leading to a widening of the overall ER diameter, in knockout cells incubated with oleic acid to induce LD biogenesis. This may indicate an accumulation of lipid cargo within the ER prior to droplet formation [40]. Mutations in the *BSCL2* gene encoding Seipin result in a severe form of human lipodystrophy, named Berardinelli-Seip congenital generalized lipodystrophy type 2 (BSCL2), a disorder marked by severe insulin resistance and near-total loss of adipose tissue [274]. A group of dominant motor neuropathies, such as hereditary motor neuropathy and Silver syndrome, have also been linked to Seipin dysfunction [275], highlighting its broad physiological relevance. Although small molecules directly targeting Seipin are currently unavailable, downstream pathways affected by Seipin deficiency have been pharmacologically targeted with promising results. Loss of Seipin function in neurons is known to impair the peroxisomal proliferator-activated receptor gamma (PPARγ) signaling, triggering a cascade of detrimental effects, including tau hyperphosphorylation, synaptic dysfunction, impaired neurogenesis, and neuroinflammation [276–281]. In mouse models lacking neuronal Seipin (nKO), reduced PPARγ expression contributes to decreased AMPA glutamate receptor expression and synaptic plasticity, leading to cognitive deficits [279]. The PPAR γ agonist, rosiglitazone, has been shown to restore AMPA receptor levels, rescue hippocampal long-term potentiation, improve spatial memory and ameliorate anxiety- and depression-like phenotypes in these mice [277, 279]. Seipin loss also promotes tau pathology. Enhanced activity of GSK3β and Akt/mTOR signaling in Seipin-nKO hippocampi drives tau hyperphosphorylation

and aggregation, processes that are reversed by treatment with PPARγ agonists, GSK3β or mTOR inhibitors [276]. Seipin-deficient mice also show increased vulnerability to Aβ-induced neurotoxicity, a process associated with slightly elevated levels of pro-inflammatory cytokines (IL-6, TNF-α). Again, PPARγ agonists mitigate these inflammatory responses conferring neuroprotection [281].

Independently of Seipin function, the sorting nexin protein Snx14, an ER-resident protein associated with cerebellar ataxia SCAR20, localizes to ER-LD contacts following FA treatment and promotes LD maturation [282]. Accordingly, Snx14-deficient cells exhibit deficits in LD morphology and, conversely, its overexpression promotes LD biogenesis and increases ER-LD contacts [282]. Another critical component of ER-LD MCS is the interaction between transport protein 1 (FATP1) and diacylglycerol acyltransferase 2 (DGAT2). FATP1, residing in the ER, interacts with DGAT2 on the LD surface to coordinate triglyceride biosynthesis [283]. This interaction enhances lipid storage and drives LD expansion, with mutations in the DGAT2 gene implicated in axonal neuropathies [284]. In detail, a heterozygous missense mutation in DGAT2 was identified in two individuals of the same family with autosomal-dominant CMT2. Genomic and functional studies revealed that the missense mutation (p.Y223H) in DGAT2 impairs triglyceride synthesis and, when overexpressed in zebrafish, disrupts axon branching in the peripheral nervous system [284]. The small GTPase Rab18 also orchestrates ER-LD MCS by recruiting the NAG-RINT1-ZW10 (NRZ) complex and their associated SNARE proteins (Syntaxin18, Use1, BNIP1) [39]. This association supports LD growth and triglyceride accumulation, particularly in lipid-demanding cells such as adipocytes [39]. Dysregulation of Rab18 activity has been linked to Warburg Micro syndrome, a neurodevelopmental disorder characterized by extensive neuronal defects, including disrupted cytoskeletal architecture and impaired lysosomal function and, accordingly, Rab18-deficient neurons exhibit severe synaptic abnormalities [285]. Other proteins such as ORP5, VPS13A, and MOSPD2 also contribute to ER-LD interactions by mediating lipid transfer and tethering. ORP5, for example, binds PI4P on LD to facilitate phospholipid transport [286], while VPS13A supports bulk lipid transfer across the MCS [143]. MOSPD2, an ER-resident VAP family protein, uniquely attaches to LD via its CRAL-TRIO domain and plays a crucial role in LD assembly and inflammatory response regulation [287].

