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The functional universe of membrane contact sites

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Abstract

Organelles compartmentalize eukaryotic cells, enhancing their ability to respond to environmental and developmental changes. One way in which organelles communicate and integrate their activities is by forming close contacts, often called ‘membrane contact sites’ (MCSs). Interest in MCSs has grown dramatically in the past decade as it is has become clear that they are ubiquitous and have a much broader range of critical roles in cells than was initially thought. Indeed, functions for MCSs in intracellular signalling (particularly calcium signalling, reactive oxygen species signalling and lipid signalling), autophagy, lipid metabolism, membrane dynamics, cellular stress responses and organelle trafficking and biogenesis have now been reported.

The exchange of signals and metabolites between cellular compartments was thought to occur primarily by two broad mechanisms: diffusion or active transport through the cytoplasm, and vesicular trafficking. A third mechanism, the exchange of signals and metabolites at regions where organelles are in direct contact, was thought to be limited to contacts between the endoplasmic reticulum (ER) and the plasma membrane or contacts between the ER and the mitochondria, both of which have roles in calcium signalling and lipid trafficking. However, discoveries in the past decade have revealed that the roles of regions at which organelles interact are markedly more widespread in cell physiology and cellular stress responses than had been appreciated. It is now clear that interorganelle contacts are ubiquitous; every organelle forms functional contacts with at least one other organelle and often with more than one[1,2].

Regions of close contact between organelles have been termed ‘membrane contact sites’ (MCSs) and, as the study of MCSs matures, the focus is shifting from cataloguing MCSs

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The authors contributed equally to all aspects of the article.

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and tethers to understanding their functions. With this shift in mind, this Review focuses on our emerging understanding of the functions of MCSs, starting with a brief discussion of MCS formation and membrane tethering at contacts before discussing the various functions of MCSs (FIG. 1). Although MCSs have been defined in previous reviews[3–6], here we define MCSs as regions at which two organelles physically interact, resulting in the function of either organelle or both organelles being affected. We use the term ‘membrane contact sites’ in this Review because it is widely used, but it should be noted that similar contacts also form between membraneless organelles (called ‘biomolecular condensates’) and membrane-bound organelles[7].

Types of MCSs and their tethering

Identifying and quantifying MCSs is complex because distinguishing functional MCSs from non-functional, stochastic interactions between compartments is technically challenging; the challenges of, and methods for, visualizing MCSs are discussed in Supplementary Box 1 and have been comprehensively reviewed[6]. While MCSs have long been noted in studies of cell architecture using electron microscopy, more recent systematic characterizations of MCSs in live cells using fluorescence microscopy have revealed that all organelles form contacts with other compartments[1,2]. MCS-like contacts are also likely to exist between membranes within organelles that have internal membranes, such as mitochondria, chloroplasts, and multivesicular bodies (MVBs). These intraorganelle MCSs probably play critical roles in the exchange of proteins, lipids and signals within mitochondria[8] and other organelles with internal membranes.

Diversity of MCSs

There is considerable diversity in the shape and composition of MCSs. For example, the distance between organelles at MCSs can be fixed or variable. Indeed, the distance between the ER and the plasma membrane at MCSs in some mammalian cells is 19–22 nm[9], while the range between these organelles is broader in *Saccharomyces cerevisiae* (17–57 nm)[10]. The stability of contact sites also differs, with some MCSs lasting less than 1 s and others, such as the specialized contacts in muscle cells, persisting for the lifetime of the cell[11,12]. These muscle MCSs, which were the first to be characterized at a molecular level, form between invaginations of the plasma membrane and the ER and are essential for coupling muscle excitation and contraction[11,12].

MCSs can have different primary functions, and organelles can form more than one type of functionally distinct MCS. There are also a growing number of instances in which two organelles are connected simultaneously by two functionally different types of MCSs. For example, there are probably two types of ER–mitochondria and ER–endosome contacts, namely those that mediate organelle fission and those that serve as sites of signalling and lipid exchange[13,14]. Similarly, there are contacts between the ER and the plasma membrane that may be sites of cholesterol exchange between these organelles and that are functionally distinct from those that facilitate store-operated calcium entry[15]. Two functionally distinct types of vacuole–mitochondria contacts also exist in yeast[16]; their functions are not known

but they may facilitate the exchange of different lipids or other hydrophobic metabolites between these organelles.

Another type of MCS, intraorganelle contact sites, forms between two regions of the same organelle, for example between different parts of the ER[17] or between two compartments of the same organelle, which has been found for mitochondria[18–20] (FIG. 2Aa). Lipid droplets can also form a unique type of MCS with the ER, which we call ‘bridging contacts’, that may be stabilized by the ER protein seipin[21] (FIG. 2Ab). Specifically, lipid droplets have a core of storage lipids surrounded by a phospholipid monolayer that can be continuous with the outer leaflet of the ER bilayer. These structures are considered MCSs because the organelles remain distinct but closely apposed despite sharing a membrane. Lipid droplets also form standard MCSs with the ER, some mediated by a Ras-related protein Rab18 (RAB18)-containing tether[22] (FIG. 2Ab).

Finally, some MCSs appear to form between three organelles. For example, the ER is present at contacts between mitochondria and the plasma membrane in yeast[23]. There are also three-way contacts between the ER, lipid droplets and vacuoles in yeast, and it has been proposed that the structural yeast protein Mdm1, which resides in the ER, can simultaneously interact with vacuoles and lipid droplets[24] (FIG. 2Ac). Similar three-way junctions have been observed between Rab5-positive early endosomes, mitochondria and the ER[25]; lipid droplets, peroxisomes and the ER[26]; mitochondria, chloroplasts and peroxisomes[27]; and the ER, lipid droplets and the Golgi complex in primary human hepatocytes infected with hepatitis C virus[28].

Tethers at MCSs

Tethers maintain MCSs, but there has been some controversy over which proteins should count as bona fide tethers[29]. We think that any protein or complex of proteins that increases the affinity of one organelle for another should be considered a tether, and we use this definition for tethers throughout this article. As the list of MCS tethers has increased, three trends have emerged with important implications for the study of contact sites. First, almost all tethering proteins have functions at MCSs in addition to tethering. For example, oxysterol-binding protein both tethers the ER to the Golgi complex and transfers lipids between these organelles[30]. Distinguishing the role of a protein in tethering from its other functions at MCSs can therefore be complex. Second, most MCSs are probably maintained by multiple tethers. For example, a recent study showed that in yeast at least seven proteins or complexes mediate ER–plasma membrane tethering[31]. Third, a growing number of tethers can localize to multiple MCSs, and some tethers transfer between MCSs during stress or nutritional change. For example, the membrane-anchored lipid-binding proteins (Lams; also known as Ltc proteins) in yeast[32] and vacuolar protein sorting-associated protein 13A (VPS13A) and VPS13C in mammalian cells[33,34] localize to multiple MCSs, and yeast nucleus–vacuolar junction protein 2 (Nvj2) relocalizes from nucleus–vacuole contacts to ER–Golgi complex contacts in *S. cerevisiae* during ceramide-induced ER stress[35] (FIG. 2B). Together, these developments reveal that many tethers are mobile and that most MCSs are probably maintained by multiple tethers. The remainder of this Review describes the main cellular functions of MCSs.

Roles in lipid exchange

Non-vesicular lipid transport is facilitated by lipid transport proteins (LTPs), which have domains that can bind one or more lipid monomers in hydrophobic pockets or grooves and transfer the lipids between membranes. Although most LTPs are soluble, some have transmembrane domains and many bind MCS-localized membrane proteins such as vesicle-associated membrane protein (VAMP)-associated proteins (VAPs) in the ER. Thus, MCSs play important roles in intracellular lipid trafficking by serving as sites of non-vesicular lipid exchange between organelles. Indeed, LTPs from numerous families are enriched at MCSs and, because their roles in non-vesicular lipid trafficking at MCSs have been reviewed recently[36], we focus on providing an overview of this topic.

Although the mechanism and functions of lipid exchange at MCSs remain poorly understood, five functions of lipid transfer at MCSs have been proposed. First, lipid transfer at MCSs may regulate lipid-based signalling pathways, particularly those pathways modulated by phosphoinositide levels (see later). Second, it may alter the organization of lipids in membranes. For example, the sterol-transporting LTP Lam6 (also known as Ltc1) operates at contacts between the ER and the vacuole in yeast and is necessary to generate sterol-enriched domains in the vacuole, probably by transferring sterols to the vacuole from the ER[32]. A third role for lipid exchange at MCSs is to channel lipids to specific compartments, such as mitochondria[37] (discussed in the following section). Fourth, lipid exchange at MCSs may allow lipids to be transferred between organelles when vesicular trafficking is compromised. For example, ceramides are transferred from the ER to the Golgi complex by both vesicular and non-vesicular mechanisms, and both yeast and mammalian cells upregulate non-vesicular transport when vesicular transport is blocked[38,39], probably at ER–Golgi complex MCSs[35]. Indeed, it has been proposed that lipid exchange at MCSs may be an evolutionarily ancient mechanism of lipid exchange between organelles that preceded vesicular transport[40]. Finally, it has been proposed that lipid transport at MCSs provides lipids for the biogenesis of mitochondria and chloroplast membranes; these organelles probably obtain most lipids by non-vesicular transport pathways that operate at MCSs. Although the mechanism of this transport is not well understood, it seems to occur via redundant pathways in yeast, which may transfer lipids to mitochondria via mitochondria–ER and mitochondria–vacuole MCSs[41–44].

The transport of lipids against a concentration gradient at some MCSs may be driven by LTPs that use the difference in phosphatidylinositol 4-phosphate (PI4P) concentration in the two membranes at an MCS to drive the transport of a second lipid. This mechanism was shown for oxysterol-binding protein (FIG. 3), which can exchange cholesterol and PI4P at MCSs between the ER and the trans-Golgi network[30]. It was subsequently shown for other lipids and LTPs, including oxysterol-binding protein-related protein 5 (ORP5) and ORP8, which exchange PI4P and phosphatidylserine at the plasma membrane, and Nir2, which may exchange phosphatidic acid and phosphatidylinositol at the plasma membrane[45–50] (FIG. 3). This form of lipid exchange at MCSs, sometimes called ‘countertransport’, may consume a large fraction of PI4P in cells to drive lipid transport[51].

Although it is widely assumed that the enrichment of soluble LTPs at MCSs increases transport efficiency by reducing the time it takes for LTPs to diffuse between organelles, this may not always be true. A recent study in yeast investigated the role of ER–plasma membrane MCSs in facilitating sterol transport from the ER to the plasma membrane using a strain in which the size of these MCSs was markedly reduced[31]. Remarkably, the rate of ER–plasma membrane sterol transport was not decreased in this strain, suggesting that, at least in some cases, MCSs may not increase non-vesicular lipid transport rates. Whether this is true for other types of lipids and MCSs remains to be determined.

Metabolic channelling at MCSs

MCSs allow metabolites to be efficiently transferred between cellular compartments. Although this transfer has been best characterized for Ca^{2+}, the metabolism of lipids and other hydrophobic metabolites at MCSs may allow a form of substrate channelling that occurs when metabolites are passed directly between enzymes. Channelling can increase reactions rates by reducing the time it takes metabolites to move from one enzyme to the next. It can also prevent metabolites from being consumed by off-pathway reactions. Lipid exchange at MCSs can also channel lipids to particular organelles or enzymes.

Calcium channelling

Ca^{2+} entry into cells through the plasma membrane is regulated by the Ca^{2+} concentration of the ER lumen and, hence, is named ‘store-operated Ca^{2+} entry’[33]. This process requires the interaction of stromal interaction molecule 1 (STIM1), a Ca^{2+} sensor in the ER, and the plasma membrane channel calcium release-activated calcium channel protein 1 (ORAI1)[52]. When Ca^{2+} levels decrease in the ER, Ca^{2+} dissociates from the luminal domain of STIM1, which is likely to be dimeric in resting cells, and the protein forms multimeric clusters that activate ORAI1 channels[53] at the plasma membrane; activated ORAI1 channels allow the Ca^{2+} pump sarcoplasmic reticulum/ER Ca^{2+}-ATPase (SERCA) in the ER to take Ca^{2+} into the ER (FIG. 4a). The channelling of Ca^{2+} via this mechanism, which involves plasma membrane–ER MCSs, allows the Ca^{2+} to directly enter the ER without increasing the Ca^{2+} concentration in the cytoplasm outside the MCS. Some cell types, namely pancreatic acinar cells and oocytes, can also channel Ca^{2+} directly from the ER lumen out of cells at ER–plasma membrane contacts, allowing a form of Ca^{2+} channelling across cells termed ‘tunnelling’[54]. Ca^{2+} channelling also occurs at ER–mitochondria MCSs (see later).

STIM1 is thought to be constitutively enriched at ER–plasma membrane MCSs owing to its interaction with the microtubule positive end-tracking protein EB1, which positions STIM1 at the tip of ER tubules in the cell periphery[55,56]. Activated STIM1 clusters appear at MCSs marked by the known ER–plasma membrane tethers extended synaptotagmin 2 (E-Syt2) and E-Syt3 (referred to here as E-Syt2/3) and GRAM domain-containing protein 2A (GRAMD2A)[15]. However, cells lacking all three E-Syt proteins do not show impaired store-operated Ca^{2+} entry[57], indicating that there are additional ER–plasma membrane tethers. Phosphoinositides are also critical for the formation of ER–plasma membrane contacts and their regulation. Specifically, E-Syts interact with the plasma membrane via C2 domains that interact with phosphoinositides, mainly phosphatidylinositol 4,5-bisphosphate

($PI(4,5)P_2$)[57]. This binding occurs at resting cytoplasmic Ca^{2+} concentrations (for E-Syt2/3) and is enhanced at elevated Ca^{2+} concentrations, particularly for E-Syt1 (REFs[57,58]).

Channelling lipids and other metabolites

The channelling of lipids at MCSs can be driven by lipid consumption. For example, the non-vesicular transport of ceramide from the ER (where it is produced) to the Golgi complex at MCSs does not require energy but is driven by the conversion of ceramide into sphingomyelin in the Golgi complex[59]. There is growing evidence that channelling can also be driven by lipid production at MCSs. This phenomenon has been shown for phosphatidylserine, which is produced in the ER at ER–mitochondria MCSs and channelled to mitochondria[60]. Channelling at ER–plasma membrane MCSs also ensures that there is sufficient phosphatidylinositol (produced in portions of the ER that contact the plasma membrane) for phosphatidylinositol kinases in the plasma membrane to produce PI4P[61]. In both cases where lipid production at MCSs drives channelling, the channelling is likely to be driven by the enrichment of the transported lipid at the MCSs.

Channelling can also occur when an LTP is enriched at an MCS. For example, relocalizing oxysterol-binding protein homologue 6 (Osh6), the LTP that transports phosphatidylserine, to ER–vacuole MCSs, channels phosphatidylserine from the ER to the vacuole in *S. cerevisiae*[62]. Similarly, artificially relocalizing CERT, the mammalian LTP that transports ceramide, to ER–mitochondria MCSs in mammalian cells promotes the transfer of ceramide from the ER to mitochondria, where it induces apoptosis[40].

Other lipids produced at MCSs are likely to be channelled between organelles. Indeed, the production of fatty acids and fatty acyl-CoA molecules at MCSs, including lipid droplet–peroxisome MCSs[63], lipid droplet–mitochondria MCSs[64], MCSs between the inner membrane and the outer membrane of mitochondria and ER–plastid MCSs[65,66], is thought to promote their transfer at these sites in yeast, mammalian cells and plant cells. It has also been suggested that the products of fatty acid β-oxidation in yeast peroxisomes are channelled to mitochondria at MCSs between these organelles, where they are degraded by the Krebs cycle[2]. It is also likely that small molecules other than lipids and calcium are exchanged at MCSs. For example, evidence suggests that iron is directly transferred between mitochondria and endosomes at transient contacts formed between these organelles in erythroid cells[67,68].

MCSs and cell signalling

MCSs play multiple roles in signalling. One is to serve as signalling hubs that assemble proteins in response to stress and altered nutritional status, particularly at ER–mitochondria contacts. The roles of MCSs in cellular stress responses are discussed later. MCSs also facilitate signalling by allowing the targeted, interorganelle exchange of Ca^{2+}, reactive oxygen species (ROS), lipids and possibly other signalling molecules. MCSs also enable signalling enzymes in one compartment to interact directly with substrates in a second compartment. Finally, MCSs facilitate signalling by maintaining phosphoinositide levels at those required for signalling events that may occur at these sites or outside them.

