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Tether Together: ER-Mitochondria Contact Sites and Their Role in ALS

Writing Assignment - Review article

Lay summary

You probably heard of the neurological disease ALS, perhaps you even know someone who has this disease. ALS stands for amyotrophic lateral sclerosis and causes dysfunction of nerve cells in the brain and spinal cord that control muscle movement. The result is that ALS patients are not able to control voluntary muscle activity. Voluntary muscle movements enable you to speak, walk and chew. It all starts in the brain, with a type of nerve cells called motor neurons. These motor neurons extend from the brain to the spinal cord and to the muscles all throughout the body. Motor neurons will degenerate (die) over time and will stop sending information to muscle cells, which will weaken over time and start to twitch. Eventually, the brain is not able to control these muscles anymore and the voluntary movement thereof. ALS is a progressive disease, which means the symptoms get worse, until eventually the patient will die within a few years after diagnosis. At the moment, there is no cure for ALS and treatments are not very effective in a way that they do not arrest disease progression. In order to find a cure, we need to understand what is happening inside the cells of ALS patients. Every cell possesses organelles, the little organs that regulate all the functions a cell needs to exert. So does the nucleus, for example, enclose the DNA with all the genomic information. In this review, I focussed on the organelles endoplasmic reticulum (ER) and mitochondria. The ER is a very large organelle and is mostly responsible for the important task of protein synthesis, folding, modification and transport. Proteins perform almost all functions in cells and in the body, regulating essential processes. Mitochondria are the so-called “powerhouses” of the cell, providing energy for other organelles and proteins to exert their functions. The ER and mitochondria can come into very close proximity and make contact. Various proteins regulate these ER-mitochondria contact sites (ERMCSs), these proteins are called “tethers”. In various research studies, it came to light that ERMCSs are sometimes disrupted in ALS patients. This does not come as a surprise, as ERMCSs regulate important cellular processes that are disrupted in ALS. Some of these functions include the regulation of calcium concentrations in the cell, lipid (fat-like molecules) metabolism, cellular stress responses, the breakdown of damaged proteins or organelles (autophagy) and transport of various compounds throughout neurons. Over the past decades, more and more information about the important roles of ERMCSs in neurons, and more specifically in ALS patient neurons, has been brought to light. In this review I will discuss the functions of ERMCSs and how their disruption is contributing to ALS.

Abstract

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by degeneration of both upper and lower motor neurons in several parts of the central nervous system. Degeneration of the motor neurons eventually results in patient death within a few years of prognosis. Currently, there is no treatment that cures ALS. Finding new therapeutics is challenging, as many cellular processes are disrupted in ALS including Ca^{2+} homeostasis, lipid metabolism, the unfolded protein response (UPR), autophagy and axonal transport. It was shown that communication between the endoplasmic reticulum (ER) and other organelles plays an important role herein. More specifically, disrupted communication at the interface between the ER and mitochondria has been linked to ALS pathology. These so-called ER-mitochondria contact sites (ERMCSs) are maintained by tether proteins or complexes. Over the past years, mutations in tethers have been extensively linked to several neurodegenerative diseases. Thus, conserving the ERMCSs and its tethers is essential for proper cellular functioning. This review will focus on the architecture of ERMCSs, the molecular processes executed at these sites, and how ALS-related mutations that disrupt the contact site can lead to ALS pathology.

Introduction

ALS is a fatal neurodegenerative disease that affects both upper and lower motor neurons in the cerebral cortex, brain stem and spinal cord. Loss of motor neurons leads to muscle atrophy and muscle weakness, including respiratory muscles. This leads to death within a few years of disease onset. ALS has a middle to late age onset and is the most common adult motor neuron disease. Only about 10% of ALS cases is found to be familial, and although ALS is believed to have a large genetic component, many gene variants causing the disease remain unknown. The vast majority of ALS cases is therefor categorized as sporadic. ALS is a complex disease, indicated to play a role in many cellular processes and with a large heterogeneity between patients. Making matters even more complex; there is no cure for the disease at the moment. Current treatment options exist solely of symptom management (1, 2).

Many cellular processes are disrupted in ALS, such as Ca^{2+} homeostasis, lipid metabolism, the UPR, autophagy and axonal transport (3). Communication between the ER and other organelles was found to play an important role in these processes. At specific membrane contact sites (MCSs), processes such as lipid and ion transfer and homeostasis, signalling and organelle division and localization are maintained, as has been described previously in other reviews (4-7). Specifically disrupted communication between the ER and the mitochondria has been linked to the pathogenesis of ALS previously (8). These ERMCSs are typically spaced at 10-30 nm and maintained by tether proteins or complexes (9). A common, well described family of tethering proteins are the vehicle-associated membrane protein (VAMP-) associated proteins (VAPs). In the past two decades an increased interest has been taken in VAPs, because they have been associated with several neurodegenerative diseases including ALS, Charcot-Marie-Tooth (CMT), frontotemporal dementia (FD), Alzheimer's disease (AD) and Parkinson's disease (PD) (10).

Many reviews have been written on VAPs and their role in neurodegenerative diseases (10, 11), on ER contact sites with other organelles (6, 12), on the ERMCS and its role in ALS (13, 14), and on targeting the ERMCS as a new drug target in ALS (15). However, the influence of ALS-associated mutations on the ERMCSs in patient samples was yet to be described. Recently published articles led to new insights in the importance of the ERMCS in patient-derived ALS samples (16-18).

In this review I will first shortly discuss the ER and mitochondria as organelles, after which I will move on to the molecular composition of ERMCSs. This section includes some recently uncovered molecular mechanisms maintaining the ERMCSs, that were proved to be essential for proper mitochondrial functioning. Moreover, I will discuss the cellular functions of the tethers maintaining the ERMCSs, going into detail on the importance of these tethers in neurons afterwards. There, I will discuss how these contact sites are crucial for some neuronal functionalities and can result in ALS. Finally, ALS-associated mutations and their effect on, and interaction with, the ERMCS are discussed. Moreover, in this final section I will briefly touch upon the subject of how recently developed human ALS models can accelerate our understanding of disrupted ERMCSs and how this links to ALS pathology in humans.

The ER and Mitochondria are essential organelles

The ER is the largest membrane-bound organelle in eukaryotic cells. The ER consists of an extensive network of cisternae and tubules, extending throughout the whole cell. As a major site of protein synthesis, -transport, -folding, calcium storage and lipid synthesis and transportation, the ER is a multi-functional organelle (19-22). In neuronal axons, the ER is largely made up of smooth tubular ER and shares many functions with this organelle in non-neuronal cells, but is specifically important for release of Ca^{2+} ions leading to an action potential or release of neurotransmitters (23, 24).

Mitochondria are special organelles, in the sense that they have two membranes; an inner- and outer mitochondrial membrane (OMM). They are constantly dividing and fusing organelles and are well-known for their role in energy metabolism. However, many studies have now confirmed that mitochondria also play essential roles in cell-cycle progression, signalling in the apoptotic cascade, development of certain tissues and production of reactive oxygen species (25-28). As described in several reviews, neurons critically depend on mitochondria. The central nervous system has an exceptionally high metabolic rate, and metabolites produced by mitochondria are essential to form and maintain neural connections. Also, the correct localization, maintenance of the right amount and the health of the mitochondria are essential for proper neural operation. On the other hand, mitochondrial dysfunction can lead to abnormal neural functioning as seen in aging and in metabolic- and neurodegenerative diseases, and overall decreases neuronal survival rates (29, 30). Mitochondrial dysfunction is one of the earliest pathophysiological events in ALS (31). Structurally altered and aggregated mitochondria were one of the first changes observed in ALS patient motor neurons (32). Likewise, ER stress has been demonstrated to play a key role in early pathogenesis of ALS, leading to activation of the UPR and ultimately to cell death (33-36).

The ER makes functional contact sites with numerous organelles

The ER can interact with other organelles through vesicular and non-vesicular transport. The main route of protein and lipid transfer is to enclose or integrate the macromolecules into a vesicle and fuse with the acceptor membrane (37). However, it was shown that non-vesicular transport is for example crucial for intracellular lipid trafficking (38). Non-vesicular transport between two opposed membranes can take place through direct communication between organelles at MCSs. Especially the ER is known to form extensive contacts with many organelles. A characterizing feature of ER-MCSs is that they lack ribosomes (12). Besides mitochondria, the extensive ER tubular network makes contact with other membrane bound organelles such as the Golgi apparatus, plasma membrane, peroxisomes, endosomes, lipid droplets but also with membraneless organelles like processing bodies (PBs) and stress granules (6, 39).

ER contact sites can both be durable and transient. ER-endosome contacts for example are maintained during trafficking of the organelles (6, 40). ER-mitochondria contact sites must also be maintained for a substantial amount of time, because the ER regulates mitochondrial positioning for fission (molecular mechanism reviewed by Wu *et al* (6)). PBs make both transient and stable interactions with the ER. PB fission is mediated by contact sites with the ER, and ER-tubule abundance leads to an increased number of PBs (39). Altogether, MCSs are known to regulate several essential cellular processes, such as organelle division and localization, but also lipid and ion transfer and homeostasis and signalling, as has been described previously in other reviews (4-7).

