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Structure and function of ER membrane contact sites with other organelles

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Abstract

The endoplasmic reticulum (ER) is the largest organelle in the cell, and its functions have been studied for decades. The past several years have provided novel insights into the existence of distinct domains between the ER and other organelles, known as membrane contact sites (MCSs). At these contact sites, organelle membranes are closely apposed and tethered, but do not fuse. Here, various protein complexes can work in concert to perform specialized functions such as binding, sensing and transferring molecules, as well as engaging in organelle biogenesis and dynamics. This Review describes the structure and functions of MCSs, primarily focusing on contacts of the ER with mitochondria and endosomes.

The endoplasmic reticulum (ER) is the largest membrane-bound organelle in eukaryotic cells and performs a variety of essential cellular functions, including protein synthesis and processing, lipid synthesis, and calcium (Ca^{2+}) storage and release. It consists of multiple structural domains that are interconnected and contiguous (FIG. 1a). The largest domain of the ER flattens around the cell nucleus to form the double membrane bilayer barrier, termed the nuclear envelope. Branching out of the outer nuclear membrane is the peripheral ER, which consists of two structural domains: flat membrane cisternae (also known as sheets) and tubules. ER sheets are covered with ribosomes for the synthesis, translocation and folding of membrane, luminal and secreted proteins. ER tubules are branched and spread throughout the cytosol. They associate with significantly fewer ribosomes and are therefore considered ‘smooth’ ER[1–6]. The tubular ER network forms abundant membrane contact sites (MCSs) with other organelles and with the plasma membrane (FIG. 1a). This Review describes and compares the structure, factors and functions found at ER MCSs with two very different cytosolic organelles: mitochondria and endosomes (TABLE 1). ER MCSs with the Golgi, peroxisomes and lipid droplets will only be discussed briefly, because these MCSs have additional complexities stemming from the fact that their biogenesis begins on the ER membrane itself, and thus they are not entirely autonomous organelles (see FIG. 1a and TABLE 1).

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Competing interests statement

The authors declare no competing interests.

The structure of MCSs

The combined efforts of electron microscopy and live-cell fluorescence microscopy have revealed the structures of ER MCSs with mitochondria and endosomes. Studies using electron microscopy and tomography have captured features of these contact sites at nanometre resolution (FIG. 1b,c). One defining feature of all MCSs is that ribosomes are excluded from the ER membrane at the interface with the partner organelle membrane[6–11]. The absence of ribosomes from these locations implies that there are specialized ER proteins that maintain the structure of contact sites and thereby prevent the ribosome-bound translocation machinery from diffusing into these regions.

Electron micrographs have also been used to measure the distance between the ER and the apposing organelles. The gap distances are quite similar: 3–15 nm for ER–endosome[7] and 6–15 nm for ER–mitochondria[8,9]. Such short tethering distances allow channelling of smooth ER materials, such as lipids and Ca^{2+} (discussed below). Electron microscopy and tomography also revealed the frequency of membrane contact. Typically, the ER network will interact at multiple small and discrete positions with an individual organelle[9,10,12–14] (FIG. 1b,c). When these contacts are cumulatively analysed, mammalian ER MCSs cover about 2–5% of the surface area of an average mitochondrion[15,16] and 3–5% of the surface of an endosome[7,10,11]. These multiple discrete contact sites could be functionally redundant, or they may each mediate different activities.

MCSs regulate organelle dynamics

Multi-colour live-cell fluorescence microscopy has been used to visualize how organelle trafficking affects the integrity of inter-organelle MCSs (FIG. 2a). Strikingly, once bound, the endosomes and mitochondria appear to be tightly tethered to the ER, but do not fuse with it. Tethering is maintained when the organelles traffic, even over very long distances. As a result of this tethering, the moving organelles will ‘drag’ ER tubules with them[10,17] (FIG. 2a,b). At any given time, dozens of endosomes and mitochondria bound to ER tubules traffic within the cell. Therefore, it can be expected that the overall dynamics and structure of the ER are influenced by these events.