Ca^{2+} signalling at ER–mitochondria MCSs

Mitochondrial Ca^{2+} levels control many aspects of mitochondrial function, including oxidative phosphorylation, ROS generation and signalling, and apoptosis. ER–mitochondria MCSs play a central role in regulating mitochondrial Ca^{2+} levels by modulating Ca^{2+} movement between the ER and mitochondria. The efficient ER–mitochondria transfer of Ca^{2+} requires the enrichment of ER Ca^{2+} channels called 'inositol 1,4,5-trisphosphate receptors (IP3Rs)' at ER–mitochondria MCSs[69] and creates Ca^{2+} concentrations at MCSs that are much higher than those in the surrounding cytoplasm[70] (FIG. 4a). Ca^{2+} exchange between the ER and mitochondria is facilitated by an ER–mitochondria tether formed by voltage-dependent anion-selective channel protein (VDAC) in mitochondria, IP3Rs in the ER and the chaperone 75-kDa glucose-regulated protein (GRP75; also known as stress-70 protein, mitochondrial)[71]. Furthermore, in muscle cells, the ryanodine receptor, a calcium channel in the ER, also allows calcium signalling between the ER and mitochondria at MCSs in addition to its well-established role in calcium channelling at ER–plasma membrane contacts[72].

There is growing evidence that Ca^{2+} signalling at ER–mitochondria MCSs is linked to ROS signalling at these sites. For example, recent work suggests that Ca^{2+} signalling at these MCSs helps to regulate a signalling pathway mediated by the ROS-regulated oxidoreductases thioredoxin-related transmembrane protein 1 (TMX1) and TMX3, which in turn controls a signalling pathway that regulates nuclear factor of activated T cells (NFAT1), a transcription factor involved in the proliferation of melanoma cell lines[73]. Studies also suggest a link between Ca^{2+} signalling at ER–mitochondria MCSs and the metabolism of cholesterol and other lipids[74], although the underlying mechanisms are not well understood. Ca^{2+} signalling at these junctions also promotes apoptosis and has been linked to diseases, including neurodegenerative diseases, cancers and type 2 diabetes; these aspects of Ca^{2+} signalling at ER–mitochondria MCSs are discussed in the section 'MCSs and stress'.

A number of ER–mitochondria tethers, in addition to the VDAC–GRP75–IP3R tether that affects mitochondrial Ca^{2+} uptake through ER–mitochondria MCSs in mammalian cells, have been identified or proposed[75], but the full repertoire remains to be determined. The most recent tethering protein to be described is PDZ domain-containing protein 8 (PDZD8)[76]. PDZD8 is homologous to Mmm1, one of four proteins in an ER–mitochondria tethering complex in *S. cerevisiae*, called the ER–mitochondria encounter structure (ERMES)[77], which was not thought to be present in mammals.

ROS signalling at ER–mitochondria MCSs

ROS are formed by oxidative metabolism and by environmental stresses such as the exposure of cells to oxidants or ultraviolet light. ROS can reversibly modify proteins and alter their function, similarly to phosphorylation. In many cases, ROS modify the oxidation state of cysteine residues in proteins or cause them to form thiol adducts. Recently, ER–mitochondria MCSs were shown to be periodically exposed to the ROS H_2O_2 produced by mitochondria; fluctuations in the levels of this H_2O_2 pool are induced by, and increase, Ca^{2+} signalling at ER–mitochondria contacts[78]. ROS modulate a number of proteins that

mediate or regulate Ca^{2+} signalling at ER–mitochondria MCSs, including the Ca^{2+} channels IP3Rs[79]. ROS signalling at ER–mitochondria MCSs has been well reviewed[80,81].

Phosphoinositide-based signalling

Phosphoinositide-based signalling occurs throughout the cell[82]. Although phosphatidylinositol is synthesized in the ER, the kinases that convert it into phosphoinositides are not[83]. Therefore, maintaining phosphoinositide levels throughout the cell requires phosphatidylinositol transport from the ER to other compartments, transport that often occurs at MCSs; the products of phosphoinositide breakdown can also be returned to the ER at these sites[83]. For example, plasma membrane phosphoinositides are critical components of the signalling cascades that are initiated by activation of cell surface receptors, such as receptor tyrosine kinases, which generate Ca^{2+} signals through the activation of phospholipase C. The products of phospholipase C-mediated $PI(4,5)P_2$ hydrolysis, namely inositol 1,4,5-trisphosphate and diacylglyceride (DAG), serve as second messengers by releasing intracellular Ca^{2+} via IP3Rs and activating protein kinase C enzymes, respectively[84]. $PI(4,5)P_2$ also promotes the activity of ion channels at the plasma membrane and promotes endocytosis[85]. Therefore, restoring levels of $PI(4,5)P_2$ in the plasma membrane and removing DAG are essential for maintaining and regulating these signalling pathways. Here we discuss two examples of how MCSs help maintain levels of phosphopinositides and DAG.

Maintenance of plasma membrane $PI(4,5)P_2$.—To replenish $PI(4,5)P_2$ pools in the plasma membrane, phosphatidylinositol has to be transferred from the ER to the plasma membrane, where it is sequentially phosphorylated by phosphatidylinositol 4-kinases and phosphatidylinositol 4-phosphate 5-kinases (FIG. 3). Although the mechanism of phosphatidylinositol transfer from the ER to the plasma membrane is not well understood, in mammalian cells NIR2 operating at ER–mitochondria MCSs delivers phosphatidylinositol from the ER to the plasma membrane[86] and phosphatidic acid from the plasma membrane to the ER[87]. Phospholipid transfer protein C2CD2L (also known as TMEM24) can also transfer phosphatidylinositol from the ER to the plasma membrane to replenish $PI(4,5)P_2$ in pancreatic β-cells stimulated with elevated levels of glucose[88]. DAG generated by phospholipase C-mediated $PI(4,5)P_2$ hydrolysis at the plasma membrane can be transported by E-Syts to the ER (FIG. 4a), where it is used to synthesize other lipids[89,90]. DAG transport out of the plasma membrane may also attenuate DAG-mediated signalling.

$PI(4,5)P_2$ metabolism and PI4P transport.—In mammalian cells, the LTPs ORP5 and ORP8 use the PI4P gradient between the plasma membrane and the ER (high PI4P level in the plasma membrane and low level in the ER) to transport phosphatidylserine from the ER to the plasma membrane at contacts between these organelles using the countertransport mechanism described in the section ‘Roles in lipid exchange’[47] (FIG. 3). Osh6 and Osh7 perform the same function in yeast[48]. This finding raises the question of how cells direct PI4P to the plasma membrane to maintain the PI4P gradient, since PI4P in the plasma membrane is converted into PI(4,5)P and transported out of the plasma membrane to the ER, where it is dephosphorylated by the ER-localized phosphoinositide phosphatase Sac1. This question was addressed in two studies that found that when PI4P or $PI(4,5)P_2$

levels in the plasma membrane are low, ORP5 and ORP8 disengage from the plasma membrane and stop transporting PI4P out of it. In addition, ORP8 also prevents the over-accumulation of $PI(4,5)P_2$ in the plasma membrane by decreasing the availability of PI4P for phosphatidylinositol 4-phosphate 5-kinases[91], and by directly transporting $PI(4,5)P_2$ out of the plasma membrane[50]. Sac1 may also decrease PI4P levels in the plasma membrane by hydrolysing it at ER–plasma membrane MCSs (discussed in the next section).

Signalling by enzymes working in trans

Some signalling at MCSs is not mediated by the exchange of small molecules but instead occurs when an enzyme in one organelle acts on a substrate in a second organelle, also called ‘working in trans’ (FIG. 4b). One example of working in trans is the dephosphorylation of several tyrosine kinase receptors by ER-localized protein-tyrosine phosphatase 1B (PTP1B)[92]. Dephosphorylation of these receptors, which is critical for limiting their signalling functions, occurs at MCSs at which the ER-localized phosphatase acts in trans to dephosphorylate receptors in the plasma membrane or in endosomes after internalization[93]. It is unclear which endocytic compartment is the most important target of PTP1B, but the endocytosis of tyrosine kinase receptors has a major role in their exposure to the phosphatase[60].

In yeast, Sac1, which is located in the ER, may control plasma membrane PI4P levels by in trans dephosphorylation (FIG. 4b). Indeed, Sac1 can dephosphorylate PI4P in the plasma membrane to phosphatidylinositol in trans in cells and Sac1 in ER-derived vesicles can dephosphorylate PI4P in liposomes in trans[94]. Sac1 may also operate in trans at ER–trans-Golgi network MCSs, and its activity is enhanced by interacting with the adaptor protein FAPP1 (also known as pleckstrin homology domain-containing family A member 3) at these junctions[95]. However, the idea that Sac1 operates in trans at MCSs has been questioned because the in trans activity seems too low to be physiologically relevant[30,96].

Regulation of membrane dynamics

MCSs generate subdomains within organelle membranes that regulate many membrane-based processes in cells and affect the physical properties and shapes of membranes, as well as the trafficking, intracellular positioning and function of organelles.

Organelle fission and fusion

Mitochondria continuously undergo fission and fusion, which helps optimize their function[97]. ER–mitochondria MCSs regulate mitochondrial fission[13]. Preceding mitochondrial fission, the ER wraps around mitochondria at future division sites in yeast and mammalian cells[13], where it is likely to have both regulatory and mechanical roles. A regulatory role is suggested by the finding that after the ER wraps around mitochondria, inner membrane scission precedes outer membrane scission[98] and mitochondrial nucleoids accumulate and replicate at MCSs before fission[11,99]. In mammalian cells, the ER also has a role in actin-dependent mitochondrial constriction during division, which is probably a mechanical process. Actin–myosin complexes assemble at ER–mitochondria contacts, a process that requires inverted formin 2 and spire 1c, actin nucleating proteins that localize

to ER–mitochondria MCSs[100,101]. Subsequently, dynamin-related protein 1 (DRP1) and other adaptor proteins are recruited to contact sites to further constrict mitochondria and complete fission[102–106]. ER–mitochondria contacts have recently been associated with sites of mitochondrial fusion, but whether MCSs play a direct role in fusion remains to be determined[107].

ER–endosome contacts also mediate endosome fission. ER tubules wrap around the base of budding regions of endosomes and determine the timing and location of fission[108]. These contacts have important roles in protein sorting in endosomal compartments (see later).

Establishing diffusion barriers

MCSs can affect the diffusion of proteins and lipids in contacting organelles. In budding yeast, specialized ER–plasma membrane MCSs form an ER diffusion barrier in the bud neck that connects mother and daughter cells[109]. The barrier helps regulate the asymmetric inheritance of cellular components, promoting the retention of ageing factors in the mother cell. There is evidence that barrier formation requires sphingolipids, the GTPase Bud1 (also known as Ras-related protein Rsr1) and plasma membrane-associated cytoskeletal structures formed by septins[110,111]. The binding of septins by the yeast homologue of VAP, VAMP-associated protein Scs2, is necessary for the formation of the diffusion barrier[112], and septins may specifically slow the diffusion of proteins that localize to ER–plasma membrane MCSs but not that of other ER or plasma membrane proteins[113]; however, it is possible that septins could play similar roles at other MCSs.

Contacts between the ER and the plasma membrane also restrict protein diffusion in plasmodesmata, narrow channels that link adjacent cells in plants[114,115]. Cells connected by plasmodesmata have a single ER tubule that runs through each plasmodesma, linking the ERs in connected cells. The ER tubule in plasmodesmata forms extensive contacts with the plasma membrane that restrict the diffusion of ER and cytoplasmic proteins between cells[116].

A diffusion barrier, termed the ‘nucleus–vacuolar junction’ (NVJ), is also present at MCSs between the vacuole and the nucleus in yeast. This MCS forms a barrier in the vacuole membrane that excludes the vacuolar ATPase complex and may have a lipid composition that affects diffusion[117,118]. The functional role of this diffusion barrier remains unknown.

Autophagosome formation

Autophagy is a process of regulated degradation that eliminates damaged or unnecessary cellular components by transporting them to lysosomes, where they are destroyed (FIG. 5). In multicellular organisms, the formation of autophagosomes (organelles that first engulf material targeted for degradation) requires the generation of MCSs between a subdomain of the ER, termed the ‘omegasome’, and a cup-shaped membrane, known as the isolation membrane or phagophore, that expands and eventually closes to form the autophagosome (FIG. 5a). Analogous MCSs form in yeast between growing autophagosomes and specialized subdomains of the ER called ‘ER exit sites’, where vesicles in the secretory pathway also form[119,120].

Where autophagosomes initially form, and the source of the proteins and lipids needed for autophagosome expansion, remains controversial[5,121]. In mammals, autophagosome nucleation may occur at ER–mitochondria MCSs[122] and ER–plasma membrane MCSs[123]. E-Syt2 and E-Syt3 at ER–plasma membrane contacts may help generate the phosphatidylinositol 3-phosphate (PI3P) needed for autophagosome formation by recruiting the PI3P-synthesizing phosphatidylinositol 3-kinase catalytic class 3 complex[123].

A number of proteins localize to contacts between the ER and the forming autophagosomes. The ER proteins VAPA and VAPB bind the serine/threonine-protein kinase ULK1 and focal adhesion kinase family kinase-interacting protein of 200 kDa (FIP200) on forming autophagosomes and are required for proper autophagosome biogenesis[124]; ULK1 forms a complex with FIP200 (together with autophagy-related protein 13 (ATG13) and ATG101) that regulates autophagosome formation[125]. ULK1 and FIP200 differ considerably from most other proteins that interact with VAPs, which often bind lipids. VMP1 is another ER protein that is present at ER–autophagosome contacts. The role of VMP1 at MCSs is not clear, but the number of ER–autophagosome contacts increases in cells depleted of this protein[126]. This increase may occur because VMP1 promotes SERCA activity, but how changes in ER calcium levels affect ER–autophagosome MCS formation is not known. It has also been suggested that VMP1 modulates lipid metabolism in cells[127], which may affect ER–autophagosome MCS formation.

Phosphatidylinositol synthase is also enriched at ER–autophagosome contact sites and may be required to generate a pool of PI3P necessary for autophagosome biogenesis[128]. Phosphatidylinositols and other lipids are probably transferred from the ER to autophagosomes at MCSs by the LTP ATG2 (REF.[129]). Lipids could also be transferred from the ER to autophagosomes via thin membranous tubules that may connect these organelles at MCSs, but the nature of these tubules remains to be determined[130,131].

Although ER–autophagosome MCSs may play a primary role in autophagosome biogenesis, many studies suggest that growing autophagosomes simultaneously contact other organelles, including mitochondria, endosomes, the Golgi complex and lysosomes (in mammalian cells) or vacuoles (in yeast)[119,132]; autophagosomes may acquire lipids via these MCSs.

Endosomal cargo sorting

There is growing evidence that MCSs help sort cargo proteins into transport vesicles in endosomal trafficking pathways, which connects endocytic vesicles with the trans-Golgi network and lysosomes; MVBs are part of these trafficking pathways[133]. Membrane proteins on the surface of MVBs can be recycled to the trans-Golgi network or transferred into intralumenal vesicles (ILVs) within the MVB. These vesicles and their contents are degraded when MVBs fuse with lysosomes. ER–MVB contacts help sort epidermal growth factor receptors from the surface of MVBs into ILVs; the ER-localized PTP1B interacts with epidermal growth factor on the surface of MVBs at MCSs and is necessary for sorting the receptor into ILVs[134]. PTP1B is also necessary for the formation of ILVs, which is initiated by a member of the endosomal sorting complexes required for transport (ESCRT) family, ESCRT-0. PTP1B probably regulates ILV formation by dephosphorylating components of ESCRT-0, namely hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) and

signal transducing adapter molecule 2 (STAM2), on the surface of MVBs at ER–MVB contact sites[135].

ER–endosome MCSs also have roles in cargo sorting in the endosome-to-Golgi retrieval pathway, which returns proteins to the trans-Golgi network from endosomes. The retromer complex, which sits on the surface of endosomes, has a central role in cargo sorting into the retrieval pathway, as VAPs in the ER bind the retromer component sorting nexin 2 (SNX2), forming MCSs that regulate the sorting of trans-Golgi network proteins into the retrieval pathway[136].

ER–endosome contacts also regulate the sorting of cargo, such as mannose 6-phosphate receptor, into endosomal transport vesicles that are formed by scission of budding domains in endosomes[108]. Spastin on the surface of the ER binds the ESCRT-III protein IST1 homologue at ER–endosome contacts and drives endosomal tubule fission by an unknown mechanism[137]. Since mutations in the gene that encodes spastin cause the neurodegenerative disease hereditary spastic paraplegia, this study suggests that ER–endosome contacts and the proper sorting of cargo into endosomal transport compartments may play a role in the axonal degradation that occurs in this disease.