The ER contact site tethers

In order to coordinate these cellular processes, the ER and the opposed organelle have to come into close contact with one another. Electron Microscopy (EM) has shown that MCSs are typically spaced at 10-30 nm (9). The organelles at the MCSs are not fused together, but rather tethered to one another by a dedicated protein or protein complex. One of these tethers is the VAP tether. Mammals have two closely related VAP genes that generally share a 63% sequence identity; VAPA and VAPB (10, 41). VAPs are integral membrane proteins and found across all eukaryotic organisms (10). They are mainly present in the ER, but have also been found in the Golgi, the ER–Golgi intermediate compartment, the plasma membrane, recycling endosomes and tight and neuromuscular junctions (10, 42-46). The VAPB tether is strongly associated with ALS and mediates the communication between the ER and the protein tyrosine phosphatase interacting protein-51 (PTPIP51) in the OMM, forming an ERMCS (47). VAP is an important tether, partly because it has been associated with several neurodegenerative diseases, among which ALS (as reviewed by Dudás *et al* (10)). The most well-known ALS-related VAP mutation is the VAPB P56S missense mutation, but at least four other VAPB mutations were linked to ALS (11, 48). Beside the VAPs, other tethering proteins like Mfn2, IP_3R, Bap31, FATP1, junctophilins, STIM1, E-Syt1-3, PTP1B and PDZD8 have been confirmed as ER-localized mammalian tethers (49-57).

The ERMCS tethers and their functions

VAPB-PTPIP51

Cell fractionation studies in the late seventies and early eighties of the previous century showed that components of the ER copurified with mitochondria, suggesting a physical link between them (58, 59). Later, EM tomography reconstruction in situ suggested that contact between mitochondria and the ER plays an important role in maintaining cellular Ca^{2+} levels (60). Over the past decades, several tethering proteins at the ERMCS have been identified, including tethers that are involved in calcium transport between the ER and mitochondria. A well-known tether pair that is involved in Ca^{2+} signalling and lipid transfer is the VAPB-PTPIP51 tether (61, 62). Depletion of either VAPB or PTPIP51 results in delayed Ca^{2+} uptake by the mitochondria. Moreover, EM and confocal microscopy show that a modification in PTPIP51 or VAPB results in decreased ER contact with mitochondria (47). VAPs play an important role in the UPR, autophagy, membrane trafficking, and lipid transfer and metabolism (10, 63, 64). The VAPB-PTPIP51 tether complex mediate the ERMCSs and therefor the health of the mitochondria. VAPs are tail-anchored proteins and insert into the ER membrane post-translationally. VAPs contain an N-terminal major sperm protein (MSP) domain, a central amphipathic coiled-coil region, and a C-terminal hydrophobic transmembrane domain. The MSP domain has an immunoglobulin fold and the coiled-coil motif is often found in proteins involved in vesicular transport, like VAMP and SNARE proteins (65). The C-terminal transmembrane domain contains a GxxxG motif that could be responsible for the homo- and heterodimerization of the VAP protein (66).

VAPs interact with two protein groups in general: SNARE and FFAT motif-containing proteins. VAPA binds mainly to different proteins in the SNARE family (43). The FFAT motif is a short linear motif which comprises the consensus sequence EFFDAxE, used by proteins to target the ER membrane, although some FFAT motifs contain a serine or threonine at position 4, instead of an aspartic acid (67, 68). The PTPIP51 protein also contains a FFAT motif. It is often proposed that the FFAT motif interacts with the MSP domain of the VAP protein, forming a tether at the ERMCS (62, 68). Di Mattia *et al* proposes that phosphorylation of the serine or threonine residue at position 4 of the FFAT motif can act as a reversible switch that mediates the interaction with VAPs. They show that when the residue is not phosphorylated, the FFAT motif is not recognized by its binding partner on the ER. The phosphorylation of the FFAT motif could act as a molecular switch to form ERMCSs. Nonetheless, the kinases and phosphatases regulating this process are still unknown, and the functional characterization of this motif was performed on STARD3, a protein that mediated the contact between the ER and endosomes (68). Moreover, often performed are *in vitro* binding experiments that only use short PTPIP51 fragments containing the FFAT motif, not the other domains of the protein. Also, the FFAT motif sequence is unconventional as many known VAP interactors contain only one or no phenylalanine at all in the FFAT motif (69). However, it was still surprising when it was shown that this long-thought FFAT motif in PTPIP51 is not essential for VAPB binding. Using EM and full-length PTPIP51 protein Mórotz *et al* showed that not the FFAT motif, but rather the coiled-coil domain is essential for PTPIP51 ERMCS signalling functions, at least *in vitro*. Deletion of the FFAT domain showed little effect on the PTPIP51-VAPB binding, but the deletion and mutation of the coiled-coil domain affected this binding (70). Still, this study was only performed with PTPIP51 and should be repeated with other known VAPB binding partners to confirm that the coiled-coil domain is essential in the formation of ERMCS with VAPB.

A study by Obara *et al* performed in COS7 cells, using 3D EM with high-speed molecular tracking of VAPB, established that subdomains of the ER at the contact site dramatically deform and match local mitochondrial curvature. An understanding of the nanoscale structure and regulation of ERMCSs was still missing, because ERMCSs are highly sensitive to experimental disruption. Moreover, live cell experiments demonstrated VAPB tethers to be highly dynamic; the tethers can make and leave the ERMCS within seconds. The dynamic subdomains are increased during nutrient stress, suggesting that ERMCSs can be altered under physiological conditions. This study proposes that the dynamic landscape of VAPB is a crucial component of ERMCSs homeostasis. Obara *et al* also studied the ALS-related VAPB P56S mutation and its impact at the ERMCS. Normally, VAPB tethers are highly dynamic, but VAPB P56S proteins were mainly immobile. The ERMCS had restricted pools of VAPB P56S molecules that, unlike wild-type (WT) VAPB, did not reach the edges of the contact sites. The restricted pools showed low diffusion rates with the ER and surrounding, possibly resulting in a more stable ERMCS, impacting contact site functionality (71).

Mfn2-Mfn2/Mfn1

Mitofusin 2 (Mfn2) is primarily an OMM protein, known for its role in mitochondrial fusion (72). However, Mfn2 also localizes to the membrane of the ER and can hetero- or homodimerize with Mfn1 or Mfn2 in the OMM respectively. In 2008, De Brito & Scorrano suggested that Mfn2 tethers the ER to mitochondria and that this tether is required for efficient Ca^{2+} uptake by the mitochondria (73). Using *Mfn2*$^{-/-}$ mouse embryonic fibroblasts (MEFs) and fluorescence microscopy, they found that the ER and mitochondria did not colocalize together. However, the confocal resolution power was around 270 nm, a lot bigger than the average tether distance (10-30 nm). But using electron tomography, the researchers confirmed that in *Mfn2*$^{-/-}$ MEFs did not come into close enough contact to form a tether, whereas the ER and mitochondria in WT MEFs were able to come into close

proximity. This observation was confirmed by the significant lower and slower Ca^{2+} uptake by *Mfn2*$^{-/-}$ mitochondria, after an equal amount of Ca^{2+} release, than by WT mitochondria. Again, eight years later in 2016, the Scorrano group validated this result using EM and proximity experiments between the ER and mitochondria based on GFP fluorescent probes (49). More studies confirm the idea that Mfn2 acts as a physical tether between the ER and mitochondria, by identifying cofactors of Mfn2 in HeLa cells, MEFs, HEK293 cells and mouse melanocytes (74-76). However, there has been an intense debate on the function of Mfn2 after several other groups showed that the knockdown of Mfn2 increased ERMCS formation and Ca^{2+} trafficking from the ER to mitochondria in HEK293 cells and MEFs (77-79). These results suggest Mfn2 does not act as a physical, essential and/or critical tether at the ERMCS, but rather a spacer. Interestingly, both Scorrano *et al* as well as Cosson- and Filadi *et al* make use of *Mfn2* knockouts in MEFs.

A recent study in HeLa cells describes that Mfn2 is also involved in the formation and longevity of mitochondrial-associated ER membranes (MAMs) during ER stress, besides its role in Ca^{2+} uptake. Notably, the findings of Gottschalk *et al* suggest both a spacer and tethering role for Mfn2, and hypothesize that ATP transport into the ER lumen as well as ER Ca^{2+} transport to the mitochondria is mediated through specialized MAMs, which are dependent on Mfn2 expression. For example, ER stress and the activation of the UPR cost ATP, which is supplied by mitochondria via these Mfn2-dependent MAMs (80). Altogether, more research should be performed to clarify the role of Mfn2 at the ERMCS, preferably in human neuronal cells, where ERMCSs have been proved to play an important role.

IP3R-VDAC

Ca^{2+} is released from the ER through the tetrameric inositol 1,4,5-triphosphate receptor (IP3R) channel and taken up by the mitochondria via the voltage-dependent anion-selective channel (VDAC) in the OMM (81-83). The interaction between IP3R and VDAC is regulated by glucose-related protein 75 (GRP75) and deglycase (DJ-1) (83, 84). Szabadkai *et al* showed that GRP75, a molecular chaperone, tethers the ligand-binding domain of the IP3 receptor to VDAC. Together, the tetramer complex IP3R-VDAC-GRP75-DJ-1 regulates the Ca^{2+} transfer from the ER to mitochondria. Interestingly, Wilson & Metzakopian (14) claim in their review that complete loss of IP3R does not result in a physically different ERMCS, whereas Bartok *et al* (85) found that loss of IP3R alters the structure of the ERMCS. More research needs to be conducted on the exact function of the IP3R-VDAC tether; does it act as functional or physical tether?