MCS-bound organelles in motion

The tubular ER network is very dynamic and constantly rearranges its structure along the microtubule cytoskeleton. In animal cells, these rearrangements, referred to as ER sliding, occur in both directions and are mediated by the molecular motors dynein and kinesin[18]. Mitochondria and endosomes are also very dynamic organelles that traffic along microtubules. As the ER, endosomes and mitochondria are all dynamic organelles, it is unclear how stable MCSs are maintained as the organelles move. One explanation could be that endosomes and mitochondria traffic using the same motors as the ER — kinesin-1 and dynein[18–22] — so that the joined organelles can travel together. In addition, a few proteins have been identified that may contribute to maintaining ER MCS stability during organelle trafficking. Miro, for example, is an outer mitochondrial membrane component and one of the proteins enriched at ER–mitochondrial contact sites[23]. It is linked to dynein through the cytosolic factor Milton[21,24] and additionally contains multiple Ca^{2+}-binding domains that

could be regulated by Ca^{2+} fluxes at the ER–mitochondria interface[23,25] (see below). Importantly, the yeast orthologue of Miro, Gem1, is also enriched at ER–mitochondrial contact sites[23], and its deletion has been shown to regulate the assembly and disassembly of the yeast ER–mitochondrial encounter structure (ERMES) complex, which maintains ER–mitochondria MCSs (also discussed below)[16].

Regulation of organelle trafficking by MCSs

The formation of the MCSs does not only affect the structure of the ER itself but has also been implicated in regulating the trafficking and localization of both endosomes and mitochondria. Following budding from the plasma membrane, endosomes traffic along microtubules towards the microtubule organizing centre (MTOC) and mature on the way, transitioning from early endosomes to late endosomes, to finally fuse with the lysosome[26]. A large percentage of endosomes maintain contact with the ER as they traffic (FIG. 2b,c). In fact, approximately 53% of all early endosomes, and a staggering 99% of late endosomes, remain in contact with the ER during trafficking. Thus, contacts between the endosomes and the ER are very pervasive and increase as endosome maturation progresses[10]. Notably, it has been shown that the composition of the ER–late endosome MCS is not constant, and various proteins that are implicated in regulating the trafficking of late endosomes can be recruited to these sites. Consequently, the observed abundance of MCSs between late endosomes and ER can be involved in regulating localization of late endosomes within the cell.

Cholesterol levels affect the composition of ER–late endosome MCSs, and this affects late endosome trafficking and localization (FIG. 2d). Late endosomes accumulate near the microtubule plus ends (at the cell periphery) when cholesterol levels are low, or at the minus ends (at the cell centre) when levels of cholesterol are high. As endosomes mature, they travel to the centre of the cell to fuse with the lysosome and degrade cargo; disrupting late endosome trafficking can result in lysosomal storage disorders[27,28]. This mechanism is mediated by a cholesterol-sensing protein, oxysterol-binding-related protein 1L (ORP1L), which is a member of the highly conserved and ubiquitous oxysterol-binding-related protein family. ORP1L contains an oxysterol-binding-related domain (ORD) that has been shown to be capable of binding sterols *in vitro*[29] and localizes to the surface of late endosomes. The current model of how cholesterol influences localization of late endosomes indicates that when the ORP1L ORD domain senses cholesterol in the late endosome membrane, ORP1L acquires a conformation that allows its interaction with a complex that includes RAB7 GTPase (a marker of late endosomes associated with their membranes), RILP (RAB-interacting lysosomal protein), the HOPS (homotypic fusion and vacuole protein sorting) late endosome tethering complex, and the dynactin ($p150^{Glued}$)–dynein motor complex, resulting in minus-end-directed late endosome trafficking[28,30] (FIG. 2d, left panel). Conversely, at low cholesterol levels, the ORP1L FFAT (diphenylalanine in an acidic tract) domain is free to bind to the ER-resident protein VAP (VAMP-associated protein) instead of the RAB7–RILP–p150 complex, which reduces dynein-facilitated trafficking towards microtubule minus ends[28] (FIG. 2d, right panel).