Finally, it has been shown that the ER membrane protein transmembrane and coiled-coil domains protein 1 (TMCC1), which has a central role in forming ER–endosome MCSs, is required for the ER-driven fission of endosome budding; this fission is necessary for the sorting of mannose 6-phosphate receptor into transport vesicles[14].

Organelle trafficking and positioning

Time-lapse imaging of multiple organelles simultaneously suggests that MCSs affect the transport and positioning of many organelles at once[1,107]. In some cases, the motor-based transport of one organelle will move organelles that are connected to it by MCSs. For example, in *Aspergillus nidulans*, the endosome-associated protein PxdA binds peroxisomes, generating MCSs that allow the peroxisomes to hitchhike on endosomes as they are moved by the microtubule-based motors dynein 1 and kinesins[138]. ER–endosome and ER–mitochondria MCSs are also maintained during the trafficking of organelles along microtubules, allowing these organelles to be transported together[139]. In other cases, MCSs can prevent vesicles from being transported by anchoring them to an immobile organelle; for example, the ER-localized protein E3 ubiquitin-protein ligase RNF26 forms a complex that binds proteins on endosomal compartments, preventing these endosomes from being transported[140]. Proteins that determine whether organelles are attached to motor proteins or to other organelles via MCSs have been identified. For example, the cholesterol sensor ORP1L determines whether endosomes form MCSs with the ER or interact with motor proteins[141]. ORP1L links endosomes to dynactin motor complexes and, in response to lower cellular cholesterol levels, undergoes a conformational change that causes it to disengage from dynactin and interact with VAPs in the ER, forming ER–endosome MCSs. Similarly, the ER protein protrudin, which is necessary for neurite outgrowth, forms ER–late endosome contacts that facilitate the transfer of kinesin 1, a microtubule motor, from protrudin to FYVE and coiled-coil domain-containing protein 1 (FYCO1) on late endosomes[142].

Finally, in budding yeast, the inheritance of mitochondria, lipid droplets and peroxisomes is regulated by MCSs. Some peroxisomes and lipid droplets are transferred into daughter cells by the motor myosin 2 (Myo2), while others are retained in the mother by contact with the ER, ensuring that some are retained by the mothers[143,144]. Similarly, mitochondrial inheritance is regulated by at least three mitochondrial MCSs with the ER and plasma membrane that are mediated by tethers that contain mitochondrial Myo2 receptor-related protein 1 (Mmr1)[145], the nuclear migration protein Num1 (REF.[23]) or the mitochondrial F-box protein Mfb1 (REF.[146]). These tethers ensure that functional mitochondria are inherited by both cells.

Organelle subpopulations

Evidence also indicates that MCSs can establish functionally distinct subpopulations of organelles. In yeast, a subpopulation of functionally distinct lipid droplets, which may mobilize stored lipids in response to environmental cues, is formed by specialized MCSs with the ER that are absent from other lipid droplets[147,148]. Similarly, in interscapular brown adipose tissue, mitochondria that form MCSs with lipid droplets are functionally distinct from mitochondria that are not in contact with lipid droplets[149]. Mitochondria associated with lipid droplets have a distinct protein and lipid composition and exhibit increased ATP synthesis and reduced β-oxidation of fatty acids compared with mitochondria that do not make these contacts. Lipid droplet–mitochondria association also increases the production of triacylglycerides, lipids that are stored in lipid droplets. It seems likely that many other examples of organelle subpopulations that are generated by MCSs will be identified in the future. The creation of MCS-mediated subpopulations might also be a mechanism for segregating functional organelles from non-functional organelles.

MCSs in stress

Much of the work on MCSs has focused on the ways in which they help cells adapt to cellular stress responses, including by facilitating the integration of stress responses in two compartments that are connected by MCSs. For example, ER–mitochondria contact increases in response to ER stress[150]. During stress, chaperones in the ER increase Ca^{2+} signalling at ER–mitochondria MCSs, which stimulates oxidative phosphorylation in mitochondria and, if the ER stress is prolonged, leads to apoptosis[151]. MCSs also alleviate stress caused by a build-up of toxic lipids in an organelle by facilitating lipid movement between, and coordinating nutrient sensing pathways that respond to changes in lipid levels within, organelles connected by MCSs. MCSs also have important but still poorly understood roles in autophagosomal-based degradation pathways, which help cells adapt to changing environments (FIG. 6).

Lipid stress

Cellular lipid stress occurs when lipids accumulate to toxic levels or when environmental changes require substantial changes in lipid metabolism. For example, during phosphate starvation in plants, most of the phospholipids in cells are converted into galactoglycerolipids[152]. This conversion requires substantial non-vesicular lipid movement between the ER, mitochondria and chloroplasts, which probably occurs at MCSs; phosphate

starvation increases the size of contacts between pairs of these organelles, which probably facilitates lipid transport[66,153]. In this and the other cases discussed here, lipid stress seems to change or enhance existing contacts rather than result in the generation of new contacts. However, contacts generated solely to alleviate lipid stress may be discovered.

Some lipid intermediates, such as DAG, fatty acids and ceramide, are essential building blocks for other membrane lipids but become toxic when overabundant. To prevent this toxicity, cells store excess lipids in lipid droplets[154–156]. Lipid droplets make extensive contacts with the ER and with other organelles, including lysosomes (or vacuoles in yeast), mitochondria and peroxisomes, which probably facilitates interorganelle lipid movement[156]. Indeed, three-way contacts between the ER, lipid droplets and vacuoles in yeast are important in preventing fatty acid stress[24,157,158]. The yeast protein Mdm1 (FIG. 2Ac) is enriched at these contacts and helps prevent the accumulation of DAG by facilitating the formation of triacylglycerol by interacting with a fatty acid acyl-CoA synthase that produces a triacylglycerol precursor[24]. Lipid storage in lipid droplets also prevents toxic fatty acid accumulation during starvation-induced autophagy. Indeed, the autophagic degradation of membranous organelles generates fatty acids that are stored in lipid droplets before being delivered to mitochondria at lipid droplet–mitochondria MCSs[159].

Also in yeast, ER–Golgi complex MCSs help prevent ceramide accumulation in the ER (the formation of these contacts requires Nvj2 (FIG. 2B)), which may directly transfer ceramides and other lipids from the ER to the Golgi complex[35]. Other MCSs in yeast may sense and respond to stress caused by changes in the lipid composition of the plasma membrane. Specifically, changes in sterol homeostasis that perturb the function of the plasma membrane activate the target of rapamycin complex 2 (TORC2)-regulated kinase Ypk1 (REF.[160]); Ypk1 phosphorylates sterol-transporting proteins at ER–plasma membrane MCSs, allowing them to restore sterol homeostasis in the plasma membrane. TORC2 and Ypk1 are probably not enriched at ER–plasma membrane MCSs, although some of their targets are. There is also evidence that phosphatidylinositol kinase-mediated signalling at ER–plasma membrane MCSs activates a TORC2–AKT signalling cascade during membrane stress, allowing Ca^{2+}-regulated lipid biogenesis in the ER to counterbalance changes in plasma membrane lipid composition to maintain membrane integrity[161].

Mechanical stress

In *Arabidopsis thaliana*, the ER–plasma membrane MCS-localized protein synaptotagmin 1 (SYT1; also known as SYTA), an orthologue of the E-Syts, is induced in leaf cells undergoing mechanical stress, where it is enriched[162]. Cells lacking SYT1 are more sensitive to mechanical stress[162]. SYT1 at MCSs may help stabilize cortical microtubules and actin filaments near the plasma membrane, making the membrane resistant to mechanical deformation. SYT1 also helps cells resist other stresses that disturb the plasma membrane, including freezing and ionic stress[163–165].

Nutrient stress

MCSs have roles in some forms of selective autophagy that are induced by nutritional stress in yeast. For example, in *S. cerevisiae*, the ERMES tether is required for mitophagy, a type

of selective autophagy by which mitochondria are degraded in yeast subjected to nitrogen starvation[166]. The role of ERMES-mediated ER–mitochondria contacts in mitophagy is tied to their role in mitochondrial fission, which precedes mitophagy; mitochondria must be separated before their engulfment by the isolation membrane. The ability of the ERMES to transfer lipids between the ER and mitochondria[167] is also likely to contribute to its role in mitophagy. In mammalian cells, FUN14 domain-containing protein 1 (encoded by *FUNDC1*) is thought be enriched at ER–mitochondria MCSs and to be required for mitochondrial fission in response to hypoxic stress[168]. The tumour suppressor promyelocytic leukaemia protein (PML) is also enriched at these contact sites in normal cells, where it represses autophagy; loss of the protein from MCSs in response to hypoxia and nutrient deprivation promotes autophagy, suggesting a connection between mitophagy regulation and cancer development[169].

ER–mitochondria contacts have also been suggested to play a role in pexophagy, the degradation of peroxisomes by autophagy, which can be induced in yeast by nitrogen starvation[170]. This study found that pexophagy is dependent on an interaction of the peroxisomal membrane protein Pex11 with the ER–mitochondria tether ERMES[171]. ER–mitochondria contacts may be important for positioning peroxisomes so that they can interact with the autophagosomal machinery.

Another form of selective autophagy in yeast occurs at the NVJ. Carbon and nitrogen starvation induce a process termed ‘piecemeal microautophagy of the nucleus’ (FIG. 5b). This process, which is initiated at the NVJ and activated by the target of rapamycin kinase nutrient-sensing pathway, causes parts of the nuclear membrane that are in contact with the vacuole to be pinched off and internalized into the vacuole for degradation[172].

The NVJ plays additional roles in the cellular response to changes in nutritional status. It expands when yeast cells are grown with a non-fermentable carbon source or subjected to ER stress, and it is also a site of lipid droplet growth[157]. This growth requires Mdm1, which mediates MCS formation and facilitates triacylglycerol production at regions where lipid droplets make contact with the NVJ and may help coordinate lipid droplet biogenesis with cellular nutritional status[24]. The Mdm1 homologue sorting nexin 14 (encoded by *SNX14*) has a similar function in higher eukaryotes[173].

Finally, in yeast, ER–plasma membrane and ER–vacuole MCSs play important roles in regulating the target of rapamycin pathway. Sterol transport proteins at these junctions create membrane domains in the plasma membrane and vacuole that regulate TORC1 and TORC2 signalling, allowing the coordination of stress response pathways and sterol homeostasis[174].

Oxidative stress

ROS are generated by oxidative processes that occur naturally, primarily in mitochondria, the ER and peroxisomes. Although there are numerous pathways for reducing the level of ROS, excessive levels that damage proteins and lipids can occur. The roles of ER–mitochondria MCSs in responding to ROS have been extensively studied and reviewed[72,80]. ROS-mediated ER stress at ER–mitochondria contacts seems to require protein kinase RNA-like ER kinase (PERK), an important regulator of the unfolded protein response[175,176].

A number of redox-active proteins are enriched at ER–mitochondria contacts in mammalian cells and are regulated by ROS, including the oxidoreductases TMX1 and ERO1-like protein-α (ERO1α), and SERCA[177–179]. Elevated ROS levels at these contacts activate mitogen-activated protein kinase signalling and decrease mitochondrial motility, which is important for proper mitochondrial inheritance and quality control by reducing the inheritance of damaged mitochondria[180]. An overabundance of ER–mitochondria MCSs and impaired ROS metabolism at these sites have been linked to the mitochondrial dysfunction that occurs in obesity[181].

The roles of other MCSs in the cellular response to oxidative stress are less well understood, but a recent study found that peroxisome–lipid droplet contacts are positively regulated by oxidative stress[63]. Contacts between mitochondria and endosomes also form in response to oxidative stress, and it has been suggested that defects in the formation of such contacts could impair mitochondrial quality control during the onset of amyotrophic lateral sclerosis by an unknown mechanism[25].

ER–mitochondria contacts may also help coordinate lipid metabolism in the ER with the synthesis of the ubiquitous lipid antioxidant coenzyme Q in mitochondria, where this lipid is required for oxidative phosphorylation. Two studies found that coenzyme Q biosynthesis occurs at ER–mitochondria MCSs in yeast, and in mammals the mitochondrial fusogen mitofusin 2 is required to maintain coenzyme Q levels[182–184]. These findings suggest that coenzyme Q biosynthesis may be coordinated with ER lipid or redox metabolism, but this remains to be determined.

ER stress

The roles of ER–mitochondria contacts in the cellular response to ER stress and apoptosis have been extensively studied and recently reviewed[185]. Ca^{2+} release from the ER and uptake by mitochondria, a process that occurs at ER–mitochondria MCSs, can initiate apoptosis[186]. Ca^{2+} exchange between the ER and mitochondria is facilitated by the VDAC–GRP75–IP3R tethers[71] described earlier. Numerous proteins regulate Ca^{2+} signalling in mitochondria, and hence apoptosis, by modulating the activity of the Ca^{2+} channels in these tethers or by regulating other ER–mitochondria tethers and proteins that regulate ER Ca^{2+} levels, such as SERCA. For example, in response to ER stress, ER-resident chaperones, including calnexin, calreticulin and heat shock protein 70 family protein 5 (often called BiP) and redox-modulated proteins, such as TMX1 and ERO1α (see the previous section), increase Ca^{2+} flux at these junctions; this Ca^{2+} signalling induces oxidative phosphorylation and, if prolonged, apoptosis[151,177,187].

A number of apoptosis-regulating proteins modulate Ca^{2+} signalling at ER–mitochondria MCSs. For example, B cell lymphoma 2 (BCL-2) and other related antiapoptotic proteins bind and inhibit IP3Rs, reducing Ca^{2+} signalling at ER–mitochondria MCSs, and overexpression of BCL-2 can increase the number of ER–mitochondria contacts and reduce Ca^{2+} exchange, inhibiting apoptosis[188,189]. Induction of the expression of BCL-2-interacting killer (BIK) disrupts BCL-2–IP3R interactions, increasing calcium transfer to mitochondria, which promotes apoptosis; it also increases the number of contacts[190].

The tumour suppressor p53 (cellular tumour antigen p53) becomes enriched at ER–mitochondria contacts during ER stress, where it is thought to promote calcium influx into mitochondria by binding and stimulating SERCA, which promotes apoptosis[191]. Another role for p53 at ER–mitochondria MCSs is to negatively regulate PML enrichment at these sites; PML increases Ca^{2+} exchange between the ER and mitochondria, which promotes apoptosis[192] or autophagy[169] during ER stress. Fetal and adult testis-expressed transcript protein (encoded by *FATE1*) also localizes to, and disrupts, ER–mitochondria MCSs, reducing the sensitivity of mitochondria to proapoptotic signals[193]. ER stress-dependent activation of PERK at ER–mitochondria contacts increases contact and Ca^{2+} exchange between these organelles and induces apoptosis, although the mechanism remains to be determined[175,176,194]. PERK also regulates ER–plasma membrane contacts and calcium signalling at these contacts during ER stress; it increases these MCSs by promoting the enrichment of the ER–plasma membrane tethers STIM1 and E-Syt1 at these MCSs[195].

In summary, MCSs allow cells to integrate signalling events and the cellular stress response in different cellular compartments. Much of the work on MCSs and stress has focused on the regulation of Ca^{2+} signalling at ER–mitochondria contacts, but the study of MCSs and the stress response is expanding to other MCSs and signalling pathways.

Conclusions and perspectives

As the field has matured beyond describing new MCSs and tethers and begins to unravel the function of MCSs beyond facilitating lipid exchange and calcium signalling, including enabling cells to adapt to stress, the study of MCSs has moved to centre stage in cell biology. Much of the early work on MCSs focused on ER–plasma membrane and ER–mitochondria contacts, in part because of the availability of methods to study calcium signalling at these sites. New tools for visualizing and studying MCSs, including electron tomography, probes that fluoresce when they interact at contact sites and advanced techniques in super-resolution microscopy[6] (Supplementary Box 1), have revealed that MCSs are ubiquitous and that organelles can work together to respond to stress in surprising ways. For example, MCSs between the ER and various compartments in the endosomal system appear to have unexpected, important roles in membrane trafficking, metabolism and signalling in endosomal compartments[108]. We have only just begun to understand how MCSs modulate membrane remodelling events, and they may play a bigger role in vesicular trafficking than is currently realized. How MCSs function as signalling and metabolic hubs, integrate signals, allow the propagation of information and facilitate cell adaptation to stress is also unclear. Finally, as MCSs play critical roles in intracellular Ca^{2+} homeostasis, they may play equally important roles in the intracellular channelling of other important metals, such as zinc and iron[196,197].