Bap31-Fis1/Tom40

B-cell receptor-associated protein-31 (Bap-31) resides in the ER, and is a membrane chaperone that is able to interact with the mitochondrial fission protein-1 (Fis-1). Together, Bap-31 and Fis-1 act as a recruiter and activator of procaspase 8 and the transmittance of pro-apoptotic signals from the mitochondria to the ER. However, Bap-31 and Fis-1 also form an interaction in non-apoptotic cells, suggesting a physical tethering role as well as apoptotic signalling role for the Bap-31-Fis-1 complex (51, 86). An important co-factor of the Bap-31-Fis-1 tether is the multifunctional sorting protein phosphofurin acidic cluster sorting protein-2 (PACS-2). PACS-2 assures mitochondrial coupling to the ER. In the absence of PACS-2, Bap-31 is cleaved, mitochondria detach from the ER, caspase-3 is activated and eventually the cell undergoes apoptosis (87). Nevertheless, questions on the exact mechanism of action of PACS-2 remain to be resolved. The function of PACS-2 is very variable, depending on the cell type and type of co-factors used in the experiment (88). Bap-31 has also been reported to mediate autophagy, mitochondrial oxygen consumption and -homeostasis through interaction with translocase of the outer mitochondrial membrane 40 (Tom40) (89). Altogether,

these data suggest that Bap-31 can act as an ERMCS tether, transferring apoptotic signals between the ER and mitochondria.

PDZD8-?

After sequence and structure prediction, the PDZD8 protein was identified as possible ERMCS localizing protein. Expression of PDZD8 in *Saccharomyces cerevisiae* showed that PDZD8 localized to the ER. Super-resolution imaging in the Neuro2a (N2a) cell line showed that PDZD8 localized in the ER and at ERMCSs. That PDZD8 was present in the ER, ERMCSs, but not in the mitochondrial fraction was confirmed by subcellular fractionation of the N2a cells. The suggestion that PDZD8 is indeed necessary to make ER-mitochondrial contacts was raised after an experiment in HeLa cells. In control cells, 16.5% of the ER was in contact with mitochondria, but in PDZD8 knockout cells, only 2.1% of the ER was still in contact with mitochondria. Moreover, in NIH3T3 cells upon knockdown of PDZD8 Ca^{2+} import into mitochondria is impaired, which was almost completely rescued by expressing a synthetic ERMCS tether, proposing that PDZD8 is needed for Ca^{2+} transfer from the ER into mitochondria (90). Supporting these findings, a recent study by Hewitt *et al* in *Drosophila melanogaster* adult brains has found that PDZD8 also plays an essential role in the regulation of mitochondrial quality control. Reduction of PDZD8-tethered ERMCSs leads to increased rates of mitophagy and less excess mitochondrial calcium storage, which could be a mechanism to protect aging neurons. Partial knockdown of PDZD8 was namely enough to rescue age-associated decline in locomotion (91). Interestingly, Elbaz-Alon *et al* identified Rab7-GTP, a late endosome protein, and Protrudin, an ER transmembrane protein, as PDZD8 interaction partners. These proteins localize to an ER-late endosome contact site, where mitochondria are also recruited to form a three-way contact (57). However, the role of PDZD8 in mitophagy requires more investigation and the mitochondrial binding partner of PDZD8 should be identified.

ESYT1-SYNJ2BP

A very recent study by Janer *et al* combined proximity labelling, confocal microscopy and subcellular fractionation in human fibroblast cells and found that the ER-residing protein ESYT1 forms a tether complex with the OMM protein Synaptojanin 2 binding protein (SYNJ2BP). Knockout of either ESYT1 or SYNJ2BP resulted in a reduction of ERMCSs, impaired the Ca^{2+} transfer from ER to mitochondria and altered the mitochondrial membrane lipid composition. Expression of WT ESYT1, SYNJ2BP or a synthetic tether rescued these deficiencies. Altogether, an essential tethering role for ESYT1-SYNJ2BP was revealed, having an influence on cellular homeostasis. However, the molecular mechanisms regulating the functions of the ESYT1-SYNJ2BP tether are yet to be discovered (92).

The discussed tethers are present only between the ER and mitochondria. As reviewed by Kornmann, the proteins at the ERMCS are not conserved and can vary in molecular composition (92). But a question that arises is why so many tethers are present at a contact site, some of which even have the same functions, like Ca^{2+} transfer for example. Numerous tether complexes are already known, however there is still controversy in the results of experiments on these complexes. More research should be conducted on ERMCSs, or contact sites in general, to get a better understanding of their functions. An issue here is that many protein complexes at the ERMCSs have multiple roles, for example tethering and Ca^{2+} transfer, so it is difficult to assign specific functions to a tether. Moreover, the techniques used to study MCSs require optimization for accurate readout of the nanoscale and complex contact sites (techniques to study MCSs are reviewed by Wilson & Metzakopian (14)).

ERMCSs functioning in neurons

In neurons, ERMCSs and tether complexes can play very neuron-specific roles. Loss of synaptic function is one of the key aspects of PD, frontotemporal dementia (FTD) and ALS. Gómez-Suaga *et al* shows that the VAPB-PTPIP51 tether is one of the regulators of synaptic functioning. The localization of VAPB and PTPIP51 in rat hippocampal neurons was studied with super resolution structured illumination microscopy (SIM). The researchers found that VAPB and PTPIP51 are present in both the presynaptic axon and postsynaptic dendrite. Moreover, performing a proximity ligation assay (PLA) revealed that VAPB and PTPIP51 are closely associated with each other in the synaptic region. Upon electrical field stimulation (neuronal activity stimulation), the amount of ERMCSs are increased as well as the amount of VAPB-PTPIP51 interactions. This indicated that VAPB-PTPIP51 plays a role in synaptic functioning. Indeed, upon knockdown of either VAPB or PTPIP51, the active dendritic spine numbers were decreased, and synaptic activity as a whole was reduced after electrical field stimulation. Altogether, this study revealed a new role for VAPB-PTPIP51 tethers at ERMCSs in neurons as regulator of synaptic activity. Mutations to VAPB-PTIP51 affecting the ERMCSs can lead to synaptic dysfunction, which could play a role in ALS pathology (93).

In 2021, the importance of the Mfn2 tether in neuronal outgrowth was shown by Casellas-Díaz *et al*. In order to facilitate neuronal outgrowth, an increased amount of energy is needed. During the period of neurite outgrowth, there is an increase in ER-mitochondria colocalization, mitochondrial mass and Mfn2 expression. Knockout of Mfn2 resulted in less ER-mitochondria colocalization. Mitochondrial-Mfn2 expression had no effect on the length of the neurons, whereas expression of ER-Mfn2 partially rescued the growth defect upon Mfn2 knockout. This study suggests that ER-targeted Mfn2-dependent ERMCS formation is necessary for neuronal maturation (94). Whether Mfn2 is a ERMCS tether, a spacer or both, Mfn2 is an important regulator of the ERMCS.

The PDZD8 tether is proposed to be involved in the regulation of dendritic Ca^{2+} transport in neurons. Dendritic Ca^{2+} dynamics play essential functions including processing synaptic inputs and synaptic plasticity. A PDZD8 knockdown in dendrites of mouse cortical pyramidal neurons did not alter the structure and localization of dendritic ER and mitochondria. After a synaptic stimulation, Ca^{2+} release from the ER was coupled to mitochondrial Ca^{2+} import in control neurons. In PDZD8 knockdown neurons on the other hand, mitochondrial Ca^{2+} import was reduced. As PDZD8 knockdown does not alter either ER or mitochondrial localization, Hirabayashi *et al* proposes that the reduced Ca^{2+} import by mitochondria is due to the loss of the contact site between the ER and mitochondria mediated by the PDZD8 tether (90). A study with *Drosophila melanogaster* adult neurons confirms this idea; PDZD8 knockdown resulted in less ERMCSs and, interestingly, increased mitophagy. Together, this caused a decline in age-associated impaired locomotor activity and an increased lifespan. On the contrary, increasing ERMCSs with a synthetic tether led to disruption of axonal mitochondrial transport and synapse formation, stimulated age-related decline in locomotion and reduced lifespan. A PDZD8 knockdown was even able to rescue locomotor defects in a fly model of Alzheimer's disease expressing Amyloid β42 (Aβ42). Hewitt *et al* proposes an important role for the PDZD8 tether in neurons as a regulator of mitochondrial quality control and turnover, and more generally in neuronal homeostasis (91).