START domain-containing protein 3 (STARD3; also known as MLN64) is another candidate that could sense sterol at ER–late endosome MCSs and provide regulation of late endosome

trafficking by means of altering the protein composition and function of the MCSs. Similar to ORP1L, STARD3 is capable of binding to cholesterol *in vitro* through its conserved START protein domain (see further discussion of START family members in lipid section below)[31] and contains an FFAT motif that is capable of interacting with ER VAP proteins[7]. Overexpression of STARD3 results in accumulation of late endosomes at the perinuclear region and enrichment of actin patches on late endosomes, which may play a part in budding domain formation or in late endosome positioning[32]. Conversely, STARD3 knockdown results in late endosome scattering to the cell periphery and loss of actin patches on late endosomes[32]. The specific cytoskeletal machinery that interacts with STARD3 and regulates late endosome positioning is yet to be discovered, but it seems that STARD3 functions by influencing late endosome association with the actin cytoskeleton.

Yet another protein that functions at ER–late endosome contact sites to regulate late endosome trafficking is protrudin[33,34]. It has been demonstrated that by localizing to MCSs, protrudin promotes plus-end-directed trafficking of late endosomes in neurite outgrowths[34]. Protrudin is an ER transmembrane protein that interacts with VAP. It binds to RAB7 on the late endosome and also contains a FYVE domain, which allows it to bind to phosphatidylinositol-3-phosphate (PtdIns(3)P), a lipid that is enriched on the endosomal membrane[34]. Overexpression of protrudin and RAB7 causes ER to wrap around late endosomes, and these late endosomes accumulate in the cell periphery. Conversely, protrudin depletion results in perinuclear accumulation of late endosomes[34]. Protrudin functions in this regulation of late endosome positioning via recruitment of kinesin-1 to contact sites, followed by transfer of this motor onto FYCO1 (FYVE and coiled-coil domain-containing protein). FYCO1 localizes on the late endosome membrane (via RAB7 and PtdIns(3)P) and functions as an adaptor to link kinesin-1 to late endosomes. Kinesin-1 association with late endosomes promotes plus-end-directed late endosome trafficking[34–36] (FIG. 2e). An interesting possibility is that protrudin–RAB7 and VAP–ORP1L could localize together to ER–late endosome contact sites and drive anterograde trafficking (that is, towards plus ends of microtubules) of late endosomes by coordinating the recruitment of kinesin-1 and the release of dynein, respectively[34].

MCSs can also be involved in regulating mitochondrial trafficking. One notable example has been described for budding yeast, in which ER–mitochondria MCSs are important for proper inheritance of mitochondria by a daughter cell upon division. This mechanism is mediated by mitochondrial Myo2p receptor- related 1 (Mmr1), a putative tether that localizes to MCSs between mitochondria and cortical ER[12]. More specifically, Mmr1 is found at both the ER and mitochondrial surfaces of the MCSs in the bud tip. Deletion of this tether perturbs the proper anchorage of mitochondria in the forming bud, leading to their slippage out of the bud and, consequently, defects in mitochondrial (but not ER) inheritance by the daughter cell[12].

In sum, it appears that MCSs formed between ER and endosomes as well as mitochondria can influence the dynamics, intracellular trafficking and localization of these organelles. This can be mediated by the regulated recruitment of various proteins and protein complexes, which alter the molecular composition and functions of these MCSs.