Progress in the study of MCSs has bought new challenges for the field. For example, because most MCSs are below the limit of resolution of light microscopy, visualizing them in live cells and measuring dynamics remain challenging and are likely to require advances in super-resolution microscopy. There is also a pressing need for standardized ways to quantify contacts and to understand how metabolite channelling and cell signalling is affected by membranes in close apposition at contact sites, which will require the

reconstitution of MCSs in vitro. Understanding the structure and function of contacts with more than two organelles is also of interest.

It also seems likely that MCS dysfunction plays important roles in a number of diseases, especially in those that are characterized by defects in metabolism, but the underlying details remain to be determined. To date, most investigations into the roles of MCS dysfunction in disease have focused on ER–mitochondria contacts, which are thought to have important roles in diabetes, cancer and neurodegenerative disorders. A list of MCS-enriched proteins that are thought to directly alter MCS function in disease is given in TABLE 1. Given the ubiquity of MCSs and the growing evidence that they have numerous functions in cells, it is surprising that defects in MCSs other than ER–mitochondria contacts have not been implicated in diseases. This fact is probably because we know much less about most MCSs than we do about ER–mitochondria contacts, owing to the need to develop tools to visualize and manipulate other MCSs. As we learn more about the functions of MCSs in metabolism and signalling, it seems likely we will discover that they play important roles in many metabolic disorders.

Pathogen-driven diseases can also be promoted by MCSs because some pathogens exploit host MCSs. For example, proteins from human herpesvirus and a hepacivirus may travel from the ER to mitochondria via MCSs[198]. Furthermore, a number of plant viruses alter ER–plasma membrane contacts at plasmodesmata to facilitate viral transfer between cells. Specifically, viral proteins called ‘movement proteins’, which make possible viral transfer between cells, interact with SYT1 at ER–plasma membrane contacts in plasmodesmata and promote the formation of viral replication sites adjacent to these structures[162,199]. Some pathogens propagate in membranous compartments and generate MCSs with the compartments to acquire host lipids. For example, *Chlamydia trachomatis* is an obligate intracellular bacterial pathogen that resides in a membrane-bound structure called an ‘inclusion’. MCSs form between the inclusion and the ER, and the MCSs have proteins from the host (including the VAPs and ceramide-transporting LTP CERT) as well as the pathogen (the protein IncD)[200,201].

In short, as we learn more about each MCS, in the next few years, we are likely to witness many exciting discoveries regarding how MCSs function in cells to respond to environmental challenges and how they are dysregulated in disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

Multivesicular bodies (MVBs)
Endocytic compartments containing internal luminal vesicles

Store-operated calcium entry
The regulated entry of Ca^{2+} into cells in response to the depletion of Ca^{2+} in the endoplasmic reticulum

ER stress
An accumulation of unfolded proteins in the endoplasmic reticulum (ER) that affects ER function

Ceramides
Lipids used to generate complex sphingolipids, one of the major types of lipid in cellular membranes

Phosphatidylinositol kinases
Kinases that phosphorylate phosphatidylinositol on the inositol moiety

Inositol 1,4,5-trisphosphate receptors (IP3Rs)
Endoplasmic Ca^{2+} channels activated by inositol 1,4,5-trisphosphate, an important signalling molecule formed by the cleavage of phosphatidylinositol 4,5-bisphosphate

ER–mitochondria encounter structure (ERMEs)
An endoplasmic reticulum (ER)–mitochondrial tethering complex found in yeasts

Sphingolipids
A major type of lipids found in cellular membranes

Septins
A group of GTP-binding proteins that can assemble into cytoskeletal-like structures

Interscapular
The region between the shoulder blades

Brown adipose tissue
A type of adipose tissue that serves as a site of thermogenesis

Galactoglycerolipids
A family of glycerolipids that contain one or more sugars linked directly to the glycerol moiety

Selective autophagy
A degradative pathway in which particular organelles or aggregates are degraded in lysosomes and vacuoles in development and in response to nutrient stress

Unfolded protein response
stress response pathways induced by endoplasmic reticulum stress

TDP-43
TAR DNA-binding protein 43 (TDP-43) is a 43-kDa RNA and DNA-binding protein that is pathologically linked to amyotrophic lateral sclerosis and frontotemporal dementia

Presenilin

A membrane protein thought to contribute to the development of Alzheimer disease

Chorea-acanthocytosis

A rare neurological disorder that affects body movement

α-Synuclein

A protein predominantly expressed in neurons that can cluster into insoluble aggregates in Parkinson disease and other neurogenerative disorders

References

1. Valm AM et al. Applying systems-level spectral imaging and analysis to reveal the organelle interactome. Nature 546, 162–167 (2017). [PubMed: 28538724] By simultaneously visualizing six organelles (the ER, Golgi complex, lysosomes, peroxisomes, mitochondria and lipid droplets), this study shows how organelles make numerous contacts that affect membrane dynamics.
2. Shai N et al. Systematic mapping of contact sites reveals tethers and a function for the peroxisome-mitochondria contact. Nat. Commun. 9, 1761 (2018). [PubMed: 29720625] This study uses split fluorophores to systematically investigate organelle contacts in *S. cerevisiae* and shows that most organelles make contacts with more than one organelle.
3. Prinz WA Bridging the gap: membrane contact sites in signaling, metabolism, and organelle dynamics. J. Cell. Biol. 205, 759–769 (2014). [PubMed: 24958771]
4. Helle SC et al. Organization and function of membrane contact sites. Biochim. Biophys Acta 1833, 2526–2541 (2013). [PubMed: 23380708]
5. Cohen S, Valm AM & Lippincott-Schwartz J Interacting organelles. Curr. Opin. Cell. Biol. 53, 84–91 (2018). [PubMed: 30006038]
6. Scorrano L et al. Coming together to define membrane contact sites. Nat. Commun. 10, 1287 (2019). [PubMed: 30894536]
7. Ma W & Mayr C A membraneless organelle associated with the endoplasmic reticulum enables 3′UTR-mediated protein-protein interactions. Cell 175, 1492–1506 (2018). [PubMed: 30449617]
8. Schorr S & van der Laan M Integrative functions of the mitochondrial contact site and cristae organizing system. Semin. Cell Dev. Biol. 76, 191–200 (2018). [PubMed: 28923515]
9. Fernandez-Busnadiego R, Saheki Y & De Camilli P Three-dimensional architecture of extended synaptotagmin-mediated endoplasmic reticulum-plasma membrane contact sites. Proc. Natl Acad. Sci. USA 112, E2004–E2013 (2015). [PubMed: 25787254]
10. West M, Zurek N, Hoenger A & Voeltz GKA 3D analysis of yeast ER structure reveals how ER domains are organized by membrane curvature. J. Cell Biol. 193, 333–346 (2011). [PubMed: 21502358]
11. Lewis SC, Uchiyama LF & Nunnari J ER-mitochondria contacts couple mtDNA synthesis with mitochondrial division in human cells. Science 353, aaf5549 (2016). [PubMed: 27418514]
12. Dickson EJ Endoplasmic reticulum-plasma membrane contacts regulate cellular excitability. Adv. Exp. Med. Biol. 997, 95–109 (2017). [PubMed: 28815524]
13. Friedman JR et al. ER tubules mark sites of mitochondrial division. Science 334, 358–362 (2011). [PubMed: 21885730] This study shows that ER-mitochondria MCS's mark sites where mitochondrial division will subsequently occur in mammalian cells and in *S. cerevisiae*.
14. Hoyer MJ et al. A novel class of ER membrane proteins regulates ER-associated endosome fission. Cell 175, 254–265 (2018). [PubMed: 30220460]
15. Besprozvannaya M et al. GRAM domain proteins specialize functionally distinct ER-PM contact sites in human cells. Elife 7, e31019 (2018). [PubMed: 29469807]
16. Gonzalez Montoro A et al. Vps39 interacts with Tom40 to establish one of two functionally distinct vacuole-mitochondria contact sites. Dev. Cell 45, 621–636 (2018). [PubMed: 29870720]

17. Wang S, Tukachinsky H, Romano FB & Rapoport TA Cooperation of the ER-shaping proteins atlastin, lunapark, and reticulons to generate a tubular membrane network. Elife 5, e18605 (2016). [PubMed: 27619977]
18. Glancy B et al. Power grid protection of the muscle mitochondrial reticulum. Cell Rep. 19, 487–496 (2017). [PubMed: 28423313]
19. Picard M et al. Trans-mitochondrial coordination of cristae at regulated membrane junctions. Nat. Commun. 6, 6259 (2015). [PubMed: 25687472]
20. Wong YC, Peng W & Krainc D Lysosomal regulation of inter-mitochondrial contact fate and motility in Charcot-Marie-Tooth type 2. Dev. Cell. 50, 339–354 (2019). [PubMed: 31231042]
21. Wang H et al. Seipin is required for converting nascent to mature lipid droplets. Elife 5, e16582 (2016). [PubMed: 27564575]
22. Xu D et al. Rab18 promotes lipid droplet (LD) growth by tethering the ER to LDs through SNARE and NRZ interactions. J. Cell. Biol. 217, 975–995 (2018). [PubMed: 29367353]
23. Lackner LL, Ping H, Graef M, Murley A & Nunnari J Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. Proc. Natl Acad. Sci. USA 110, E458–E467 (2013). [PubMed: 23341591]
24. Hariri H et al. Mdm1 maintains endoplasmic reticulum homeostasis by spatially regulating lipid droplet biogenesis. J. Cell Biol. 218, 1319–1334 (2019). [PubMed: 30808705]
25. Hsu F et al. Rab5 and Alsin regulate stress-activated cytoprotective signaling on mitochondria. Elife 7, e32282 (2018). [PubMed: 29469808]
26. Joshi AS et al. Lipid droplet and peroxisome biogenesis occur at the same ER subdomains. Nat. Commun. 9, 2940 (2018). [PubMed: 30054481]
27. Oikawa K, Hayashi M, Hayashi Y & Nishimura M Re-evaluation of physical interaction between plant peroxisomes and other organelles using live-cell imaging techniques. J. Integr. Plant Biol. 61, 836–852 (2019). [PubMed: 30916439]
28. Lin CC et al. Apolipoprotein J, a glucose-upregulated molecular chaperone, stabilizes core and NS5A to promote infectious hepatitis C virus virion production. J. Hepatol. 61, 984–993 (2014). [PubMed: 24996046]
29. Eisenberg-Bord M, Shai N, Schuldiner M & Bohnert M A tether is a tether is a tether: tethering at membrane contact sites. Dev. Cell 39, 395–409 (2016). [PubMed: 27875684]
30. Mesmin B et al. A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP. Cell 155, 830–843 (2013). [PubMed: 24209621] This study demonstrates that OSBP can use the difference in P14P levels in the ER and Golgi to drive cholesterol transport to the Golgi.
31. Quon E et al. Endoplasmic reticulum-plasma membrane contact sites integrate sterol and phospholipid regulation. PLOS Biol. 16, e2003864 (2018). [PubMed: 29782498]
32. Murley A et al. Ltc1 is an ER-localized sterol transporter and a component of ER-mitochondria and ER-vacuole contacts. J. Cell Biol. 209, 539–548 (2015). [PubMed: 25987606]
33. Kumar N et al. VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. J. Cell Biol. 217, 3625–3639 (2018). [PubMed: 30093493]
34. Munoz-Braceras S, Tornero-Ecija AR, Vincent O & Escalante R VPS13A, a closely associated mitochondrial protein, is required for efficient lysosomal degradation. Dis. Model. Mech 12, dmm036681 (2019). [PubMed: 30709847]
35. Liu LK, Choudhary V, Toulmay A & Prinz WA An inducible ER-Golgi tether facilitates ceramide transport to alleviate lipotoxicity. J. Cell Biol. 216, 131–147 (2017). [PubMed: 28011845] This study shows that yeast cells can generate MCSs that prevent the toxic accumulation of ceramide in the ER by facilitating non-vesicular ceramide exit from the ER when vesicular transport out of the ER is blocked.
36. Wong LH, Gatta AT & Levine TP Lipid transfer proteins: the lipid commute via shuttles, bridges and tubes. Nat. Rev. Mol. Cell Biol. 20, 85–101 (2019). [PubMed: 30337668]
37. Osman C, Voelker DR & Langer T Making heads or tails of phospholipids in mitochondria. J. Cell Biol. 192, 7–16 (2011). [PubMed: 21220505]
38. Hanada K et al. Molecular machinery for non-vesicular trafficking of ceramide. Nature 426, 803–809 (2003). [PubMed: 14685229]

39. Funato K & Riezman H Vesicular and nonvesicular transport of ceramide from ER to the Golgi apparatus in yeast. J. Cell Biol. 155, 949–959 (2001). [PubMed: 11733544]
40. Jain A & Holthuis JCM Membrane contact sites, ancient and central hubs of cellular lipid logistics. Biochim. Biophys Acta. 1864, 1450–1458 (2017).
41. John Peter AT et al. Vps13-Mcp1 interact at vacuole-mitochondria interfaces and bypass ER-mitochondria contact sites. J. Cell Biol. 216, 3219–3229 (2017). [PubMed: 28864540]
42. Lang AB, John Peter AT, Walter P & Kornmann B ER-mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. J. Cell Biol. 210, 883–890 (2015). [PubMed: 26370498]
43. Elbaz-Alon Y et al. A dynamic interface between vacuoles and mitochondria in yeast. Dev. Cell 30, 95–102 (2014). [PubMed: 25026036]
44. Honscher C et al. Cellular metabolism regulates contact sites between vacuoles and mitochondria. Dev. Cell 30, 86–94 (2014). [PubMed: 25026035]
45. de Saint-Jean M et al. Osh4p exchanges sterols for phosphatidylinositol 4-phosphate between lipid bilayers. J. Cell Biol. 195, 965–978 (2011). [PubMed: 22162133]
46. Kim YJ, Hernandez ML & Balla T Inositol lipid regulation of lipid transfer in specialized membrane domains. Trends Cell Biol. 23, 270–278 (2013). [PubMed: 23489878]
47. Chung J et al. PI4P/phosphatidylserine countertransport at ORP5- and ORP8-mediated ER-plasma membrane contacts. Science 349, 428–432 (2015). [PubMed: 26206935]
48. Moser von Filseck J et al. Phosphatidylserine transport by ORP/Osh proteins is driven by phosphatidylinositol 4-phosphate. Science 349, 432–436 (2015). [PubMed: 26206936]
49. Moser von Filseck J, Vanni S, Mesmin B, Antonny B & Drin G A phosphatidylinositol-4-phosphate powered exchange mechanism to create a lipid gradient between membranes. Nat. Commun. 6, 6671 (2015). [PubMed: 25849868]
50. Ghai R et al. ORP5 and ORP8 bind phosphatidylinositol-4, 5-biphosphate (PtdIns(4,5) P 2) and regulate its level at the plasma membrane. Nat. Commun. 8, 757 (2017). [PubMed: 28970484]
51. Mesmin B et al. Sterol transfer, PI4P consumption, and control of membrane lipid order by endogenous OSBP. EMBO J. 36, 3156–3174 (2017). [PubMed: 28978670]
52. Putney JW Introduction. Adv. Exp. Med. Biol. 993, 3–13 (2017). [PubMed: 28900906]
53. Hirve N, Rajanikanth V, Hogan PG & Gudlur A Coiled-coil formation conveys a STIM1 signal from ER lumen to cytoplasm. Cell. Rep. 22, 72–83 (2018). [PubMed: 29298434]
54. Petersen OH, Courjaret R & Machaca K Ca^{2+} tunnelling through the ER lumen as a mechanism for delivering Ca^{2+} entering via store-operated Ca^{2+} channels to specific target sites. J. Physiol. 595, 2999–3014 (2017). [PubMed: 28181236]
55. Grigoriev I et al. STIM1 is a MT-plus-end-tracking protein involved in remodeling of the ER. Curr. Biol. 18, 177–182 (2008). [PubMed: 18249114]
56. Chang CL, Chen YJ, Quintanilla CG, Hsieh TS & Liou J EB1 binding restricts STIM1 translocation to ER-PM junctions and regulates store-operated Ca^{2+} entry. J. Cell Biol. 217, 2047–2058 (2018). [PubMed: 29563214]
57. Giordano F et al. PI(4,5)P2-dependent and Ca^{2+}-regulated ER-PM interactions mediated by the extended synaptotagmins. Cell 153, 1494–1509 (2013). [PubMed: 23791178]
58. Chang CL et al. Feedback regulation of receptor-induced Ca^{2+} signaling mediated by e-syt1 and nir2 at endoplasmic reticulum-plasma membrane junctions. Cell Rep. 5, 813–825 (2013). [PubMed: 24183667]
59. Kumagai K & Hanada K Structure, functions and regulation of CERT, a lipid-transfer protein for the delivery of ceramide at the ER-Golgi membrane contact sites. FEBS Lett. 593, 2366–2377 (2019). [PubMed: 31254361]
60. Kannan M, Lahiri S, Liu LK, Choudhary V & Prinz WA Phosphatidylserine synthesis at membrane contact sites promotes its transport out of the ER. J. Lipid Res. 58, 553–562 (2017). [PubMed: 28119445] This study demonstrates that phospholipid synthesis at MCSs promotes non-vesicular lipid transport at MCSs.