ERMCSs are also involved in trafficking of the ER and mitochondria in neurons. Mitochondria are particularly important in the brain, because the brain is one of the most energy-demanding tissues of the human body, and the mitochondria are the main source of energy in the cell (95). Miro is responsible for proper transportation and distribution of mitochondria into the axons and dendrites. In *Drosophila* neurons with a mutation in *Miro*, mitochondria are not being transported into axons and dendrites, causing a lack in presynaptic mitochondria. Neurotransmitter release and acute Ca^{2+}

buffering were eventually impaired (96). The mechanism by which Miro enables mitochondrial transport, is by facilitating the attachment of kinesin 1 to mitochondria, and kinesin 1 on its turn transports mitochondria towards the synapse. Taken together the facts that Miro localizes to the ERMCS, that Miro is a Ca^{2+} sensor and that the ER was shown to be co-transported with mitochondria, supports the idea that Miro may sense Ca^{2+} exchange at the ERMCS to regulate transport of both organelles in response to physiological stimulation (3, 97, 98).

Also neuronal fitness is regulated by ERMCSs. As described in the previous chapter, PACS-2 is an important co-factor for proper functioning of the Bap-31-Fis-1 tether. Knockdown of PACS-2 in mouse primary hippocampal neurons resulted in neuronal degeneration 16 hours after knockdown. The activation of Caspase-3 was detected in these neurons, indicating that the apoptosis pathway was activated. This proved that proper functioning of ERMCSs is critical for neuronal fitness (99).

Altogether, maintaining the ERMCSs plays an important role in maintaining cellular health. Ca^{2+} exchange between the organelles to generate ATP, phospholipid exchange and synthesis, intracellular trafficking of the mitochondria and ER, facilitation of stress responses and the UPR, autophagy, mitochondrial biogenesis, and the formation of the inflammasome are all processes the ERMCS is involved in (3). Strikingly, all of these functions regulated by ERMCSs are affected in AD, PD, FTD and ALS. This is not a coincidence, as recent studies have shown now that ERMCSs are disrupted in such diseases (3, 100). But in order to maintain the ERMCSs, the tethers must function properly.

ALS-associated mutations and the impact on ERMCSs

C9orf72

A hexanucleotide (GGGGCC) expansion in the first intron of the *C9orf72* gene causes around 40% of the cases of familial ALS, and up to 7% of sporadic cases (101, 102). Altered Ca^{2+} homeostasis has been linked to induction of the UPR and autophagy, which are both hallmarks of ALS. Dafinca *et al* wanted to explore whether there is a link between *C9orf72*-related ALS and disrupted Ca^{2+} homeostasis and ER stress. They generated human motor- and cortical neurons from induced pluripotent stem cells (iPSC), derived from skin fibroblasts of ALS patients carrying *C9orf72* hexanucleotide expansions. In this study, they showed that loss of Ca^{2+} homeostasis is associated with an increase in ER stress and cell death. A marker for ER stress is the poly-A-binding protein (PABP), which was found in increased levels in stress granules within the motor- and cortical neurons of *C9orf72* patients. Elevated levels of PABP-positive stress granules suggest altered autophagy and proteasomal degradation, possibly explaining neuronal degeneration in *C9orf72* patients. The neuronal cells of these patients also showed increased levels of aggregates containing SQST1/p62, a marker of the UPR and autophagy. Altogether, Dafinca *et al* revealed a pathogenic link between ER Ca^{2+} dysregulation, ER stress leading to the release of apoptotic factors, the formation of stress granules, and loss of proteostasis, all contributing to the ALS pathogenesis. Interestingly, they found that *C9orf71* patients with over 1000 hexanucleotide expansions had a more drastic phenotype. Nevertheless, more research linking the number of repeats with the severity of phenotypes is required (103).

Recently, Gómez-Suaga *et al* also performed a study in iPSCs from ALS patients carrying an expansion in the *C9orf72* gene. Bidirectional translation of the hexanucleotide repeat generates dipeptide repeat proteins (DPRs), some of which are neurotoxic. Previously, the targets for the DPRs were unknown, but Gómez-Suaga *et al* revealed that the VAPB-PTPIP51 tether is disrupted in *C9orf72* patients by the DPRs. Using PLA to quantify the VAPB-PTPIP51- and IP3-VDAC interactions in iPSC cortical neurons showed that both VAPB-PTPIP51 and IP3-VDAC numbers were reduced in *C9orf72*

patient neurons. PLA revealed that toxic DPRs disrupt VAPB-PTPIP51- and IP3-VDAC complexes, and SIM confirmed that ERMCSs were reduced. Monitoring mitochondrial Ca^{2+} levels in SH-SY5Y cells revealed that ER-mitochondria Ca^{2+} exchange was disturbed because VAPB-PTPIP51 tethers were disrupted by mutant *C9orf72*-derived DPRs. Moreover, immunoblotting experiments proved activation of glycogen synthase kinase-3β (GSK-3β), a known VAPB-PTPIP51- and ER-mitochondria interaction inhibitor, in mutant *C9orf72*-derived DPR-transfected SH-SY5Y cells. In conclusion, mutant *C9orf72*-derived DPRs disrupt the IP3 receptor-mediated Ca^{2+} delivery from the ER to mitochondria, disrupt the ERMCSs, and this disruption may involve the activation of GSK-3β, a negative regulator of the VAPB-PTPIP51 tether. The proper delivery of Ca^{2+} at the ERMCSs is involved in key neuronal functions that are damaged in ALS (16).

VAPB-PTPIP51 tether complex

That the ERMCSs play a large role in ALS pathogenesis is becoming more and more obvious. However, many studies looking into the role of ERMCSs in ALS are performed in cell- and transgenic ALS models. But these cell- and transgenic mouse models do not always mimic human disease pathology, for example in a C9orf72 transgenic mouse model the mice didn't show motor neuron loss, but hippocampal loss (104). Studies in human ALS models are not performed often, thus evidence that ER-mitochondrial signalling is truly altered in humans is currently lacking. To overcome this knowledge gap, Hartopp *et al* studied the VAPB-PTPIP51 tether in post-mortem ALS spinal cords and compared them to control spinal cords. Comparing protein expression levels of control and patient spinal cord tissues on immunoblots showed that VAPB protein expression levels, but not PTPIP51 expression levels, are reduced in patient spinal cords. To examine whether this would affect the VAPB-PTPIP51 interaction, PLAs were used to quantify this interaction. PLAs revealed that VAPB-PTPIP51 interactions were significantly reduced in ALS spinal cord motor neurons compared to healthy controls. Hartopp *et al* proposes that reduced VAPB levels contribute to the reduced VAPB-PTPIP51 interaction in ALS patients. Yet, the mechanisms leading to the disruption of the VAPB-PTPIP51 tether remain largely unknown. Perhaps does GSK-3β activation play an important role herein. Despite this unknown mechanism, Hartopp *et al* presented the first evidence that the VAPB-PTPIP51 tether is perturbed in human ALS patients, proposing a possible therapeutic target (17).

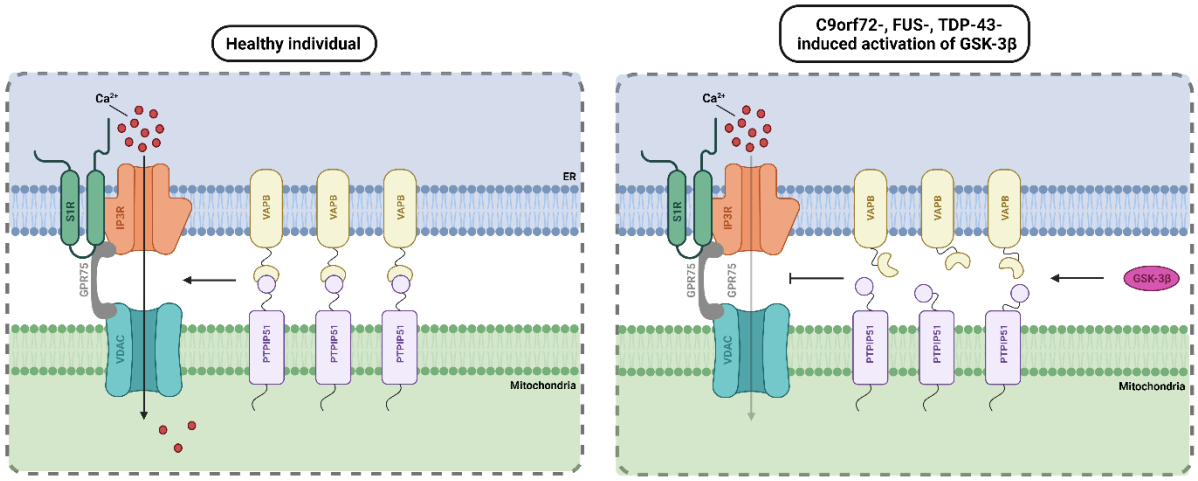

Figure 1.1: ALS-associated genes and their impact on the ERMCS. ***Left:*** *The situation at the ERMCS in a healthy individual is shown.* ***Right:*** *In individuals with ALS caused by C9orf72, FUS or TDP-43, GSK-3β is activated, leading to a reduced amount of VABP-PTPIP51 complexes. This reduction has a negative effect on the IP3R-mediated Ca^{2+} influx into mitochondria.*