MSCs in lipid biosynthesis and exchange

Phospholipids, sterols and the precursors for sphingo-lipids and cardiolipin are largely synthesized at the ER and need to be transported to other membrane-bound cellular compartments. Lipids can be transferred from the ER to other organelles by vesicular transport. However, mounting evidence suggests that rapid transport also occurs by non-vesicular transport mechanisms at positions where the ER membrane is closely apposed to other membrane- bound compartments[37–39]. The short (<30 nm) distance between the ER and other membranes at MCSs provides an excellent hub for non-vesicular transfer. Non-vesicular transport of lipids at MCSs requires machinery that can extract a lipid molecule out of the outer leaflet of the originating membrane, shield it in a hydrophobic pocket from the cytosolic aqueous environment, bridge the cytosolic gap between the apposing membranes to allow the transfer of the molecule between membranes and finally insert it into the outer leaflet of the target membrane.

It is likely that multiple factors work together to coordinate lipid transfer. In the 1960s, potential lipid- transfer proteins (LTPs) were first detected *in vitro* as soluble factors that accelerated the transfer of lipids between mitochondria and ER-derived microsomes[38]. However, it took many more years to actually identify the proteins involved. Now, LTPs are much better understood, and in this Review, three major protein families will be discussed: the oxysterol-binding protein-related protein family[40]; the START (steroidogenic acute regulatory protein-related lipid transfer domain) protein family[31]; and the synaptotagmin-like mitochondrial protein (SMP)/tubular lipid-binding protein (TULIP) super-family[41]. Members of these protein families contain a hydrophobic pocket that is possibly capable of binding a lipid monomer, and, importantly, many contain domains that can recruit them to ER MCSs. Below, we discuss the role of tight coupling between particular organelles and the ER in the process of lipid biosynthesis and exchange, thus emphasizing the importance of the formation of MCSs as well as the role of LTPs and their recruitment to MCSs in this fundamental pathway.

ER–mitochondria

Biosynthetic enzymes that coordinate synthesis of major cellular phospholipids are localized to both the ER membrane and the mitochondrial matrix. As an example, phosphatidylserine synthesized at the ER can be altered by mitochondria-localized phospholipid synthase to generate phosphatidylethanolamine, which can be converted to phosphatidylcholine by ER-localized enzymes[42,43] (FIG. 3a). In addition, the precursor for mitochondrial-specific cardiolipin, phosphatidic acid, is synthesized at the ER and must be transferred to the mitochondria for modification[42,43]. Directional transfer of these biosynthetic precursors between the ER and the outer and inner mitochondrial membranes would be facilitated by rapid non-vesicular lipid transfer at the ER–mitochondria interface[39]. Ideally, this machinery would also monitor levels of precursors at each organelle to balance the synthesis and transfer process, thereby regulating the proper composition for each individual organelle.

In yeast, ERMES is the primary candidate for tethering non-vesicular transfer of lipids between ER and mitochondria[23] (FIG. 3a). Fluorescence microscopy has revealed that all ERMES complex components localize to punctate structures at contact sites between the ER

and mitochondria[14,16,23]. Three of the four components of the yeast ERMES complex contain SMP domains and thus are members of the SMP/TULIP family of LTPs[14,41,44]. Structural analysis of other SMP/TULIP protein family members suggests that SMP domains are capable of binding to lipids to shield them from an aqueous environment[41,45]. It remains to be tested whether ERMES directly binds to and transfers lipids itself, or whether it functions as a structural tether to facilitate transfer of lipids by other proteins. In addition, there have been conflicting reports as to whether ERMES complex depletion in yeast alters the lipid transfer rates between the ER and mitochondria[14,46–50]. ERMES may not be the only machinery facilitating lipid transfer from ER to mitochondria. In fact, recent evidence has shown that the MCSs between the yeast vacuole and mitochondria can function as a circuitous route for lipid transfer, which occurs via a vCLAMP (vacuole and mitochondria patch) complex[51,52]. Thus, even in the absence of ERMES, phospholipids could traffic through an alternative route from the ER via the vacuole to the mitochondria. Interestingly, when ERMES is depleted, the contact between mitochondria and vacuole at vCLAMP expands[51,52]. Elimination of both vCLAMP and ERMES contact sites leads to significant defects in phospholipid transfer to mitochondria[51,52]. These findings suggest that lipid trafficking at ER–mitochondria MCSs can be rescued by vacuole–mitochondria MCSs.