61. Kim YJ, Guzman-Hernandez ML & Balla T A highly dynamic ER-derived phosphatidylinositol-synthesizing organelle supplies phosphoinositides to cellular membranes. Dev. Cell 21, 813–824 (2011). [PubMed: 22075145]
62. Maeda K et al. Interactome map uncovers phosphatidylserine transport by oxysterol-binding proteins. Nature 501, 257–261 (2013). [PubMed: 23934110]
63. Chang CL et al. Spastin tethers lipid droplets to peroxisomes and directs fatty acid trafficking through ESCRT-III. J. Cell Biol. 218, 2583–2599 (2019). [PubMed: 31227594]
64. Schuldiner M & Bohnert M A different kind of love - lipid droplet contact sites. Biochim. Biophys Acta 1862, 1188–1196 (2017).
65. Kerner J & Hoppel C Fatty acid import into mitochondria. Biochim. Biophys Acta. 1486, 1–17 (2000). [PubMed: 10856709]
66. Michaud M & Jouhet J Lipid trafficking at membrane contact sites during plant development and stress response. Front. Plant Sci. 10, 2 (2019). [PubMed: 30713540]
67. Sheftel AD, Zhang AS, Brown C, Shirihai OS & Ponka P Direct interorganellar transfer of iron from endosome to mitochondrion. Blood 110, 125–132 (2007). [PubMed: 17376890]
68. Das A, Nag S, Mason AB & Barroso MM Endosome-mitochondria interactions are modulated by iron release from transferrin. J. Cell Biol. 214, 831–845 (2016). [PubMed: 27646275]
69. Rizzuto R, Brini M, Murgia M & Pozzan T Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science 262, 744–747 (1993). [PubMed: 8235595] This study demonstrates that high Ca2+ levels at ER–mitochondria MCSs are transiently generated next to the ER-localized Ca2+ channel and are sensed by contacting mitochondria.
70. Csordas G et al. Imaging interorganelle contacts and local calcium dynamics at the ER-mitochondrial interface. Mol. Cell 39, 121–132 (2010). [PubMed: 20603080] This study provides direct evidence that high-Ca2+ domains exist between the ER and mitochondria at MCSs and shows that these organelles must be tethered for Ca2+ signalling.
71. Szabadkai G et al. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. J. Cell Biol. 175, 901–911 (2006). [PubMed: 17178908]
72. Csordas G, Weaver D & Hajnoczky G Endoplasmic reticulum-mitochondrial contactology: structure and signaling functions. Trends Cell Biol. 28, 523–540 (2018). [PubMed: 29588129]
73. Zhang X et al. Redox signals at the ER-mitochondria interface control melanoma progression. EMBO J. 38, e100871 (2019). [PubMed: 31304984]
74. Muallem S, Chung WY, Jha A & Ahuja M Lipids at membrane contact sites: cell signaling and ion transport. EMBO Rep. 18, 1893–1904 (2017). [PubMed: 29030479]
75. Herrera-Cruz MS & Simmen T Over six decades of discovery and characterization of the architecture at mitochondria-associated membranes (MAMs). Adv. Exp. Med. Biol. 997, 13–31 (2017). [PubMed: 28815519]
76. Hirabayashi Y et al. ER-mitochondria tethering by PDZD8 regulates Ca^{2+} dynamics in mammalian neurons. Science 358, 623–630 (2017). [PubMed: 29097544]
77. Kornmann B et al. An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325, 477–481 (2009). [PubMed: 19556461] This study identifies an ER–mitochondria tethering complex in yeast that is found exclusively at these contact sites and plays a role in lipid exchange between the ER and mitochondria.
78. Booth DM, Enyedi B, Geiszt M, Varnai P & Hajnoczky G Redox nanodomains are induced by and control calcium signaling at the ER-mitochondrial interface. Mol. Cell 63, 240–248 (2016). [PubMed: 27397688] This study demonstrates how mitochondria-generated ROS participate in ER–mitochondria communication at MCSs and regulate Ca^{2+} signalling and oxidative phosphorylation.
79. Lock JT, Sinkins WG & Schilling WP Protein S-glutathionylation enhances Ca2+-induced Ca^{2+} release via the IP3 receptor in cultured aortic endothelial cells. J. Physiol. 590, 3431–3447 (2012). [PubMed: 22855054]
80. Yoboue ED, Sitia R & Simmen T Redox crosstalk at endoplasmic reticulum (ER) membrane contact sites (MCS) uses toxic waste to deliver messages. Cell Death. Dis. 9, 331 (2018). [PubMed: 29491367]

81. Gordaliza-Alaguero I, Canto C & Zorzano A Metabolic implications of organelle-mitochondria communication. EMBO Rep. 20, e47928 (2019). [PubMed: 31418169]
82. Behnia R & Munro S Organelle identity and the signposts for membrane traffic. Nature 438, 597–604 (2005). [PubMed: 16319879]
83. Dickson EJ & Hille B Understanding phosphoinositides: rare, dynamic, and essential membrane phospholipids. Biochem. J. 476, 1–23 (2019). [PubMed: 30617162]
84. Berridge MJ & Irvine RF Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature 312, 315–321 (1984). [PubMed: 6095092]
85. Balla T Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol. Rev. 93, 1019–1137 (2013). [PubMed: 23899561]
86. Chang CL & Liou J Phosphatidylinositol 4,5-bisphosphate homeostasis regulated by Nir2 and Nir3 proteins at endoplasmic reticulum-plasma membrane junctions. J. Biol. Chem. 290, 14289–14301 (2015). [PubMed: 25887399]
87. Kim YJ, Guzman-Hernandez ML, Wisniewski E & Balla T Phosphatidylinositol-phosphatidic acid exchange by Nir2 at ER-PM contact sites maintains phosphoinositide signaling competence. Dev. Cell 33, 549–561 (2015). [PubMed: 26028218]
88. Lees JA et al. Lipid transport by TMEM24 at ER-plasma membrane contacts regulates pulsatile insulin secretion. Science 355, eaah6171 (2017). [PubMed: 28209843]
89. Bian X, Saheki Y & De Camilli P Ca^{2+} releases E-Syt1 autoinhibition to couple ER-plasma membrane tethering with lipid transport. EMBO J. 37, 219–234 (2018). [PubMed: 29222176]
90. Saheki Y et al. Control of plasma membrane lipid homeostasis by the extended synaptotagmins. Nat. Cell Biol. 18, 504–515 (2016). [PubMed: 27065097]
91. Sohn M et al. PI(4,5)P2 controls plasma membrane PI4P and PS levels via ORP5/8 recruitment to ER-PM contact sites. J. Cell Biol 217, 1797–1813 (2018). [PubMed: 29472386]
92. Stuible M & Tremblay ML In control at the ER: PTP1B and the down-regulation of RTKs by dephosphorylation and endocytosis. Trends Cell Biol. 20, 672–679 (2010). [PubMed: 20864346]
93. Haj FG et al. Regulation of signaling at regions of cell-cell contact by endoplasmic reticulum-bound protein-tyrosine phosphatase 1B. PLOS One 7, e36633 (2012). [PubMed: 22655028]
94. Stefan CJ et al. Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. Cell 144, 389–401 (2011). [PubMed: 21295699]
95. Venditti R et al. The activity of Sac1 across ER-TGN contact sites requires the four-phosphate-adaptor-protein-1. J. Cell Biol. 218, 783–797 (2019). [PubMed: 30659099]
96. Zewe JP, Wills RC, Sangappa S, Goulden BD & Hammond GR SAC1 degrades its lipid substrate PtdIns4P in the endoplasmic reticulum to maintain a steep chemical gradient with donor membranes. Elife 7, e35588 (2018). [PubMed: 29461204]
97. Labbe K, Murley A & Nunnari J Determinants and functions of mitochondrial behavior. Annu. Rev. Cell Dev. Biol. 30, 357–391 (2014). [PubMed: 25288115]
98. Cho B et al. Constriction of the mitochondrial inner compartment is a priming event for mitochondrial division. Nat. Commun. 8, 15754 (2017). [PubMed: 28598422]
99. Murley A et al. ER-associated mitochondrial division links the distribution of mitochondria and mitochondrial DNA in yeast. Elife 2, e00422 (2013). [PubMed: 23682313]
100. Korobova F, Ramabhadran V & Higgs HN An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science 339, 464–467 (2013). [PubMed: 23349293]
101. Manor U et al. A mitochondria-anchored isoform of the actin-nucleating spire protein regulates mitochondrial division. Elife 4, 10.7554/eLife.08828 (2015).
102. Smirnova E, Shurland DL, Ryazantsev SN & van der Bliek AM A human dynamin-related protein controls the distribution of mitochondria. J. Cell Biol. 143, 351–358 (1998). [PubMed: 9786947]
103. Labrousse AM, Zappaterra MD, Rube DA & van der Bliek AMC elegans dynamin-related protein DRP-1 controls severing of the mitochondrial outer membrane. Mol. Cell 4, 815–826 (1999). [PubMed: 10619028]
104. Osellame LD et al. Cooperative and independent roles of the Drp1 adaptors Mff, MiD49 and MiD51 in mitochondrial fission. J. Cell Sci. 129, 2170–2181 (2016). [PubMed: 27076521]

105. Arasaki K et al. A role for the ancient SNARE syntaxin 17 in regulating mitochondrial division. Dev. Cell 32, 304–317 (2015). [PubMed: 25619926]
106. Lee JE, Westrate LM, Wu H, Page C & Voeltz GK Multiple dynamin family members collaborate to drive mitochondrial division. Nature 540, 139–143 (2016). [PubMed: 27798601]
107. Guo Y et al. Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. Cell 175, 1430–1442 (2018). [PubMed: 30454650]
108. Rowland AA, Chitwood PJ, Phillips MJ & Voeltz GK ER contact sites define the position and timing of endosome fission. Cell 159, 1027–1041 (2014). [PubMed: 25416943]
109. Shcheprova Z, Baldi S, Frei SB, Gonnet G & Barral Y A mechanism for asymmetric segregation of age during yeast budding. Nature 454, 728–734 (2008). [PubMed: 18660802] This study demonstrates that specialized ER–plasma membrane MCSs form a septin-dependent ER diffusion barrier in *S. cerevisiae* between mother and bud cells, which results in the asymmetric inheritance of cellular components.
110. Clay L et al. A sphingolipid-dependent diffusion barrier confines ER stress to the yeast mother cell. Elife 3, e01883 (2014). [PubMed: 24843009]
111. Luedeke C et al. Septin-dependent compartmentalization of the endoplasmic reticulum during yeast polarized growth. J. Cell Biol. 169, 897–908 (2005). [PubMed: 15967812]
112. Chao JT et al. Polarization of the endoplasmic reticulum by ER-septin tethering. Cell 158, 620–632 (2014). [PubMed: 25083872]
113. Sugiyama S & Tanaka M Distinct segregation patterns of yeast cell-peripheral proteins uncovered by a method for protein segregatome analysis. Proc. Natl Acad. Sci. USA 116, 8909–8918 (2019). [PubMed: 30975753]
114. Perez-Sancho J et al. Stitching organelles: organization and function of specialized membrane contact sites in plants. Trends Cell Biol. 26, 705–717 (2016). [PubMed: 27318776]
115. Tilsner J, Nicolas W, Rosado A & Bayer EM Staying tight: plasmodesmal membrane contact sites and the control of cell-to-cell connectivity in plants. Annu. Rev. Plant Biol. 67, 337–364 (2016). [PubMed: 26905652]
116. Nicolas WJ et al. Architecture and permeability of post-cytokinesis plasmodesmata lacking cytoplasmic sleeves. Nat. Plants 3, 17082 (2017). [PubMed: 28604682]
117. Kvam E & Goldfarb DS Nucleus-vacuole junctions in yeast: anatomy of a membrane contact site. Biochem. Soc. Trans 34, 340–342 (2006). [PubMed: 16709156]
118. Dawaliby R & Mayer A Microautophagy of the nucleus coincides with a vacuolar diffusion barrier at nuclear-vacuolar junctions. Mol. Biol. Cell 21, 4173–4183 (2010). [PubMed: 20943953]
119. Graef M, Friedman JR, Graham C, Babu M & Nunnari J ER exit sites are physical and functional core autophagosome biogenesis components. Mol. Biol. Cell 24, 2918–2931 (2013). [PubMed: 23904270]
120. Suzuki K, Akioka M, Kondo-Kakuta C, Yamamoto H & Ohsumi Y Fine mapping of autophagy-related proteins during autophagosome formation in Saccharomyces cerevisiae. J. Cell Sci. 126, 2534–2544 (2013). [PubMed: 23549786]
121. Ktistakis NT ER platforms mediating autophagosome generation. Biochim. Biophys Acta 10.1016/j.bbalip.2019.03.005 (2019).
122. Okumura K et al. Norepinephrine-induced 1,2-diacylglycerol accumulation and change in its fatty acid composition in the isolated perfused rat heart. Mol. Cell Biochem. 93, 173–178 (1990). [PubMed: 2345542]
123. Nascimbeni AC et al. ER-plasma membrane contact sites contribute to autophagosome biogenesis by regulation of local PI3P synthesis. EMBO J. 36, 2018–2033 (2017). [PubMed: 28550152]
124. Zhao YG et al. The ER contact proteins vapa/b interact with multiple autophagy proteins to modulate autophagosome biogenesis. Curr. Biol. 28, 1234–1245 (2018). [PubMed: 29628370] This study identifies proteins that link the ER and autophagosomal regulators at contact sites within growing autophagosomes.
125. Zachari M & Ganley IG The mammalian ULK1 complex and autophagy initiation. Essays Biochem. 61, 585–596 (2017). [PubMed: 29233870]

126. Zhao YG et al. The ER-localized transmembrane protein EPG-3/VMP1 regulates SERCA activity to control ER-isolation membrane contacts for autophagosome formation. Mol. Cell 67, 974–989 (2017). [PubMed: 28890335]

127. Tabara LC & Escalante R VMP1 Establishes er-microdomains that regulate membrane contact sites and autophagy. PLOS One 11, e0166499 (2016). [PubMed: 27861594]

128. Nishimura T et al. Autophagosome formation is initiated at phosphatidylinositol synthase-enriched ER subdomains. EMBO J. 36, 1719–1735 (2017). [PubMed: 28495679]

129. Valverde DP et al. ATG2 transports lipids to promote autophagosome biogenesis. J. Cell Biol. 218, 1787–1798 (2019). [PubMed: 30952800]

130. Hayashi-Nishino M et al. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat. Cell Biol. 11, 1433–1437 (2009). [PubMed: 19898463]

131. Yla-Anttila P, Vihinen H, Jokitalo E & Eskelinen EL 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy 5, 1180–1185 (2009). [PubMed: 19855179]

132. Biazik J, Yla-Anttila P, Vihinen H, Jokitalo E & Eskelinen EL Ultrastructural relationship of the phagophore with surrounding organelles. Autophagy 11, 439–451 (2015). [PubMed: 25714487]

133. Elkin SR, Lakoduk AM & Schmid SL Endocytic pathways and endosomal trafficking: a primer. Wien Med Wochenschr 166, 196–204 (2016). [PubMed: 26861668]

134. Eden ER, White IJ, Tsapara A & Futter CE. Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. Nat. Cell Biol. 12, 267–272 (2010). [PubMed: 20118922] This study shows that, at MCSs, PTP1B in the ER acts in trans on epidermal growth factor in endosomes.