SYNJ2BP

The complex in which the OMM protein SYNJ2BP plays a role as a tether was very recently discovered (92). Shortly before identifying the complex and SYNJ2BP's role in maintaining the ERMCS, increased expression levels of SYNJ2BP were detected by RT-PCR and Western blot analysis in iPSC-derived motor neurons of spinal and bulbar muscular atrophy (SBMA) patients. SBMA is a lower motor neuron disease. Moreover, H_2O_2-induced mitochondrial stress resulted in elevated SYNJ2BP expression levels in these iPSC-derived motor neurons. Within two hours, elevated levels of SYNJ2BP were detectable, which is enough time to cause oxidative- and mitochondrial damage. These experiments suggest that as a result of mitochondrial stress, SYNJ2BP protein expression levels are increased. Mitochondrial stress has been suggested as a key factor in ALS pathogenesis (105, 106). In ALS4-patient post mortem spinal cord cells, SYNJ2BP protein expression levels were, but mRNA levels were not significantly upregulated compared to healthy individuals. The regulatory mechanism by which protein expression of SYNJ2BP is upregulated while transcription levels remain the same is yet to be discovered. Mass spectrometry analysis of the proteome could perhaps aid in finding the answer to this question. Transmission EM (TEM) was used to visualize ERMCSs during SYNJ2BP overexpression in iPSC-derived motor neurons. Analysis of the data showed that SYNJ2BP overexpression increased the amount of ERMCSs, which was confirmed with PLA. Increased amounts of ERMCSs lead to more Ca^{2+} import into the mitochondria which can trigger mitochondrial swelling, increased production of reactive oxygen species (ROS), and eventually cell death (107). Knockdown of SYNJ2BP in SBMA motor neurons revealed that reduced SYNJ2BP levels diminish the amount of contact sites between the ER and mitochondria and partly make up for mitochondrial functional impairment. In short, Pourshafie *et al* showed with this study that SYNJ2BP overexpression is associated with neuronal degeneration, specifically in diseases with abnormal mitochondrial functioning. There could be a positive feedback mechanism, where mitochondrial stress increases SYNJ2BP levels, leading to increased ERMCSs, inducing Ca^{2+} influx into mitochondria, increasing mitochondrial stress even more, eventually leading to cell death and thus neuronal degeneration. Altogether, SYNJ2BP is an important regulator of the ERMCSs in both healthy individuals and ALS patients (18).

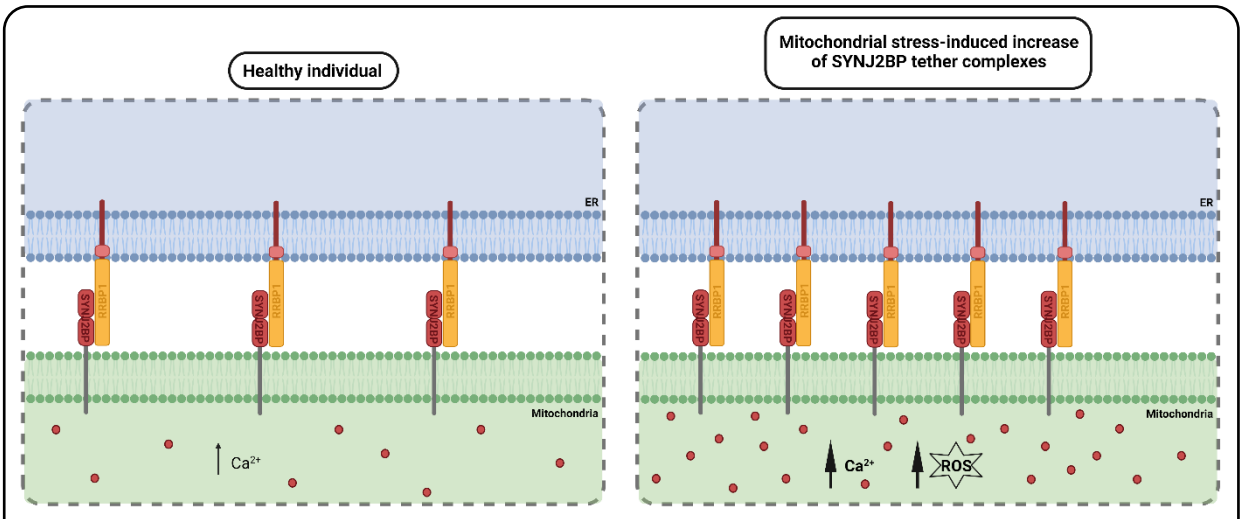

Figure 1.2: ALS-associated genes and their impact on the ERMCS. ***Left:*** *The situation at the ERMCS in a healthy individual is shown.* ***Right:*** *Mitochondrial stress increases the amount of SYNJ2NP-RRBP1 tether complexes at the ERMCS, leading to an increase in Ca^{2+}influx into mitochondria. This may lead to cytotoxic ROS production and eventually cell death.*

TDP-43

Many ALS-associated mutations are known to disrupt the ERMCS. Another example is the TAR DNA binding protein-43 (TDP-43), a major component of the insoluble aggregates in the brains of ALS patients (108). Notably, around 97% of the ALS cases concerns TDP-43 aggregation (109). In 2014, (part of) the mechanism by which both WT and mutant TDP-43 were involved in several neurodegenerative diseases was revealed by Stoica *et al*. Their study showed that overexpression of WT and mutant TDP-43 led to a reduction in ERMCSs, and that this reduction was linked to a reduction in VAPB-PTPIP51 tether complexes. Supporting these results, cytosolic Ca^{2+} levels were increased, and mitochondrial Ca^{2+} levels were reduced upon TDP-43 overexpression. The mechanism by which TDP-43 overexpression leads to reduced VAPB-PTPIP51 complexes could be through GSK-3β activation, a kinase which was strongly associated with ALS before (110). GSK-3β overexpression in this study decreased VAPB-PTPIP51 tether complex formation, confirming this hypothesis. The exact molecular mechanism by which GSK-3β affects the binding of VAPB to PTPIP51 is still unknown, but it is suggested that VAPB or PTPIP51 is phosphorylated by GSK-3β to inhibit their binding, or perhaps GSK-3β activates downstream effectors that act on the binding of VAPB to PTPIP51 (61).

Sigma-1 Receptor

In 2011, a missense mutation (E102Q) in the Sigma-1 receptor (S1R) was linked to juvenile cases of ALS (111). The S1R is an ER transmembrane protein, functions as a molecular chaperone and mainly localizes to the ERMCS. *In vitro* studies with primary embryonic motor neurons showed that motor neurons were degenerated upon S1R knockout. Moreover, in mice spinal cords, knockout of S1R reduced the amount of ERMCSs from 30% to 17%. As a result of decreased ERMCSs, calcium transfer at the ERMCS is also dysregulated (112). The S1R modulates the IP3R and thus Ca^{2+} transfer between the ER and mitochondria. This shows the importance of S1R and its role in maintaining motor neuron integrity. In 2019, Couly *et al* confirmed that protein expression of $S1R^{E102Q}$ at similar levels as WT S1R in control *Drosophila*, led to abnormal locomotor activity and abnormal eye morphology and -development. The mitochondria of $S1R^{E102Q}$-expressing flies were examined with TEM. Both the mitochondrial surface area and ATP levels were decreased in these flies. Overexpression of one of the most important interactors of S1R, IP3R, reduced the abnormal phenotype of the $S1R^{E102Q}$-flies. Since S1R is known to regulate mitochondrial ATP production, and TDP-43 mutants are known to reduce ATP production, Couly *et al* investigated whether WT S1R had favourable effects on TDP-43

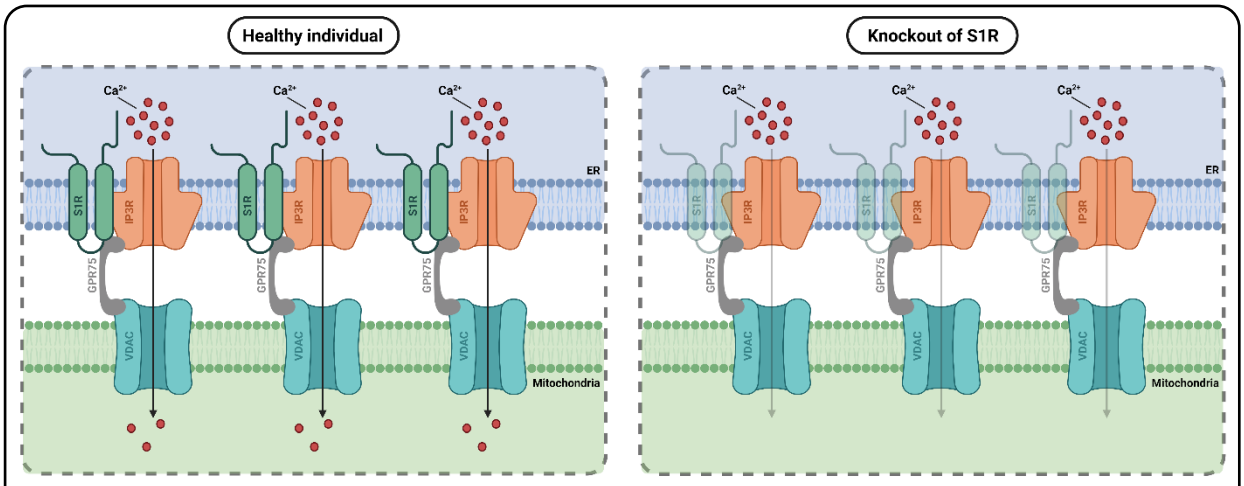

Figure 1.3: ALS-associated genes and their impact on the ERMCS. ***Left:*** *The situation at the ERMCS in a healthy individual is shown.* ***Right:*** *Knockout of S1R leads to dysregulated Ca^{2+} influx into the mitochondria, because S1R regulates the IP3R-mediated Ca^{2+} transfer.*

induced toxicity. Overexpression of WT S1R rescued 26% ATP levels that were decreased by 27% in flies expressing WT TDP-43. Altogether, Couly *et al* were the first to provide evidence that mutant S1R induces ALS pathology *in vivo*, and that WT S1R can (at least partly) rescue TDP-43 induced pathology (113). A reason why a mutation in the S1R could drive ALS pathology, is that particularly high levels of the receptor are present in motor neurons of the brainstem and spinal cord, compared other tissues (114). So mutations in S1R will mainly affect the motor neurons more than other cells.