ER–endosome

Endosomes store cholesterol and can redistribute this cholesterol from the endosomal membrane to the ER. Different protein pairs at the ER–endosome interface may be capable of sensing and regulating cholesterol at this MCS (FIG. 3b). Cholesterol enters the cell in low-density lipoprotein (LDL) particles. LDL particles bind to cell surface receptors and accumulate in late endosomes and lysosomes[53]. Studies monitoring the localization of cholesterol indicate that approximately 30% of cholesterol in late endosomes and lysosomes is directly transported to the ER[54]. This LDL-bound cholesterol (LDL-C) can then be distributed to other parts of the cell, providing membrane rigidity, fluidity and permeability. Improper export of LDL from late endosomes and lysosomes has been linked to Niemann–Pick Type C disease, which is an autosomal recessive neurodegenerative disorder characterized by accumulation of LDL-derived cholesterol in late endosomes and lysosomes[55]. Niemann–Pick C2 protein (NPC2) is a soluble LTP in the late endosome or lysosome lumen that binds to LDL-C and transfers it to NPC1, a late endosome integral membrane protein, which facilitates LDL-C export[56,57]. It is not fully understood how NPC1 exports cholesterol out of the late endosome or lysosome. ORP5 is an ER transmembrane protein and is a potential acceptor that could receive LDL-C from NPC1 (FIG. 3b). ORP5 is capable of binding sterol in a conserved pocket[29] and co-immunoprecipitates with NPC1. This has led to a model in which the two proteins function in the same complex to transfer sterol from late endosomes to the ER membrane[58]. In support of this model, depletion of NPC1 or ORP5 will prevent sterol transfer from the late endosome to the ER, and this results in sterol accumulation on the late endosome membrane[58].

ORP1L and STARD3 are other sterol-binding proteins that are present on late endosomes, but their role in sterol transfer at ER–endosome MCSs remains unclear. ORP1L and STARD3 can both interact with ER-localized VAP and form ER–endosome MCSs (see discussion above). Interestingly ORP1L is found on the same population of endosomes as

ORP5, but STARD3 is found on a less mature population of late endosomes containing a different sterol-transfer protein, ATP-binding cassette transporter 3 (ABCA3)[59]. The purpose of this separate population of STARD3 and ABCA3 double-positive late endosomes and the exact function of ABCA3 are both unknown. One idea is that ABCA3 facilitates the recycling of newly hydrolysed cholesterol back to the plasma membrane, whereas the hydrolysed cholesterol in more mature late endosome populations is transferred to the ER by NPC1–ORP5 interaction[59].

Overall, multiple complexes that bind to lipids are present at the ER–late endosome interface, and these could have cooperative or opposing roles in the regulation of sterol flux at MCSs. Although overexpression of many of these pairs will increase ER–endosome contact, no one pair is essential for MCS formation and maintenance, because their depletion does not prevent contact site formation. Furthermore, contact is already observed with early endosomes[10,17], and the above-mentioned proteins are all recruited to late endosomes. Thus, it seems that it is not the formation of the ER–late endosome MCSs per se but the regulation of their composition that affects important cellular processes such as lipid biogenesis.