135. Stuible M et al. PTP1B targets the endosomal sorting machinery: dephosphorylation of regulatory sites on the endosomal sorting complex required for transport component STAM2. J. Biol. Chem. 285, 23899–23907 (2010). [PubMed: 20504764]

136. Dong R et al. Endosome-ER contacts control actin nucleation and retromer function through VAP-dependent regulation of PI4P. Cell 166, 408–423 (2016). [PubMed: 27419871]

137. Allison R et al. Defects in er-endosome contacts impact lysosome function in hereditary spastic paraplegia. J. Cell Biol. 216, 1337–1355 (2017). [PubMed: 28389476]

138. Salogiannis J, Egan MJ & Reck-Peterson SL Peroxisomes move by hitchhiking on early endosomes using the novel linker protein PxdA. J. Cell Biol. 212, 289–296 (2016). [PubMed: 26811422] This study shows that peroxisomes can be transported in cells by being linked at MCSs to early endosomes, which are themselves moved by microtubule-dependent motors.

139. Friedman JR, Webster BM, Mastronarde DN, Verhey KJ & Voeltz GK ER sliding dynamics and ER-mitochondrial contacts occur on acetylated microtubules. J. Cell Biol. 190, 363–375 (2010). [PubMed: 20696706]

140. Jongsma ML et al. An ER-associated pathway defines endosomal architecture for controlled cargo transport. Cell 166, 152–166 (2016). [PubMed: 27368102]

141. Rocha N et al. Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 glued and late endosome positioning. J. Cell Biol. 185, 1209–1225 (2009). [PubMed: 19564404] This study shows that late endosomes can be attached to the ER via MCSs or to motor proteins by a cholesterol-regulated switch.

142. Raiborg C et al. Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth. Nature 520, 234–238 (2015). [PubMed: 25855459] This study shows that ER–late endosome MCSs regulate loading of the microtubule-dependent motor kinesin 1 onto the late endosomes, controlling their movement to the plasma membrane and, as a result, neurite outgrowth.

143. Knoblach B et al. An ER-peroxisome tether exerts peroxisome population control in yeast. EMBO J. 32, 2439–2453 (2013). [PubMed: 23900285]

144. Knoblach B & Rachubinski RA Transport and retention mechanisms govern lipid droplet inheritance in Saccharomyces cerevisiae. Traffic 16, 298–309 (2015). [PubMed: 25524182]

145. Swayne TC et al. Role for cER and Mmr1p in anchorage of mitochondria at sites of polarized surface growth in budding yeast. Curr. Biol. 21, 1994–1999 (2011). [PubMed: 22119524]

146. Pernice WM, Vevea JD & Pon LA A role for Mfb1p in region-specific anchorage of high-functioning mitochondria and lifespan in Saccharomyces cerevisiae. Nat. Commun. 7, 10595 (2016). [PubMed: 26839174]

147. Eisenberg-Bord M et al. Identification of seipin-linked factors that act as determinants of a lipid droplet subpopulation. J. Cell Biol 217, 269–282 (2018). [PubMed: 29187527]

148. Teixeira V et al. Regulation of lipid droplets by metabolically controlled Ldo isoforms. J. Cell Biol. 217, 127–138 (2018). [PubMed: 29187528]

149. Benador IY et al. Mitochondria bound to lipid droplets have unique bioenergetics, composition, and dynamics that support lipid droplet expansion. Cell Metab. 27, 869–885 (2018). [PubMed: 29617645] This study demonstrates that, in brown adipose tissue, mitochondria that from contacts with lipid droplets are metabolically different from those that do not.

150. Bravo R et al. Increased ER-mitochondrial coupling promotes mitochondrial respiration and bioenergetics during early phases of ER stress. J. Cell Sci. 124, 2143–2152 (2011). [PubMed: 21628424]

151. Gutierrez T & Simmen T Endoplasmic reticulum chaperones tweak the mitochondrial calcium rheostat to control metabolism and cell death. Cell Calcium. 70, 64–75 (2018). [PubMed: 28619231]

152. Michaud M, Prinz WA & Jouhet J Glycerolipid synthesis and lipid trafficking in plant mitochondria. FEBS J. 284, 376–390 (2017). [PubMed: 27406373]

153. Michaud M et al. AtMic60 is involved in plant mitochondria lipid trafficking and is part of a large complex. Curr. Biol. 26, 627–639 (2016). [PubMed: 26898467]

154. Listenberger LL et al. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. Proc. Natl Acad. Sci. USA 100, 3077–3082 (2003). [PubMed: 12629214]

155. Garbarino J et al. Sterol and diacylglycerol acyltransferase deficiency triggers fatty acid-mediated cell death. J. Biol. Chem. 284, 30994–31005 (2009). [PubMed: 19690167]

156. Olzmann JA & Carvalho P Dynamics and functions of lipid droplets. Nat. Rev. Mol. Cell Biol. 20, 137–155 (2018).

157. Hariri H et al. Lipid droplet biogenesis is spatially coordinated at ER-vacuole contacts under nutritional stress. EMBO Rep. 19, 57–72 (2018). [PubMed: 29146766]

158. Henne WM et al. Mdm1/Snx13 is a novel ER-endolysosomal interorganelle tethering protein. J. Cell Biol. 210, 541–551 (2015). [PubMed: 26283797]

159. Nguyen TB et al. DGAT1-dependent lipid droplet biogenesis protects mitochondrial function during starvation-induced autophagy. Dev. Cell 42, 9–21 (2017). [PubMed: 28697336]

160. Roelants FM et al. TOR complex 2-regulated protein kinase Ypk1 controls sterol distribution by inhibiting StARkin domain-containing proteins located at plasma membrane-endoplasmic reticulum contact sites. Mol. Biol. Cell 29, 2128–2136 (2018). [PubMed: 29927351]

161. Omnus DJ, Manford AG, Bader JM, Emr SD & Stefan CJ Phosphoinositide kinase signaling controls ER-PM cross-talk. Mol. Biol. Cell 27, 1170–1180 (2016). [PubMed: 26864629]

162. Perez-Sancho J et al. The Arabidopsis synaptotagmin1 is enriched in endoplasmic reticulum-plasma membrane contact sites and confers cellular resistance to mechanical stresses. Plant Physiol. 168, 132–143 (2015). [PubMed: 25792253]

163. Lee E et al. Ionic stress enhances ER-PM connectivity via phosphoinositide-associated SYT1 contact site expansion in Arabidopsis. Proc. Natl Acad. Sci. USA 116, 1420–1429 (2019). [PubMed: 30610176]

164. Schapire AL et al. Arabidopsis synaptotagmin 1 is required for the maintenance of plasma membrane integrity and cell viability. Plant Cell 20, 3374–3388 (2008). [PubMed: 19088329]

165. Yamazaki T, Kawamura Y, Minami A & Uemura M Calcium-dependent freezing tolerance in Arabidopsis involves membrane resealing via synaptotagmin SYT1. Plant Cell 20, 3389–3404 (2008). [PubMed: 19088330]

166. Bockler S & Westermann B Mitochondrial ER contacts are crucial for mitophagy in yeast. Dev. Cell 28, 450–458 (2014). [PubMed: 24530295]

167. Kawano S et al. Structure-function insights into direct lipid transfer between membranes by Mmm1-Mdm12 of ERMES. J. Cell Biol. 217, 959–974 (2018). [PubMed: 29279306]

168. Wu W et al. FUNDC1 regulates mitochondrial dynamics at the ER-mitochondrial contact site under hypoxic conditions. EMBO J. 35, 1368–1384 (2016). [PubMed: 27145933]

169. Missiroli S et al. PML at mitochondria-associated membranes is critical for the repression of autophagy and cancer development. Cell Rep. 16, 2415–2427 (2016). [PubMed: 27545895]

170. Liu X, Wen X & Klionsky DJ ER-mitochondria contacts are required for pexophagy in Saccharomyces cerevisiae. Contact (Thousand Oaks) 2, 10.1177/2515256418821584 (2018).

171. Mattiazzi Usaj M et al. Genome-wide localization study of yeast Pex11 identifies peroxisome-mitochondria interactions through the ERMES complex. J. Mol. Biol. 427, 2072–2087 (2015). [PubMed: 25769804]

172. Roberts P et al. Piecemeal microautophagy of nucleus in *Saccharomyces cerevisiae*. Mol. Biol. Cell 14, 129–141 (2003). [PubMed: 12529432]

173. Datta S, Liu Y, Hariri H, Bowerman J & Henne WM Cerebellar ataxia disease-associated Snx14 promotes lipid droplet growth at ER-droplet contacts. J. Cell Biol. 218, 1335–1351 (2019). [PubMed: 30765438]

174. Murley A et al. Sterol transporters at membrane contact sites regulate TORC1 and TORC2 signaling. J. Cell Biol. 216, 2679–2689 (2017). [PubMed: 28774891]

175. Liu ZW et al. Protein kinase RNA-like endoplasmic reticulum kinase (PERK) signaling pathway plays a major role in reactive oxygen species (ROS)-mediated endoplasmic reticulum stress-induced apoptosis in diabetic cardiomyopathy. Cardiovasc. Diabetol. 12, 158 (2013). [PubMed: 24180212]

176. Verfaillie T et al. PERK is required at the ER-mitochondrial contact sites to convey apoptosis after ROS-based ER stress. Cell Death Differ. 19, 1880–1891 (2012). [PubMed: 22705852]

177. Raturi A et al. TMX1 determines cancer cell metabolism as a thiol-based modulator of ER-mitochondria Ca^{2+} flux. J. Cell Biol. 214, 433–444 (2016). [PubMed: 27502484]

178. Gilady SY et al. Ero1alpha requires oxidizing and normoxic conditions to localize to the mitochondria-associated membrane (MAM). Cell Stress Chaperones 15, 619–629 (2010). [PubMed: 20186508]

179. Marino M et al. SEPN1, an endoplasmic reticulum-localized selenoprotein linked to skeletal muscle pathology, counteracts hyperoxidation by means of redox-regulating SERCA2 pump activity. Hum. Mol. Genet. 24, 1843–1855 (2015). [PubMed: 25452428]

180. Debattisti V, Gerencser AA, Saotome M, Das S & Hajnoczky G ROS control mitochondrial motility through p38 and the motor adaptor Miro/Trak. Cell Rep. 21, 1667–1680 (2017). [PubMed: 29117569]

181. Arruda AP et al. Chronic enrichment of hepatic endoplasmic reticulum-mitochondria contact leads to mitochondrial dysfunction in obesity. Nat. Med. 20, 1427–1435 (2014). [PubMed: 25419710]

182. Eisenberg-Bord M et al. The endoplasmic reticulum-mitochondria encounter structure complex coordinates coenzyme Q biosynthesis. Contact (Thousand Oaks) 2, 10.1177/2515256418825409 (2019).

183. Mourier A et al. Mitofusin 2 is required to maintain mitochondrial coenzyme Q levels. J. Cell Biol. 208, 429–442 (2015). [PubMed: 25688136]

184. Subramanian K et al. Coenzyme Q biosynthetic proteins assemble in a substrate-dependent manner into domains at ER-mitochondria contacts. J. Cell Biol. 218, 1353–1369 (2019). [PubMed: 30674579]

185. Simmen T & Herrera-Cruz MS Plastic mitochondria-endoplasmic reticulum (ER) contacts use chaperones and tethers to mould their structure and signaling. Curr. Opin. Cell Biol. 53, 61–69 (2018). [PubMed: 29870872]

186. Orrenius S, Zhivotovsky B & Nicotera P Regulation of cell death: the calcium-apoptosis link. Nat. Rev. Mol. Cell Biol. 4, 552–565 (2003). [PubMed: 12838338]

187. Lynes EM et al. Palmitoylation is the switch that assigns calnexin to quality control or ER Ca^{2+} signaling. J. Cell Sci. 126, 3893–3903 (2013). [PubMed: 23843619]

188. Eckenrode EF, Yang J, Velmurugan GV, Foskett JK & White C Apoptosis protection by Mcl-1 and Bcl-2 modulation of inositol 1,4,5-trisphosphate receptor-dependent Ca^{2+} signaling. J. Biol. Chem. 285, 13678–13684 (2010). [PubMed: 20189983]

189. Xu L et al. Bcl-2 overexpression reduces cisplatin cytotoxicity by decreasing ER-mitochondrial Ca^{2+} signaling in SKOV3 cells. Oncol. Rep. 39, 985–992 (2018). [PubMed: 29286126]

190. Mebratu YA et al. Bik reduces hyperplastic cells by increasing Bak and activating DAPk1 to juxtapose ER and mitochondria. Nat. Commun. 8, 803 (2017). [PubMed: 28986568]

191. Giorgi C et al. p53 at the endoplasmic reticulum regulates apoptosis in a Ca^{2+}-dependent manner. Proc. Natl Acad. Sci. USA 112, 1779–1784 (2015). [PubMed: 25624484]

192. Giorgi C et al. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science 330, 1247–1251 (2010). [PubMed: 21030605]

193. Doghman-Bouguerra M et al. FATE1 antagonizes calcium- and drug-induced apoptosis by uncoupling ER and mitochondria. EMBO Rep. 17, 1264–1280 (2016). [PubMed: 27402544]

194. Chami M et al. Role of SERCA1 truncated isoform in the proapoptotic calcium transfer from ER to mitochondria during ER stress. Mol. Cell 32, 641–651 (2008). [PubMed: 19061639]

195. van Vliet AR et al. The ER stress sensor PERK coordinates ER-plasma membrane contact site formation through interaction with filamin-A and F-actin remodeling. Mol. Cell 65, 885–899 (2017). [PubMed: 28238652]

196. Philpott CC & Jadhav S The ins and outs of iron: escorting iron through the mammalian cytosol. Free. Radic. Biol. Med. 133, 112–117 (2019). [PubMed: 30321701]

197. Kambe T, Matsunaga M & Takeda TA Understanding the contribution of zinc transporters in the function of the early secretory pathway. Int. J. Mol. Sci. 18, E2179 (2017).

198. Williamson CD & Colberg-Poley AM Access of viral proteins to mitochondria via mitochondria-associated membranes. Rev. Med. Virol. 19, 147–164 (2009). [PubMed: 19367604]

199. Levy A, Zheng JY & Lazarowitz SG Synaptotagmin SYTA forms ER-plasma membrane junctions that are recruited to plasmodesmata for plant virus movement. Curr. Biol. 25, 2018–2025 (2015). [PubMed: 26166780]

200. Derre I, Swiss R & Agaisse H The lipid transfer protein CERT interacts with the Chlamydia inclusion protein IncD and participates to ER-Chlamydia inclusion membrane contact sites. PLOS Pathog. 7, e1002092 (2011). [PubMed: 21731489] This study shows how the intracellular pathogen *Chlamydia trachomatis* generates MCSs between the ER and the membrane of the inclusion that the bacteria propagate in, by hijacking CERT and other host MCS proteins.

201. Elwell CA et al. Chlamydia trachomatis co-opts GBF1 and CERT to acquire host sphingomyelin for distinct roles during intracellular development. PLOS Pathog. 7, e1002198 (2011). [PubMed: 21909260]

202. Stoica R 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). [PubMed: 24893131] This study shows that TDP-43, which is linked to amyotrophic lateral sclerosis, regulates ER–mitochondria MCSs and cellular Ca^{2+} homeostasis.

203. Bernard-Marissal N, Medard JJ, Azzedine H & Chrast R Dysfunction in endoplasmic reticulum-mitochondria crosstalk underlies SIGMAR1 loss of function mediated motor neuron degeneration. Brain 138, 875–890 (2015). [PubMed: 25678561]

204. Nishimura AL 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). [PubMed: 15372378]

205. Area-Gomez E et al. Presenilins are enriched in endoplasmic reticulum membranes associated with mitochondria. Am. J. Pathol. 175, 1810–1816 (2009). [PubMed: 19834068]

206. Area-Gomez E et al. Upregulated function of mitochondria-associated ER membranes in Alzheimer disease. EMBO J. 31, 4106–4123 (2012). [PubMed: 22892566]

207. Zampese E et al. Presenilin 2 modulates endoplasmic reticulum (ER)-mitochondria interactions and Ca^{2+} cross-talk. Proc. Natl Acad. Sci. USA 108, 2777–2782 (2011). [PubMed: 21285369]

208. Lim Y, Cho IT, Schoel LJ, Cho G & Golden JA Hereditary spastic paraplegia-linked REEP1 modulates endoplasmic reticulum/mitochondria contacts. Ann. Neurol. 78, 679–696 (2015). [PubMed: 26201691]

209. Rampoldi L et al. A conserved sorting-associated protein is mutant in chorea-acanthocytosis. Nat. Genet. 28, 119–120 (2001). [PubMed: 11381253]

210. Ueno S et al. The gene encoding a newly discovered protein, chorein, is mutated in chorea-acanthocytosis. Nat. Genet. 28, 121–122 (2001). [PubMed: 11381254]

211. Guardia-Laguarta C et al. -Synuclein is localized to mitochondria-associated ER membranes. J. Neurosci. 34, 249–259 (2014). [PubMed: 24381286]

212. Cali T, Ottolini D, Negro A & Brini M α-Synuclein controls mitochondrial calcium homeostasis by enhancing endoplasmic reticulum-mitochondria interactions. J. Biol. Chem. 287, 17914–17929 (2012). [PubMed: 22453917]

213. Lesage S et al. Loss of VPS13C function in autosomal-recessive Parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. Am. J. Hum. Genet. 98, 500–513 (2016). [PubMed: 26942284]

214. Sano R et al. GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca^{2+}-dependent mitochondrial apoptosis. Mol. Cell 36, 500–511 (2009). [PubMed: 19917257] This study emonstrates that accumulation of the ganglioside monosialotetrahexosylganglioside at ER–mitochondria MCSs induces Ca2+-mediated apoptotic signalling that links ER stress and apoptosis in neurons.