FUS

Mutations in the fused in sarcoma (FUS) gene also contribute to a subset of familial ALS cases (115, 116). Again, both WT and mutant FUS were shown to disrupt ERMCSs. Overexpression decreases ERMCSs in both NSC34 motor neurons, which are often used as a valid model for ALS research, and in transgenic mice. Just like TDP-43, FUS overexpression decreases mitochondrial Ca^{2+} levels and ATP production and reduced the binding of VAPB to PTPIP51. Stoica *et al* also tried to examine whether FUS would bind to either VAPB or PTPIP51, however they did not detect binding of FUS to either of the proteins, suggesting that FUS is not a major binding partner of the tether complex and thus involves another regulatory protein (117). FUS was indeed found to be involved in the activation of GSK-3β, which could be the regulatory protein involved in disrupting the VAPB-PTPIP51 tether (117).

Autophagy

Autophagy is a recurring theme in ALS (118). Gomez-Suaga *et al* showed that the ERMCS also plays a role in autophagy. Loss of either VAPB or PTPIP51 causes autophagy, whereas overexpression of VAPB or PTPIP51 increases contacts and hinders autophagy. Moreover, this study is in line with other studies that show that disruption of Ca^{2+} transfer from the ER to mitochondria stimulates autophagy: they show that the inhibition of autophagy is entirely abolished by blocking IP3-receptor-mediated Ca^{2+} delivery to the mitochondria. Altogether, the VAPB-PTPIP51 tethering complex plays a role in autophagy that involves regulation of ER-mitochondria Ca^{2+} exchange at the ERMCS (119).

Conclusions

One of the difficulties in understanding the ALS disease mechanism, is the large interpatient variety. Every patient is unique and possesses different genomic mutations eventually leading to disease. However, patients share some similarities in physiological processes that are disrupted. Some of these processes can be linked to disrupted ER-mitochondria contacts. More and more research is focused on unravelling the molecular composition, functioning and how loss of the ERMCSs is linked to disease pathology. From disease models such as HeLa- or HEK293 cells, *Drosophila* or transduced mice we gained understanding of the role of ERMCSs in ALS. It was shown that the ERMCS maintains some essential cellular functions, like mitochondrial ATP production, Ca^{2+} homeostasis, autophagy, ER stress and the UPR. In neurons, ERMCSs also regulate more specialized functions, like mitochondrial axonal transport, neuronal maturation, indirect processing of synaptic inputs, synaptic plasticity, synapse formation, synaptic functioning, mitochondrial quality control and overall neuronal fitness. Over the past years, at least six well-known genes linked to ALS have been shown to damage the ERMCS, including C9orf72, VAPB, SYNJ2BP, TDP-43, S1R and FUS. This suggests that disruption of ERMCSs is considerably a common feature of ALS. But only in the last decade, a cellular model system that allows direct observation of ALS pathogenesis has been developed: fibroblast obtained iPSC-derived motor neurons possessing the patient's genome. Together with studies in post-mortem human ALS samples, this model system allowed us to get a more detailed understanding of (basic) disease mechanisms. It is essential that we understand the disease progression in humans, because if disturbed components of the ERMCS, for example, are proven to

be valid therapeutic targets for ALS, it is crucial we know whether these components are actually disrupted in humans.

Although the development of better model systems, more accurate biochemical assays and a more complete understanding of the contribution of ERMCSs to ALS, many questions remain to be answered. Why are there so many tether complexes at the ERMCS that regulate the same biological function? Do these complexes respond to different stimuli? Are some tethers more prevalent in specific tissues over others? More research should be performed in order to find more tethers, to fully understand their functions and the molecular mechanisms enabling these functions. The fact that FUS, C9orf72 and TDP-43 all lead to the activation of GSK-3β, a negative regulator of the VAPB-PTPIP51 tether complex, suggests that GSK-3β activation could play a very important regulatory role in ERMCS reduction. Future research is needed to uncover the molecular mechanisms behind GSK-3β-mediated VAPB-PTPIP51 interruption and its role in ALS pathology. The communication between organelles at contact sites adds another level of complexity to our understanding of the cell. Only when we understand the basic principles of cellular functioning, we can understand and reason what happens during the progression of complex diseases such as ALS.

References

1. R. Mejzini *et al.*, ALS Genetics, Mechanisms, and Therapeutics: Where Are We Now? *Front Neurosci* **13**, 1310 (2019).
2. N. Borgese, N. Iacomino, S. F. Colombo, F. Navone, The Link between VAPB Loss of Function and Amyotrophic Lateral Sclerosis. *Cells* **10** (2021).
3. S. Paillusson *et al.*, There's Something Wrong with my MAM; the ER-Mitochondria Axis and Neurodegenerative Diseases. *Trends Neurosci* **39**, 146-157 (2016).
4. C. Raiborg, E. M. Wenzel, H. Stenmark, ER-endosome contact sites: molecular compositions and functions. *Embo j* **34**, 1848-1858 (2015).
5. Y. Saheki, P. De Camilli, Endoplasmic Reticulum-Plasma Membrane Contact Sites. *Annu Rev Biochem* **86**, 659-684 (2017).
6. H. Wu, P. Carvalho, G. K. Voeltz, Here, there, and everywhere: The importance of ER membrane contact sites. *Science* **361** (2018).
7. M. L. Sassano, B. Felipe-Abrio, P. Agostinis, ER-mitochondria contact sites; a multifaceted factory for Ca(2+) signaling and lipid transport. *Front Cell Dev Biol* **10**, 988014 (2022).
8. G. Manfredi, H. Kawamata, Mitochondria and endoplasmic reticulum crosstalk in amyotrophic lateral sclerosis. *Neurobiol Dis* **90**, 35-42 (2016).
9. L. Scorrano *et al.*, Coming together to define membrane contact sites. *Nat Commun* **10**, 1287 (2019).
10. E. F. Dudás, M. A. Huynen, A. M. Lesk, A. Pastore, Invisible leashes: The tethering VAPs from infectious diseases to neurodegeneration. *J Biol Chem* **296**, 100421 (2021).
11. N. Borgese, F. Navone, N. Nukina, T. Yamanaka, Mutant VAPB: Culprit or Innocent Bystander of Amyotrophic Lateral Sclerosis? *Contact* **4** (2021).
12. M. J. Phillips, G. K. Voeltz, Structure and function of ER membrane contact sites with other organelles. *Nat Rev Mol Cell Biol* **17**, 69-82 (2016).
13. S. Parakh, J. D. Atkin, The Mitochondrial-associated ER membrane (MAM) compartment and its dysregulation in Amyotrophic Lateral Sclerosis (ALS). *Semin Cell Dev Biol* **112**, 105-113 (2021).
14. E. L. Wilson, E. Metzakopian, ER-mitochondria contact sites in neurodegeneration: genetic screening approaches to investigate novel disease mechanisms. *Cell Death Differ* **28**, 1804-1821 (2021).
15. S. M. Martín-Guerrero *et al.*, Targeting ER-Mitochondria Signaling as a Therapeutic Target for Frontotemporal Dementia and Related Amyotrophic Lateral Sclerosis. *Front Cell Dev Biol* **10**, 915931 (2022).
16. P. Gomez-Suaga *et al.*, Disruption of ER-mitochondria tethering and signalling in C9orf72-associated amyotrophic lateral sclerosis and frontotemporal dementia. *Aging Cell* **21**, e13549 (2022).
17. N. Hartopp *et al.*, Disruption of the VAPB-PTPIP51 ER-mitochondria tethering proteins in post-mortem human amyotrophic lateral sclerosis. *Front Cell Dev Biol* **10**, 950767 (2022).
18. N. Pourshafie *et al.*, Altered SYNJ2BP-mediated mitochondrial-ER contacts in motor neuron disease. *Neurobiol Dis* **172**, 105832 (2022).
19. A. VITALE, A. CERIOTTI, J. DENECKE, The Role of the Endoplasmic Reticulum in Protein Synthesis, Modification and Intracellular Transport. *Journal of Experimental Botany* **44**, 1417-1444 (1993).
20. I. Braakman, D. N. Hebert, Protein folding in the endoplasmic reticulum. *Cold Spring Harb Perspect Biol* **5**, a013201 (2013).
21. J. Meldolesi, T. Pozzan, The endoplasmic reticulum Ca2+ store: a view from the lumen. *Trends Biochem Sci* **23**, 10-14 (1998).
22. P. Fagone, S. Jackowski, Membrane phospholipid synthesis and endoplasmic reticulum function. *J Lipid Res* **50 Suppl**, S311-316 (2009).