ER–Golgi

The past several years have revealed that MCSs exist between the ER and the Golgi, and that they are able to regulate direct lipid transfer. A variety of LTPs have been localized to ER–Golgi MCSs (TABLE 1). All of these proteins have the ability to bind to lipids to facilitate ER–Golgi lipid transfer[60–64]. These include the ceramide-transfer protein (CERT)[61], the glycosylceramide-transfer protein Golgi-associated four-phosphate adaptor protein 2 (FAPP2; also known as PLEKHA8)[62], the phosphatidylinositol-transfer protein NIR2 (PYK2 N-terminal domain-interacting receptor 2)[63], and the cholesterol- and PtdIns(4)P-transfer protein oxysterol-binding protein (OSBP)[64,65] (FIG. 3c). All four proteins contain a pleckstrin homology (PH) domain that allows them to bind to PtdIns(4)P on the Golgi, and an FFAT motif that is capable of interacting with the ER-localized VAPs[39,66]. CERT and FAPP2 regulate ceramide and glucosylceramide transfer, respectively, at ER–Golgi MCSs[61,62,67]. NIR2 plays a part in maintaining diacylglycerol levels in the Golgi[60,63,68].

Since all of these LTPs are recruited to the Golgi by PtdIns(4)P binding, it is especially important to understand what regulates Golgi PtdIns(4)P levels. A recent elegant study has shown that OSBP regulates both PtdIns(4)P and sterol transfer at ER–Golgi MCSs. OSBP can bridge ER–Golgi MCS, because it contains a PH domain that binds to PtdIns(4)P in the Golgi and an FFAT domain that interacts with ER VAPs[64]. *In vitro* and *in vivo* data demonstrate that the OSBP ORD domain can bind to and transfer both sterol and PtdIns(4)P[64]. In the overall model (FIG. 3c), OSBP promotes anterograde sterol transfer from the ER to the Golgi and retrograde PtdIns(4)P transfer from the Golgi back to the ER[64]. This process is regulated by a feedback mechanism: OSBP dissociates from the Golgi when Golgi PtdIns(4)P levels are low, because it is no longer recruited[64]. This also disrupts direct transfer of sterol from the Golgi to the ER[64].

Collectively, proper lipid synthesis and intracellular lipid distribution seem to be tightly coupled to the existence of MCSs between various membranous organelles within the cell. These tight membrane contacts have been implicated in supporting the non-vesicular

exchange of lipids between the organelles through specialized lipid-binding and lipid-transferring proteins, the LTPs, which can specifically associate with various MCSs. Exactly how these proteins function at MCSs and how their localization is regulated are still poorly understood, and both of these questions open up exciting new avenues for future studies.

MCSs in Ca^{2+} exchange

Ca^{2+} must be transferred across membrane interfaces to propagate signals throughout the cell. The intracellular Ca^{2+} concentration is controlled by regulated opening of Ca^{2+}-permeable channels on the plasma membrane and ER. Multiple mechanisms regulate Ca^{2+} concentration to maintain extremely low Ca^{2+} levels in the cytosol, and low micromolar Ca^{2+} levels in endosomes and mitochondria (FIG. 4a). The ER lumen houses the major Ca^{2+} store in mammalian cells (FIG. 4a), and this store is released through inositol-1,4,5-trisphosphate receptor ($Ins(1,4,5)P_3R$) channels that are found throughout the ER membrane[69]. External stimuli activate receptors on the plasma membrane that subsequently activate phospholipase C (PLC). PLC cleaves phosphatidylinositol-4,5- bisphosphate ($PtdIns(4,5)P_2$) within the plasma membrane, releasing cytosolic $Ins(1,4,5)P_3$ that binds to and stimulates ER $Ins(1,4,5)P_3Rs$[70,71] and thus Ca^{2+} release from ER stores. This cascade enables the cell to convey external signals to intracellular organelles through Ca^{2+} signalling. These ER stores can release Ca^{2+} into the cytosol or onto its neighbouring organelles when higher levels are needed.

MCSs have been found to be involved in regulating this important signalling pathway by means of concentrating and directing Ca^{2+} transfer. Opening of $Ins(1,4,5)P_3R$ Ca^{2+} channels on the ER leads to an increase in local Ca^{2+} concentration, but this spike in Ca^{2+} diffuses, substantially decreasing approximately 100 nm away from the channels[72]. Thus, signalling crosstalk through the release of Ca^{2+} from the ER to other organelles can be expected to be much more efficient at tight interfaces[73,74].