215. Szado T et al. Phosphorylation of inositol 1,4,5-trisphosphate receptors by protein kinase B/Akt inhibits Ca^{2+} release and apoptosis. Proc. Natl Acad. Sci. USA 105, 2427–2432 (2008). [PubMed: 18250332]

216. Marchi S et al. Selective modulation of subtype III IP(3)R by Akt regulates ER Ca(2)(+) release and apoptosis. Cell Death Dis. 3, e304 (2012). [PubMed: 22552281]

217. Scorrano L et al. BAX and BAK regulation of endoplasmic reticulum Ca^{2+}: a control point for apoptosis. Science 300, 135–139 (2003). [PubMed: 12624178]

218. Anelli T et al. Ero1alpha regulates Ca^{2+} fluxes at the endoplasmic reticulum-mitochondria interface (MAM). Antioxid. Redox Signal. 16, 1077–1087 (2012). [PubMed: 21854214]

219. Kakihana T, Nagata K & Sitia R Peroxides and peroxidases in the endoplasmic reticulum: integrating redox homeostasis and oxidative folding. Antioxid. Redox Signal. 16, 763–771 (2012). [PubMed: 22146055]

220. Betz C et al. mTOR complex 2-Akt signaling at mitochondria-associated endoplasmic reticulum membranes (MAM) regulates mitochondrial physiology. Proc. Natl Acad. Sci. USA 110, 12526–12534 (2013). [PubMed: 23852728]

221. Bononi A et al. Identification of PTEN at the ER and MAMs and its regulation of Ca^{2+} signaling and apoptosis in a protein phosphatase-dependent manner. Cell Death Differ. 20, 1631–1643 (2013). [PubMed: 23811847]

222. Avalle L et al. STAT3 localizes to the ER, acting as a gatekeeper for ER-mitochondrion Ca^{2+} fluxes and apoptotic responses. Cell Death Differ. 26, 932–942 (2019). [PubMed: 30042492]

223. Tubbs E et al. Mitochondria-associated endoplasmic reticulum membrane (MAM) integrity is required for insulin signaling and is implicated in hepatic insulin resistance. Diabetes 63, 3279–3294 (2014). [PubMed: 24947355]

224. Thoudam T et al. PDK4 augments ER-mitochondria contact to dampen skeletal muscle insulin signaling during obesity. Diabetes 68, 571–586 (2019). [PubMed: 30523025]

225. Saxena R et al. Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. Nat. Genet. 42, 142–148 (2010). [PubMed: 20081857]

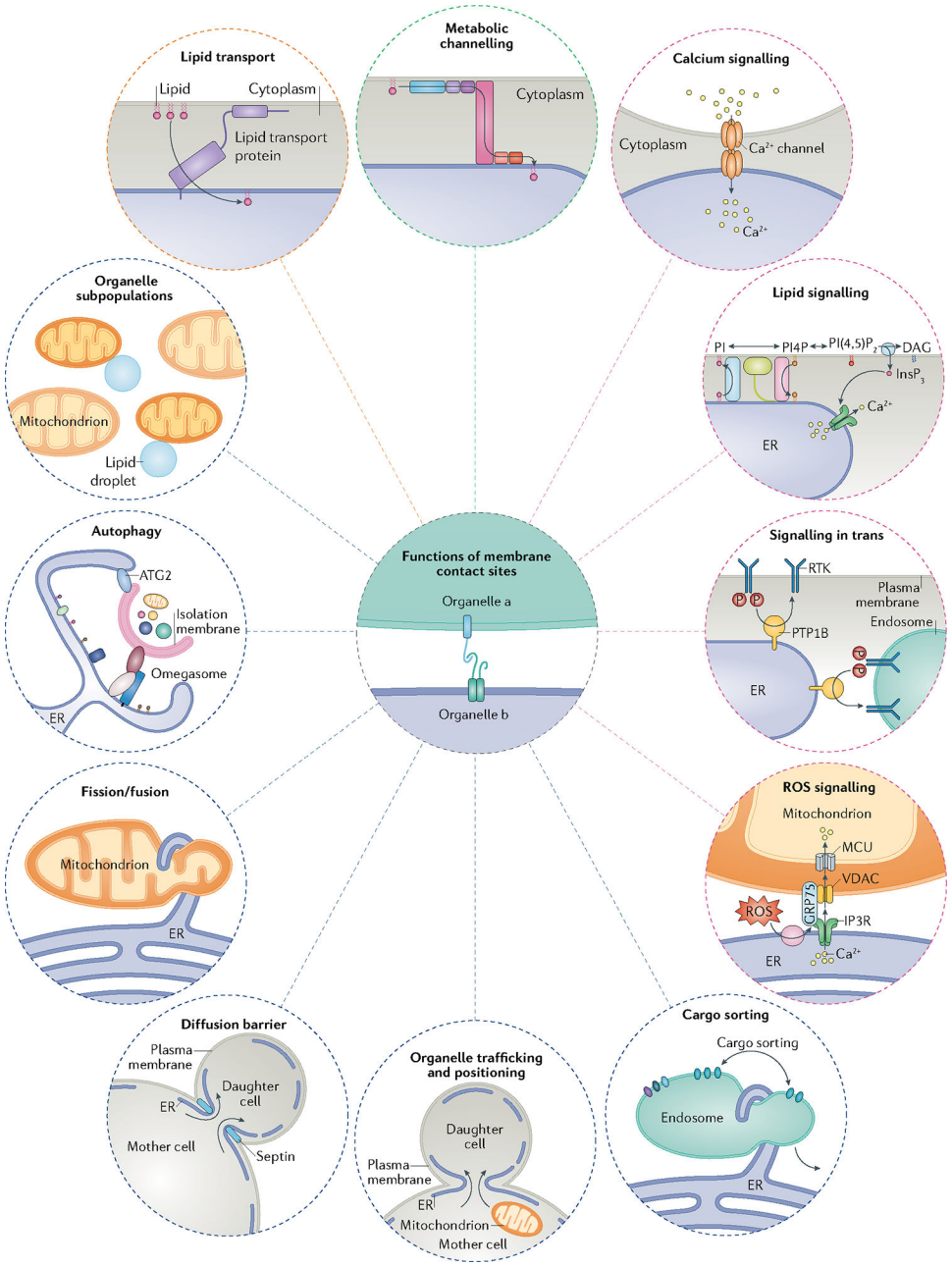

Fig. 1 |. Functions of membrane contact sites.

The different functions of membrane contact sites are each depicted by a representative image, with functions grouped into the four broad categories of signalling (pink circles), regulation of organelle membrane dynamics (blue circles), lipid transport (orange circle) and metabolic channelling (green circle). In some cases, specific examples of the function are shown, but for lipid transport, metabolic channelling and calcium signalling, a depiction of the general principle is given. ATG2, autophagy-related protein 2; DAG, diacylglycerol; ER, endoplasmic reticulum; GRP75, 75-kDa glucose-regulated protein; $InsP_3$, inositol 1,4,5-trisphosphate; IP3R, inositol 1,4,5-trisphosphate receptor; MCU, mitochondrial calcium uniporter protein; P, phosphate group; PI, phosphatidylinositol; PI4P, phosphatidylinositol

4-phosphate; $PI(4,5)P_2$, phosphatidylinositol 4,5-bisphosphate; PTP1B, protein-tyrosine phosphatase 1B; ROS, reactive oxygen species; RTK, receptor tyrosine kinase; VDAC, voltage-dependent anion-selective channel proteins.

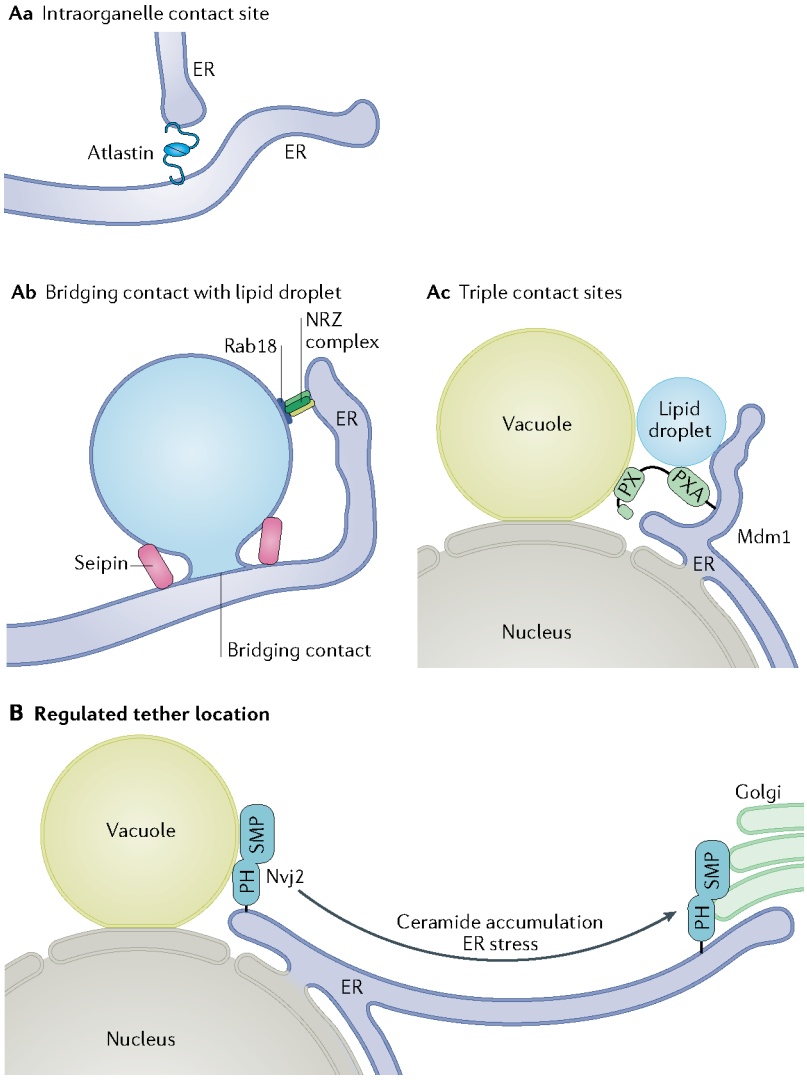

Fig. 2 |. Diversity of membrane contact sites.

A | Types of unusual membrane contact sites (MCSs). **Aa** | Intraorganelle MCSs can form between two regions or compartments of the same organelle; an endoplasmic reticulum (ER)–ER contact, formed by atlastin, dynamin-like GTPases, is depicted. **Ab** | Lipid droplets form two types of contacts: bridging contacts, where the phospholipid monolayer on the surface of the droplet is continuous with the cytoplasmic leaflet of the ER, and standard lipid droplet–ER contacts. Bridging contacts are marked, and may be stabilized by the ER protein seipin. Standard ER–lipid droplet contacts can be formed by the Rab18-dependent tether NRZ (a complex of NAG, RINT1 and ZW10; known as the Dpl1 complex in *Saccharomyces cerevisiae*), which binds to Rab18 on the surface of lipid droplets and to Q-type soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors in the ER. **Ac** | Three-way contacts can form between organelles; a contact between a lipid droplet, the ER and a vacuole in yeast is shown here. This contact is mediated by Mdm1, which contains a phosphoinositide-binding domain (PX) that binds the vacuole and a domain associated with PX domains (PXA) that binds the lipid droplet. **B** | Some tethering proteins move between MCSs in response to environmental stresses or changes in nutritional status. For

example, in yeast, during ER stress or when ceramides accumulate in the ER, Nvj2 moves from its primary location at the nucleus–vacuole junction to ER–Golgi complex contacts, increasing the number of these contacts. Nvj2 harbours a pleckstrin homology (PH) domain, which binds phosphoinositides, and a synaptotagmin-like mitochondrial lipid-binding (SMP) domain. SMP domains can transfer lipids between membranes at MCSs.

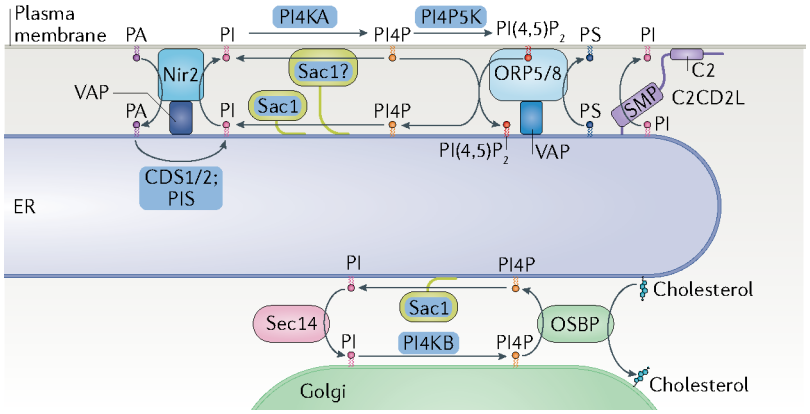

Fig. 3 |. Phosphoinositide metabolism at membrane contact sites.

The role of membrane contact sites (MCSs) in phosphoinositide-based signalling is to generate or deplete phosphoinositides required for signalling. This regulation has been best characterized at endoplasmic reticulum (ER)–plasma membrane and ER–Golgi complex MCSs, shown here, and probably also occurs at MCSs between the ER and endosomes. For example, phosphatidylinositol (PI) is generated in the ER and transported to the plasma membrane or Golgi complex, where it is converted into phosphatidylinositol 4-phosphate (PI4P) by the enzymes phosphatidylinositol 4-kinase-α (PI4KA) on the plasma membrane and phosphatidylinositol 4-kinase-β (PI4KB) on the Golgi complex in mammals. PI transport is mediated by lipid transport proteins, including Nir2 and Sec14 in yeast. Some of these proteins use countertransport, in which they move PI4P from the plasma membrane or Golgi complex to the ER and move a second lipid, such as phosphatidylserine (PS) or cholesterol, respectively, in the other direction. When PI4P reaches the ER it can be hydrolysed by the phosphatidylinositide phosphatase Sac1, which converts PI4P into PI. Sac1 may also hydrolyse PI4P in the plasma membrane or Golgi complex; this has been termed ‘working in trans’, since Sac1 is in the ER but acting on a substrate in another membrane at an MCS. On the plasma membrane, PI4P can be converted into phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) by the enzyme phosphatidylinositol 4-phosphate 5-kinase (PI4P5K). Oxysterol-binding protein-related protein 5/8 (ORP5/8) may also be able to travel from the plasma membrane to the ER. Enzymes are highlighted in blue. CDS1/2, cytidine diphosphate diacylglycerol synthase 1 and 2; C2CD2L, an ER-resident lipid transport protein (also known as TMEM24); OSBP, oxysterol-binding protein; PA, phosphatidic acid; PIS, phosphatidylinositol synthase; SMP, synaptotagmin-like mitochondrial lipid-binding domain; VAP, vesicle-associated membrane protein-associated protein.