23. S. Tsukita, H. Ishikawa, Three-dimensional distribution of smooth endoplasmic reticulum in myelinated axons. *Journal of Electron Microscopy* **25**, 141-149 (1976).
24. A. Verkhratsky, Endoplasmic reticulum calcium signaling in nerve cells. *Biol Res* **37**, 693-699 (2004).
25. D. G. Kirova *et al.*, A ROS-dependent mechanism promotes CDK2 phosphorylation to drive progression through S phase. *Dev Cell* **57**, 1712-1727.e1719 (2022).
26. N. Zamzami *et al.*, Mitochondrial control of nuclear apoptosis. *J Exp Med* **183**, 1533-1544 (1996).
27. S. Madan, B. Uttekar, S. Chowdhary, R. Rikhy, Mitochondria Lead the Way: Mitochondrial Dynamics and Function in Cellular Movements in Development and Disease. *Front Cell Dev Biol* **9**, 781933 (2021).
28. M. P. Murphy, How mitochondria produce reactive oxygen species. *Biochem J* **417**, 1-13 (2009).
29. O. Kann, R. Kovács, Mitochondria and neuronal activity. *Am J Physiol Cell Physiol* **292**, C641-657 (2007).
30. A. Mandal, C. M. Drerup, Axonal Transport and Mitochondrial Function in Neurons. *Front Cell Neurosci* **13**, 373 (2019).
31. E. F. Smith, P. J. Shaw, K. J. De Vos, The role of mitochondria in amyotrophic lateral sclerosis. *Neurosci Lett* **710**, 132933 (2019).
32. S. Sasaki, M. Iwata, Mitochondrial alterations in the spinal cord of patients with sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* **66**, 10-16 (2007).
33. A. K. Walker, J. D. Atkin, Stress signaling from the endoplasmic reticulum: A central player in the pathogenesis of amyotrophic lateral sclerosis. *IUBMB Life* **63**, 754-763 (2011).
34. L. Wang, B. Popko, R. P. Roos, The unfolded protein response in familial amyotrophic lateral sclerosis. *Hum Mol Genet* **20**, 1008-1015 (2011).
35. S. Saxena, E. Cabuy, P. Caroni, A role for motoneuron subtype-selective ER stress in disease manifestations of FALS mice. *Nat Neurosci* **12**, 627-636 (2009).
36. C. Hetz *et al.*, XBP-1 deficiency in the nervous system protects against amyotrophic lateral sclerosis by increasing autophagy. *Genes Dev* **23**, 2294-2306 (2009).
37. J. S. Bonifacino, B. S. Glick, The mechanisms of vesicle budding and fusion. *Cell* **116**, 153-166 (2004).
38. S. Lev, Non-vesicular lipid transport by lipid-transfer proteins and beyond. *Nat Rev Mol Cell Biol* **11**, 739-750 (2010).
39. J. E. Lee, P. I. Cathey, H. Wu, R. Parker, G. K. Voeltz, Endoplasmic reticulum contact sites regulate the dynamics of membraneless organelles. *Science* **367** (2020).
40. J. R. Friedman, J. R. Dibenedetto, M. West, A. A. Rowland, G. K. Voeltz, Endoplasmic reticulum-endosome contact increases as endosomes traffic and mature. *Mol Biol Cell* **24**, 1030-1040 (2013).
41. Y. Nishimura, M. Hayashi, H. Inada, T. Tanaka, Molecular cloning and characterization of mammalian homologues of vesicle-associated membrane protein-associated (VAMP-associated) proteins. *Biochem Biophys Res Commun* **254**, 21-26 (1999).
42. D. Peretti, N. Dahan, E. Shimoni, K. Hirschberg, S. Lev, Coordinated lipid transfer between the endoplasmic reticulum and the Golgi complex requires the VAP proteins and is essential for Golgi-mediated transport. *Mol Biol Cell* **19**, 3871-3884 (2008).
43. M. L. Weir, H. Xie, A. Klip, W. S. Trimble, VAP-A binds promiscuously to both v- and tSNAREs. *Biochem Biophys Res Commun* **286**, 616-621 (2001).
44. L. A. Lapierre, P. L. Tuma, J. Navarre, J. R. Goldenring, J. M. Anderson, VAP-33 localizes to both an intracellular vesicle population and with occludin at the tight junction. *J Cell Sci* **112 (Pt 21)**, 3723-3732 (1999).
45. M. Kuijpers *et al.*, Amyotrophic lateral sclerosis (ALS)-associated VAPB-P56S inclusions represent an ER quality control compartment. *Acta Neuropathol Commun* **1**, 24 (2013).

46. G. Pennetta, P. R. Hiesinger, R. Fabian-Fine, I. A. Meinertzhagen, H. J. Bellen, Drosophila VAP-33A directs bouton formation at neuromuscular junctions in a dosage-dependent manner. *Neuron* **35**, 291-306 (2002).
47. K. J. De Vos *et al.*, VAPB interacts with the mitochondrial protein PTPIP51 to regulate calcium homeostasis. *Hum Mol Genet* **21**, 1299-1311 (2012).
48. A. L. Nishimura *et al.*, A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet* **75**, 822-831 (2004).
49. D. Naon *et al.*, Critical reappraisal confirms that Mitofusin 2 is an endoplasmic reticulum-mitochondria tether. *Proc Natl Acad Sci U S A* **113**, 11249-11254 (2016).
50. A. Bartok *et al.*, IP(3) receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer. *Nat Commun* **10**, 3726 (2019).
51. R. Iwasawa, A. L. Mahul-Mellier, C. Datler, E. Pazarentzos, S. Grimm, Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction. *Embo j* **30**, 556-568 (2011).
52. N. Xu *et al.*, The FATP1-DGAT2 complex facilitates lipid droplet expansion at the ER-lipid droplet interface. *J Cell Biol* **198**, 895-911 (2012).
53. C. A. Piggott, Y. Jin, Junctophilins: Key Membrane Tethers in Muscles and Neurons. *Front Mol Neurosci* **14**, 709390 (2021).
54. J. Liou *et al.*, STIM is a Ca2+ sensor essential for Ca2+-store-depletion-triggered Ca2+ influx. *Curr Biol* **15**, 1235-1241 (2005).
55. R. Fernández-Busnadiego, Y. Saheki, P. De Camilli, Three-dimensional architecture of extended synaptotagmin-mediated endoplasmic reticulum-plasma membrane contact sites. *Proc Natl Acad Sci U S A* **112**, E2004-2013 (2015).
56. F. Giordano *et al.*, PI(4,5)P(2)-dependent and Ca(2+)-regulated ER-PM interactions mediated by the extended synaptotagmins. *Cell* **153**, 1494-1509 (2013).
57. Y. Elbaz-Alon *et al.*, PDZD8 interacts with Protrudin and Rab7 at ER-late endosome membrane contact sites associated with mitochondria. *Nat Commun* **11**, 3645 (2020).
58. G. C. Shore, J. R. Tata, Two fractions of rough endoplasmic reticulum from rat liver. I. Recovery of rapidly sedimenting endoplasmic reticulum in association with mitochondria. *J Cell Biol* **72**, 714-725 (1977).
59. P. J. Meier, M. A. Spycher, U. A. Meyer, Isolation and characterization of rough endoplasmic reticulum associated with mitochondria from normal rat liver. *Biochim Biophys Acta* **646**, 283-297 (1981).
60. C. A. Mannella, K. Buttle, B. K. Rath, M. Marko, Electron microscopic tomography of rat-liver mitochondria and their interaction with the endoplasmic reticulum. *Biofactors* **8**, 225-228 (1998).
61. R. Stoica *et al.*, ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. *Nat Commun* **5**, 3996 (2014).
62. H. K. Yeo *et al.*, Phospholipid transfer function of PTPIP51 at mitochondria-associated ER membranes. *EMBO Rep* **22**, e51323 (2021).
63. K. Kanekura, I. Nishimoto, S. Aiso, M. Matsuoka, Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). *J Biol Chem* **281**, 30223-30233 (2006).
64. Y. G. Zhao *et al.*, The ER Contact Proteins VAPA/B Interact with Multiple Autophagy Proteins to Modulate Autophagosome Biogenesis. *Curr Biol* **28**, 1234-1245.e1234 (2018).
65. A. M. Baker, T. M. Roberts, M. Stewart, 2.6 A resolution crystal structure of helices of the motile major sperm protein (MSP) of Caenorhabditis elegans. *J Mol Biol* **319**, 491-499 (2002).
66. W. P. Russ, D. M. Engelman, The GxxxG motif: a framework for transmembrane helix-helix association. *J Mol Biol* **296**, 911-919 (2000).
67. C. J. Loewen, A. Roy, T. P. Levine, A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. *Embo j* **22**, 2025-2035 (2003).