ER–mitochondria

Localized Ca^{2+} spikes released from ER by $Ins(1,4,5)P_3Rs$ stimulate mitochondrial Ca^{2+} uptake. Ca^{2+} passes through voltage-dependent anion channels (VDACs) on the outer mitochondrial membrane and mitochondrial Ca^{2+} uniporters (MCUs) on the inner mitochondrial membrane[75,76]. The low-affinity MCU requires a large, localized Ca^{2+} concentration to facilitate Ca^{2+} transfer to the mitochondrial matrix[75] (FIG. 4b). Uptake of Ca^{2+} into the mitochondrial matrix alters mitochondrial activity in several ways. For example, Ca^{2+} stimulates dehydrogenases in the tricarboxylic acid cycle, resulting in more energy production for the cell[77]. In addition, fluctuations in mitochondrial Ca^{2+} levels regulate cell death programmes[77], and the Ca^{2+} released from the ER stimulates apoptosis by opening the mitochondrial transition pore[78,79].

It has been known for some time that Ca^{2+} released from the ER can be sequestered by mitochondria[80]. Further research using a sensor targeted to the outside of mitochondria showed that upon $Ins(1,4,5)P_3$-induced Ca^{2+} mobilization, mitochondria are exposed to higher Ca^{2+} concentrations than the bulk cytosol[73,81]. Ca^{2+} transfer occurs specifically at ER–mitochondria MCSs, and it is abrogated by increasing the gap distance between the

organelles[8,81]. Evidence suggests that the mitochondrial dynamin-related family member mitofusin 2 (MFN2) localizes to both ER and mitochondria and regulates inter-organelle linkage at Ca^{2+}-transfer sites[82]. However, owing to conflicting results[15,83], further research in this field is currently striving to fully elucidate the role of MFN2 at ER–mitochondria MCSs. For example, there is an open question as to how MFN2 targeting to two different organelles is regulated.

Recent studies have identified additional regulators of Ca^{2+} transfer at ER–mitochondrial MCSs. The 75 kDa glucose-regulated protein (GRP75) is required for coupling the VDAC channel to Ins(1,4,5)P_3R channels[84] (FIG. 4b). However, as overexpression of GRP75 does not result in increased ER–mitochondria contact, it is likely that GRP75 functions at established contacts to regulate mitochondrial Ca^{2+} uptake. Promyelocytic leukaemia tumour suppressor (PML) also regulates Ins(1,4,5) P_3R activity to control the amount of Ca^{2+} at the ER– mitochondria membrane interface and facilitates mitochondrial Ca^{2+} uptake[85]. PML was recently found within mitochondria-associated membrane fractions in a complex with AKT and protein phosphatase 2A (PP2A)[85]. Active, phosphorylated AKT phosphorylates Ins(1,4,5) P_3R and inhibits Ins(1,4,5) P_3R Ca^{2+} release, protecting mitochondria from raising a Ca^{2+}-mediated apoptotic response, whereas PP2A phosphatase activity is capable of deactivating AKT by means of dephosphorylation[86,87]. The amount of phosphorylated AKT was found to be increased in PML-knockout mouse embryonic fibroblasts (MEFs)[85]. Additionally, in these knockout MEFs, mitochondrial and cytosolic Ca^{2+} did not respond as dramatically to apoptotic stimuli, but a normal response to stimuli was recovered by introducing an ER-localized PML[85]. Therefore, PML may recruit PP2A, which inactivates AKT, and regulate Ins(1,4,5)P_3R-mediated Ca^{2+} release in response to apoptotic stimuli. These findings show that ER–mitochondria MCSs are tightly regulated interfaces that easily respond to various cues, including cell stress stimuli, thereby regulating various mitochondrial functions.