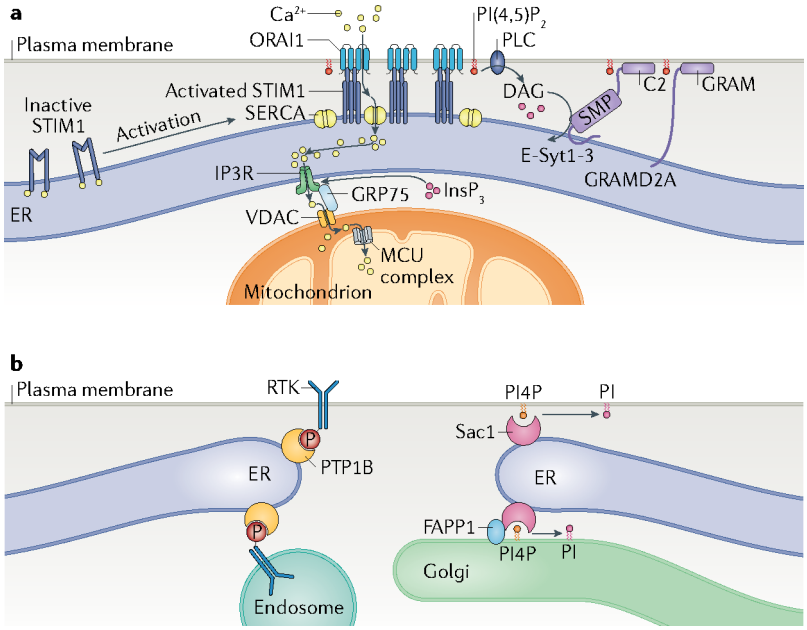

Fig. 4 |. Calcium signalling and signalling in trans at membrane contact sites.

a | Examples of Ca^{2+} signalling between the plasma membrane and endoplasmic reticulum (ER) and between the ER and mitochondria. Stromal interaction molecule 1 (STIM1) senses the Ca^{2+} concentration in the ER lumen. In the resting condition, STIM1 binds Ca^{2+} and is not enriched at ER–plasma membrane membrane contact sites (MCSs) (far left). However, when the Ca^{2+} concentration in the ER decreases, STIM1 accumulates at these contact sites, where it undergoes a conformational change and activates the Ca^{2+} channel calcium release-activated calcium channel protein 1 (ORAI1) in the plasma membrane. This activation causes Ca^{2+} entry at MCSs, which increases the local concentration of Ca^{2+}, activating the sarcoplasmic reticulum/ER Ca^{2+}-ATPase (SERCA) Ca^{2+} channel in the ER. Thus, Ca^{2+} is channelled from outside the cell to the ER lumen at ER–plasma membrane MCSs in response to ER Ca^{2+} levels, a process known as store-operated calcium entry (SOCE). Other proteins at SOCE-mediating MCSs include the three extended synaptotagmins (E-Syt1–3) and GRAM-containing protein 2A (GRAMD2A). These proteins reside in the ER and bind the plasma membrane via C2 or GRAM domains, respectively. The E-Syts have synaptotagmin-like mitochondrial lipid-binding (SMP) domains that can transfer diacylglycerol (DAG) from the plasma membrane to the ER. The DAG is derived from phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), which is hydrolysed by phospholipase C (PLC) when PLC is activated during SOCE. Hydrolysis also yields inositol 1,4,5-trisphosphate ($InsP_3$), which activates $InsP_3$ receptors (IP3Rs; Ca^{2+} channels in the ER). Ca^{2+} is channelled from the ER lumen to mitochondria, via an ER–mitochondria tether formed by voltage-dependent anion-selective channel protein (VDAC) in mitochondria, the ER Ca^{2+} channel IP3R and the chaperone 75-kDa glucose-regulated protein (GRP75). This tether allows Ca^{2+} entry into the space between the outer mitochondrial membrane and the inner mitochondrial membrane. The mitochondrial calcium uniporter (MCU) complex in the mitochondrial inner membrane allows Ca^{2+} entry into the mitochondrial matrix. **b** | Two examples of signalling in trans at MCSs, which occurs when an enzyme in one compartment operates on substrates in a second, are shown. Protein-tyrosine phosphatase

1B (PTP1B), which is in the ER, can dephosphorylate receptor tyrosine kinases (RTKs) in other organelles at ER–plasma membrane and ER–endosome MCSs. The phosphoinositide phosphatase Sac1 is a phosphatase in the ER but it can hydrolyse phosphatidylinositol 4-phosphate (PI4P) to phosphatidylinositol (PI) in the plasma membrane or Golgi complex at MCSs. In the Golgi complex, it is activated by PI4P adaptor protein 1 (FAPP1).

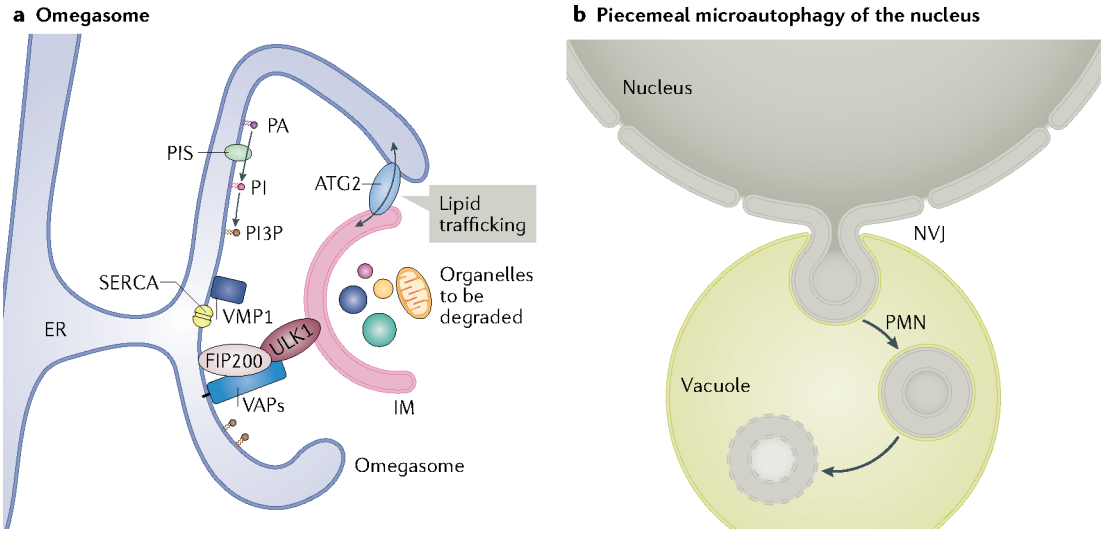

Fig. 5 |. Examples of autophagy at membrane contact sites.

a | Autophagosomes form at specialized domains of the endoplasmic reticulum (ER) called ‘omegasomes’ in mammalian cells and at analogous sites near ER exit sites in yeast. These specialized domains form a membrane contact site (MCS) with a nascent autophagosome, called an isolation membrane (IM). Lipid synthesis in the ER at these MCSs seems to play an important role in autophagosome growth, and lipid-synthesizing enzymes, such as phosphatidylinositol synthase (PIS), are enriched at these sites. This enzyme converts phosphatidic acid (PA) into phosphatidylinositol (PI), which is in turn used to produce phosphatidylinositol 3-phosphate (PI3P), which is required for autophagosome formation. The lipids may be transferred from the ER to the nascent autophagosomes by autophagy-related protein 2 (ATG2). Autophagosome formation is also facilitated by an ER–autophagosome tether formed by vesicle-associated membrane protein-associated proteins (VAPs), Unc-51-like autophagy activating kinase 1 (ULK1) and focal adhesion kinase family kinase-interacting protein of 200 kDa (FIP200). Vacuole membrane protein 1 (VMP1) is also enriched at these MCSs, where it seems to promote the activity of sarcoplasmic reticulum/ER Ca^{2+}-ATPase (SERCA) and may regulate lipid metabolism at these sites. **b** | During starvation in *Saccharomyces cerevisiae* a specialized form of selective mitophagy, called ‘piecemeal microautophagy of the nucleus’ (PMN), degrades parts of the nuclear membrane. Pieces of the nuclear membrane are removed and internalized into the vacuole (the equivalent organelle to the mammalian lysosome), where they are degraded. The process occurs at MCSs between the nucleus and the vacuole termed the ‘nucleus–vacuolar junction’ (NVJ).

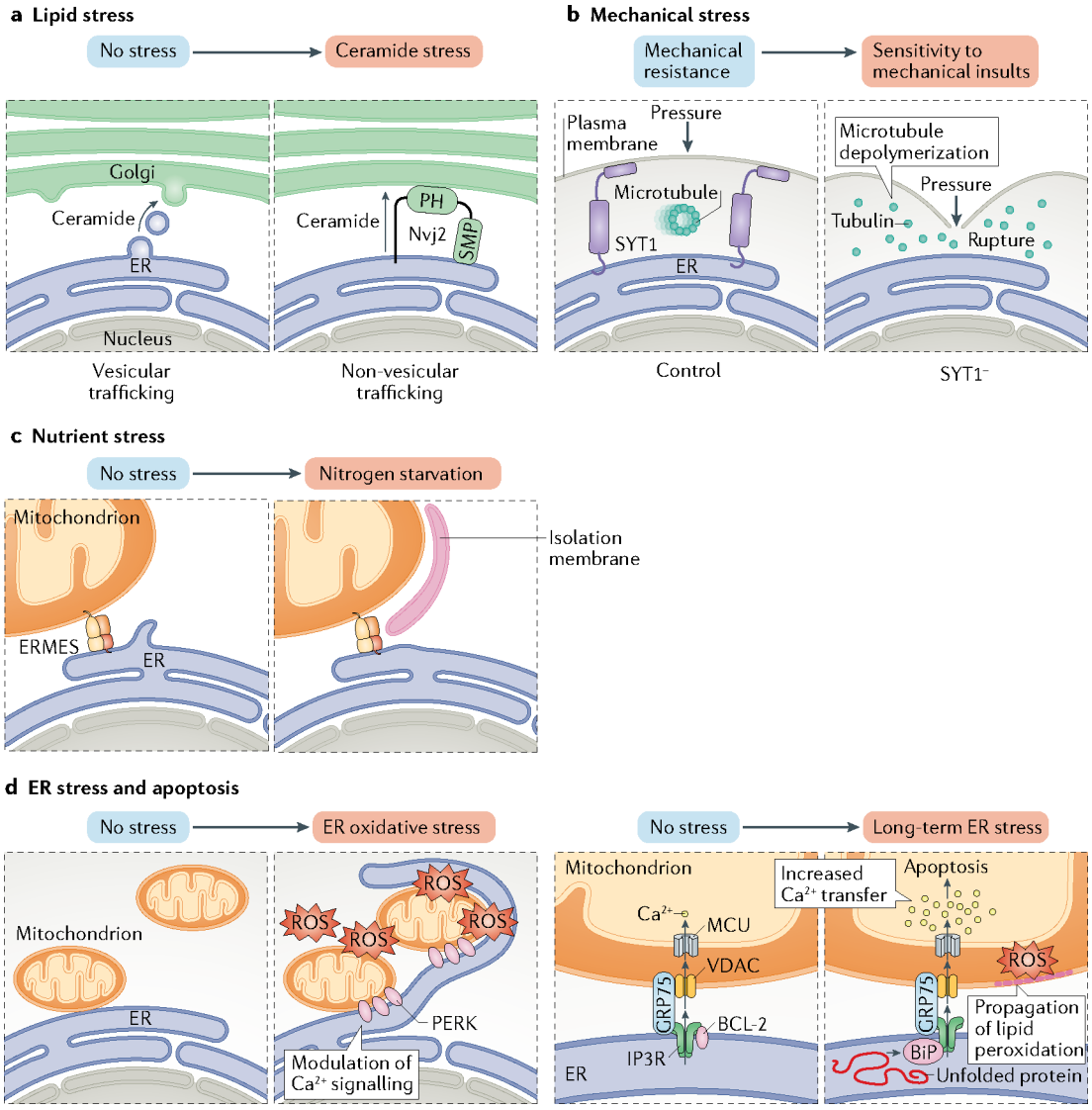

Fig. 6 |. Roles of membrane contact sites in cellular stress responses.

Membrane contact sites (MCSs) play roles in the cellular response to stress, as depicted for the main stresses discussed in this Review. Examples of the roles of MCSs for each type of stress are shown. **a** | Lipid stress. When ceramides accumulate in the endoplasmic reticulum (ER) of *Saccharomyces cerevisiae*, MCSs between the ER and the Golgi complex increase. The formation of these MCSs, which facilitate the non-vesicular movement of ceramide out of the ER to alleviate stress, requires nucleus–vacuole junction protein 2 (Nvj2), an ER-resident protein that binds the Golgi membrane via a pleckstrin homology (PH) domain. Nvj2 also contains a synaptotagmin-like mitochondrial lipid-binding (SMP) domain that may facilitate ceramide transport from the ER to the Golgi complex. **b** | Mechanical stress. In plants, synaptotagmin 1 (SYT1) and microtubules at ER–plasma membrane MCSs help stabilize the plasma membrane (mechanical resistance). During mechanical stress, SYT1 forms aggregates in leaf cells. In the absence of SYT1, microtubules fail to form and the plasma membrane is more susceptible to mechanical stress and rupture. **c** | Nutrient stress.

In *S. cerevisiae*, the ER–mitochondria encounter structure (ERMES) tethers the ER and mitochondria. During nutrient stress such as nitrogen starvation, some mitochondria are degraded by a form of selective autophagy known as mitophagy. An isolation membrane grows from sites near the ERMES, perhaps in the ER, that will eventually engulf the mitochondria and then fuse with vacuoles (the equivalent of lysosomes in yeast), where the mitochondria will be degraded. A similar process occurs in mammalian cells, although they lack the ERMES. **d** | ER stress, oxidative stress and apoptosis. During reactive oxygen species (ROS)-mediated ER stress, MCSs between the ER and mitochondria are increased, the protein composition of contacts changes, and a number of signalling pathways and stress response proteins are induced at these MCSs. One protein that becomes enriched at these sites is protein kinase RNA-like ER kinase (PERK), which reduces mitochondrial motility (left panels). When stress is prolonged, unfolded proteins in the ER and other signals increase Ca^{2+} entry into mitochondria at ER–mitochondria MCSs, promoting apoptosis (right panels). Ca^{2+} entry requires a tether composed of the ER Ca^{2+} channels, inositol 1,4,5-trisphosphate receptors (IP3Rs), voltage-dependent anion-selective channel protein (VDAC) in the mitochondrial outer membrane and 75-kDa glucose-regulated protein (GRP75). The mitochondrial calcium uniporter (MCU) in the mitochondrial inner membrane allows Ca^{2+} entry into the mitochondrial matrix. In unstressed cells, antiapoptotic proteins such as B cell lymphoma 2 (BCL-2) bind and inhibit IP3Rs, reducing Ca^{2+} signalling. Removal of BCL-2 increases Ca^{2+} entry into mitochondria, promoting apoptosis. During ER stress, ER chaperones such as heat shock protein 70 family protein 5 (BiP) bind unfolded proteins and stimulate IP3Rs, increasing Ca^{2+} signalling.

Table 1

Diseases linked to membrane contact site dysfunction

Disease	MCS	MCS-enriched proteins linked to disease
Amyotrophic lateral sclerosis	ER-mitochondria	TDP-43 (REF.[202]), σ_1 receptor[203], VAPB[204]
Alzheimer disease	ER-mitochondria	Presenilin, amyloid precursor protein[205–207]
Hereditary spastic paraplegia	ER-mitochondria	REEP1 (REF.[208])
Chorea-acanthocytosis	ER-mitochondria	VPS13A[33,209,210]
Parkinson disease	ER-mitochondria,ER-endosome	α- synuclein[211,212], VPS13C[33,213]
GM1 gangliosidosis	ER-mitochondria	GM1 (REF.[214])
Cancers	ER-mitochondria	AKT[215,216], BCL-2 (REF.[217]), ERO1α [218,219], FATE1 (REF.[193]), mTORC2 (REF.[220]), p53 (REF.[191]), PML[192], PTEN[221], STAT3 (REF.[222])
Type 2 diabetes/obesity	ER-mitochondria	Cyclophilin D[223]; mitofusin 2, IP3R1 and PACS2 (REF.[181]); PKD4, VDAC1 and GRP75 (REF.[224]); VPS13C[225]

ER, endoplasmic reticulum; ERO1α, ERO1-like protein- α; FATE1, fetal and adult testis- expressed transcript protein; GM1, monosialotetrahexosylganglioside; GRP75, 75-kDa glucose- regulated protein; IP3R1, inositol 1,4,5-trisphosphate receptor type 1; MCS, membrane contact site; mTORC2, mechanistic target of rapamycin complex 2; PACS2, phosphofurin acidic cluster sorting protein 2; PKD4, pyruvate dehydrogenase kinase 4; PML, promyelocytic leukaemia protein; REEP1, receptor expression- enhancing protein 1; STAT3, signal transducer and activator of transcription 3; TDP-43, TAR DNA-binding protein 43; VAPB, vesicle- associated membrane protein- associated protein B; VDAC1, voltage- dependent anion- selective channel protein 1; VPS13A, vacuolar protein sorting- associated protein 13A; VPS13C, vacuolar protein sorting- associated protein 13C.