68. T. Di Mattia *et al.*, FFAT motif phosphorylation controls formation and lipid transfer function of inter-organelle contacts. *Embo j* **39**, e104369 (2020).
69. S. Kors, J. L. Costello, M. Schrader, VAP Proteins - From Organelle Tethers to Pathogenic Host Interactors and Their Role in Neuronal Disease. *Front Cell Dev Biol* **10**, 895856 (2022).
70. G. M. Mórotz *et al.*, The PTPIP51 coiled-coil domain is important in VAPB binding, formation of ER-mitochondria contacts and IP3 receptor delivery of Ca(2+) to mitochondria. *Front Cell Dev Biol* **10**, 920947 (2022).
71. C. J. Obara *et al.*, Motion of single molecular tethers reveals dynamic subdomains at ER-mitochondria contact sites. *bioRxiv* 10.1101/2022.09.03.505525 (2022).
72. H. Chen *et al.*, Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development. *J Cell Biol* **160**, 189-200 (2003).
73. O. M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* **456**, 605-610 (2008).
74. C. Cerqua *et al.*, Trichoplein/mitostatin regulates endoplasmic reticulum-mitochondria juxtaposition. *EMBO Rep* **11**, 854-860 (2010).
75. A. Sugiura *et al.*, MITOL regulates endoplasmic reticulum-mitochondria contacts via Mitofusin2. *Mol Cell* **51**, 20-34 (2013).
76. T. Daniele *et al.*, Mitochondria and melanosomes establish physical contacts modulated by Mfn2 and involved in organelle biogenesis. *Curr Biol* **24**, 393-403 (2014).
77. P. Cosson, A. Marchetti, M. Ravazzola, L. Orci, Mitofusin-2 independent juxtaposition of endoplasmic reticulum and mitochondria: an ultrastructural study. *PLoS One* **7**, e46293 (2012).
78. R. Filadi *et al.*, Mitofusin 2 ablation increases endoplasmic reticulum-mitochondria coupling. *Proc Natl Acad Sci U S A* **112**, E2174-2181 (2015).
79. N. S. Leal *et al.*, Mitofusin-2 knockdown increases ER-mitochondria contact and decreases amyloid β-peptide production. *J Cell Mol Med* **20**, 1686-1695 (2016).
80. B. Gottschalk *et al.*, MFN2 mediates ER-mitochondrial coupling during ER stress through specialized stable contact sites. *Front Cell Dev Biol* **10**, 918691 (2022).
81. R. Rizzuto *et al.*, Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. *Science* **280**, 1763-1766 (1998).
82. E. Rapizzi *et al.*, Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca2+ microdomains to mitochondria. *J Cell Biol* **159**, 613-624 (2002).
83. G. Szabadkai *et al.*, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. *J Cell Biol* **175**, 901-911 (2006).
84. Y. Liu *et al.*, 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 (2019).
85. A. Bartok *et al.*, IP3 receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer. *Nat Commun* **10** (2019).
86. F. W. Ng *et al.*, p28 Bap31, a Bcl-2/Bcl-XL- and procaspase-8-associated protein in the endoplasmic reticulum. *J Cell Biol* **139**, 327-338 (1997).
87. T. Simmen *et al.*, PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. *Embo j* **24**, 717-729 (2005).
88. C. Li, L. Li, M. Yang, L. Zeng, L. Sun, PACS-2: A key regulator of mitochondria-associated membranes (MAMs). *Pharmacol Res* **160**, 105080 (2020).
89. T. Namba, BAP31 regulates mitochondrial function via interaction with Tom40 within ER-mitochondria contact sites. *Sci Adv* **5**, eaaw1386 (2019).
90. Y. Hirabayashi *et al.*, ER-mitochondria tethering by PDZD8 regulates Ca(2+) dynamics in mammalian neurons. *Science* **358**, 623-630 (2017).
91. V. L. Hewitt *et al.*, Decreasing pdzd8-mediated mito-ER contacts improves organismal fitness and mitigates Aβ(42) toxicity. *Life Sci Alliance* **5** (2022).
92. A. Janer *et al.*, ESYT1 tethers the endoplasmic reticulum to mitochondria and is required for mitochondrial lipid and calcium homeostasis. *BioRxiv* 10.1101/2022.11.14.516495 (2022).

93. P. Gómez-Suaga *et al.*, The VAPB-PTPIP51 endoplasmic reticulum-mitochondria tethering proteins are present in neuronal synapses and regulate synaptic activity. *Acta Neuropathologica Communications* **7** (2019).
94. S. Casellas-Díaz *et al.*, Mfn2 localization in the ER is necessary for its bioenergetic function and neuritic development. *EMBO Rep* **22**, e51954 (2021).
95. D. Tomasi, G. J. Wang, N. D. Volkow, Energetic cost of brain functional connectivity. *Proc Natl Acad Sci U S A* **110**, 13642-13647 (2013).
96. X. Guo *et al.*, The GTPase dMiro is required for axonal transport of mitochondria to Drosophila synapses. *Neuron* **47**, 379-393 (2005).
97. A. F. Macaskill *et al.*, Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. *Neuron* **61**, 541-555 (2009).
98. X. Wang, T. L. Schwarz, The mechanism of Ca2+ -dependent regulation of kinesin-mediated mitochondrial motility. *Cell* **136**, 163-174 (2009).
99. L. Hedskog *et al.*, Modulation of the endoplasmic reticulum-mitochondria interface in Alzheimer's disease and related models. *Proc Natl Acad Sci U S A* **110**, 7916-7921 (2013).
100. L. Xu, X. Wang, C. Tong, Endoplasmic Reticulum-Mitochondria Contact Sites and Neurodegeneration. *Front Cell Dev Biol* **8**, 428 (2020).
101. M. DeJesus-Hernandez *et al.*, Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* **72**, 245-256 (2011).
102. A. E. Renton *et al.*, A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* **72**, 257-268 (2011).
103. R. Dafinca *et al.*, C9orf72 Hexanucleotide Expansions Are Associated with Altered Endoplasmic Reticulum Calcium Homeostasis and Stress Granule Formation in Induced Pluripotent Stem Cell-Derived Neurons from Patients with Amyotrophic Lateral Sclerosis and Frontotemporal Dementia. *Stem Cells* **34**, 2063-2078 (2016).
104. J. Jiang *et al.*, Gain of Toxicity from ALS/FTD-Linked Repeat Expansions in C9ORF72 Is Alleviated by Antisense Oligonucleotides Targeting GGGGCC-Containing RNAs. *Neuron* **90**, 535-550 (2016).
105. S. Sasaki, Y. Horie, M. Iwata, Mitochondrial alterations in dorsal root ganglion cells in sporadic amyotrophic lateral sclerosis. *Acta Neuropathol* **114**, 633-639 (2007).
106. J. Zhao *et al.*, The Impact of Mitochondrial Dysfunction in Amyotrophic Lateral Sclerosis. *Cells* **11** (2022).
107. M. Bonora *et al.*, Physiopathology of the Permeability Transition Pore: Molecular Mechanisms in Human Pathology. *Biomolecules* **10** (2020).
108. T. Arai *et al.*, TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* **351**, 602-611 (2006).
109. S. C. Ling, M. Polymenidou, D. W. Cleveland, Converging mechanisms in ALS and FTD: disrupted RNA and protein homeostasis. *Neuron* **79**, 416-438 (2013).
110. S. S. Ambegaokar, G. R. Jackson, Functional genomic screen and network analysis reveal novel modifiers of tauopathy dissociated from tau phosphorylation. *Hum Mol Genet* **20**, 4947-4977 (2011).
111. A. Al-Saif, F. Al-Mohanna, S. Bohlega, A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. *Ann Neurol* **70**, 913-919 (2011).
112. N. Bernard-Marissal, J. J. Médard, H. Azzedine, R. Chrast, Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. *Brain* **138**, 875-890 (2015).
113. S. Couly *et al.*, Sigma-1 receptor is a key genetic modulator in amyotrophic lateral sclerosis. *Hum Mol Genet* **29**, 529-540 (2020).
114. T. A. Mavlyutov, M. L. Epstein, K. A. Andersen, L. Ziskind-Conhaim, A. E. Ruoho, The sigma-1 receptor is enriched in postsynaptic sites of C-terminals in mouse motoneurons. An anatomical and behavioral study. *Neuroscience* **167**, 247-255 (2010).

115. T. J. Kwiatkowski, Jr. *et al.*, Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. *Science* **323**, 1205-1208 (2009).
116. C. Vance *et al.*, Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. *Science* **323**, 1208-1211 (2009).
117. R. Stoica *et al.*, ALS/FTD-associated FUS activates GSK-3β to disrupt the VAPB-PTPIP51 interaction and ER-mitochondria associations. *EMBO Rep* **17**, 1326-1342 (2016).
118. D. K. H. Nguyen, R. Thombre, J. Wang, Autophagy as a common pathway in amyotrophic lateral sclerosis. *Neurosci Lett* **697**, 34-48 (2019).
119. P. Gomez-Suaga *et al.*, The ER-Mitochondria Tethering Complex VAPB-PTPIP51 Regulates Autophagy. *Curr Biol* **27**, 371-385 (2017).