Beyond ER-LD interfaces, LD establish contact sites with mitochondria, PO, Lys, and even other LD. These interactions regulate processes such as FA β-oxidation, lipophagy, and lipid recycling. For example, LD-PO MCS, essential for FA trafficking, are regulated by a complex interaction between Spastin, mutations of which are the most common cause for hereditary spastic paraplegia (HSP), and the peroxisomal FA transporter ATP binding cassette D member 1 (ABCD1) [60]. LD-mitochondria contacts facilitate FA transfer for ATP generation, while LD-PO interactions support the breakdown of very-long-chain FAs. Disruptions in these MCS have been linked to various diseases, including non-alcoholic fatty liver disease (NAFLD) and HSP [269].

While significant progress has been made, the molecular mechanisms underlying the dynamic behaviour of LD contact sites and their full repertoire of molecular components remain unresolved. Advanced tools, such as proximity labelling and *in vitro* reconstitution, could prove crucial in addressing these gaps. Furthermore, understanding the role of LD contact sites, particularly in metabolic stress responses across different tissues, may pave the way for targeted therapeutic interventions.

Conclusion and future perspectives

Neurodegenerative diseases and aging are complex, multifaceted biological processes characterized by a gradual loss of cell homeostasis and resilience [67, 288]. Emerging evidence recognizes that organelles do not function in isolation and a new paradigm is emerging: cells operate within an integrated network of MCSs whose dynamic regulation underpins many hallmarks of aging and age-associated diseases [65, 68, 69]. Despite significant advances, many of them discussed in this review, fundamental questions remain. Mechanistic studies have uncovered diverse molecular mediators of MCSs and revealed that their dysregulation may be either a causative driver of neurodegenerative conditions or a secondary consequence [65]. Nevertheless, substantial knowledge gaps in this field remain, largely due to methodological limitations and the incomplete translational relevance of current model organisms, which often fail to recapitulate the complex pathophysiological processes observed in humans [289, 290]. Therefore, a renewed emphasis is needed. In this context, advanced cellular reprogramming technologies offer a promising path forward. While iPSC-derived neurons have greatly enhanced our ability to model patient-specific neurodegenerative diseases [291, 292], their embryonic-like identity limits their use for studying age-related phenotypes, including neurodegenerative diseases. In contrast, transdifferentiation techniques, where somatic cells are directly converted into neurons, bypass the pluripotent state and preserve age-associated epigenetic and transcriptomic features [293, 294]. This approach enables the generation of patient-derived neurons that faithfully recapitulate the molecular and functional landscape of aging human brains [295–304]. For instance, a recent study using transdifferentiated neurons from aged

and AD patients revealed that these cells accumulate proteotoxic aggregates and exhibit constitutive lysosomal damage, lysosomal Ca^{2+} dysregulation, and increased mitochondria-Lys contacts. These findings support a model in which age-related loss of proteostasis and organelle homeostasis, especially within the endosome-lysosomal system, contributes to early pathogenesis of AD and shows how transdifferentiated neurons may serve as powerful tools for studying inter-organelle communication in human-derived cells [305]. Beyond cellular models, recent developments in systems biology and multi-omics technologies hold the potential to unravel the complexity of MCSs. Techniques such as proximity biotinylation, crosslinking mass spectrometry (XL-MS) and splitFAST systems represent particularly innovative strategies to identify and measure the dynamic recruitment of proteins at contact sites, while characterizing their proteomic rearrangements under different conditions [84, 306–311]. By capturing transient and low-affinity interactions between proteins, these techniques might reveal changes in MCS architecture and composition during aging or various age-related diseases. Ultimately, adopting a systems-level perspective that integrates spatial, functional, and biochemical information from dynamic organelle networks will be essential. Future research must not only map the full spectrum of MCS in human cells but also assign functional relevance to these networks under physiological and pathological conditions. This integrated approach, spanning novel models, high-resolution imaging, and proteomics, promises to shift our understanding of aging and neurodegenerative diseases. By unraveling and targeting the inter-organelle interactome, MCS alterations may serve as promising biomarkers for the early detection of several diseases. Moreover, this knowledge could guide the development of novel interventions able to preserve brain homeostasis and delay the onset of neurodegenerative disorders in the elderly.

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Data availability Data sharing is not applicable to this article, as no datasets were generated or analyzed during the current study.

Declarations

Ethical approval This article does not report any studies involving human participants or animals, nor does it disclose any patient data. Therefore, no additional ethical approval is required.

Consent to participate Not applicable.

Consent for publication D.A.T. and P.P have approved the final version of this manuscript and agree with its submission to Cellular and Molecular Life Sciences.

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