ER–endosome

Current research aims to better understand the purpose of endosome Ca^{2+} stores and how this storage may be regulated by MCSs. Newly formed endocytic vesicles contain material from the Ca^{2+}-rich extracellular space, so, inadvertently, Ca^{2+} concentration within the endosomal lumen is similar to that in the extracellular space. Ca^{2+} is quickly released from endosomes, suggesting that Ca^{2+} release may be required for early steps in endocytic maturation or that it may be coupled to the acidification of endosomes[88–90] (FIG. 4a). Specifically, early endosomes marked by RAB5 GTPase have a luminal Ca^{2+} concentration of around 0.5 μM, and late endosomes marked by RAB7 GTPase have a luminal Ca^{2+} concentration of around 2.5 μM. Late endosomes and lysosomes contain Ca^{2+} levels that are close to ER Ca^{2+} levels[91,92] (FIG. 4a). Notably, in late endosomes Ca^{2+} levels are known to fluctuate, and these fluctuations may be a result of the existence of abundant MCSs between late endosomes and the Ca^{2+}-rich ER[92,93].

Several lines of evidence suggest that the ER–endosome interface is a dynamic site for Ca^{2+} crosstalk between these organelles, with Ca^{2+} being released from both endosomes and the ER. Studies indicate that endosomes can release Ca^{2+} stores through both transient receptor

potential channels (TRPs) and two-pore channels (TPCs), which have homology to TRP channels[94–96] (FIG. 4c). Interestingly, stimulating Ca^{2+} release from acidic endocytic vesicles can stimulate ER Ca^{2+} mobilization[97] and vice versa, release of Ca^{2+} from the ER, induced by either $Ins(1,4,5)P_3$ or cyclic ADP-ribose (cADPR) can activate Ca^{2+} release from acidic vesicles[74]. Additional evidence indicates that ER Ca^{2+} release can stimulate increases in fluorescence of a calcium indicator in lysosomes, suggesting that ER Ca^{2+} could be sequestered into endosomes and/or lysosomes through unknown Ca^{2+}-uptake channels[98] (FIG. 4c). Together, these data reveal that substantial Ca^{2+} crosstalk occurs between the ER and the endo-lysosomal system. However, further studies are needed to determine whether Ca^{2+} transfer from the ER lumen to the endosome lumen occurs specifically at ER–endosome MCSs, and how this exchange would be regulated.

Organelle biogenesis

Mitochondria and endosomes are dynamic organelles that are constantly undergoing fission and fusion, which is important for maintaining cellular homeostasis[26,99]. Surprisingly, live-cell imaging has revealed that ER contact sites define the position of fission on mitochondria and endosomes[9,13] (FIG. 5). In addition, ER MCSs regulate the sorting and degradation of at least one endocytic cargo, the epidermal growth factor receptor (EGFR)[11].

ER–mitochondria

The central player in mitochondrial fission is the dynamin-related protein DRP1 (Dnm1 in yeast)[100–102]. These proteins oligomerize into spirals that circumscribe mitochondria and mediate their fisson[103,104]. In both yeast and mammalian cells, ER MCSs define the position at which this fission machinery will assemble and, consequently, where mitochondrial fission will occur[9,16] (FIG. 5a). Notably, the mean diameter of mitochondria is at least twofold larger than the diameter of the DRP1 (Dnm1) spiral assembly[103,105,106]. However, it has been observed that at points at which ER and mitochondria are in contact, mitochondrial membranes are constricted, and this is where DRP1 spirals preferentially assemble on mitochondria[9] (FIG. 5a). These data suggest that ER contact sites play a direct part in mitochondrial membrane constriction and facilitate the recruitment of the machinery that drives mitochondrial fission.

An important question is how this site of constriction and recruitment of fission machinery is defined. In yeast, the majority of ER-marked mitochondrial fission sites colocalize with the position of the nucleoid[16] (FIG. 5a). Synergizing nucleoid position, ER contact and fission machinery would help to ensure that upon fission, both daughter mitochondria can inherit nucleoids and ER contact sites. Several layers of tethering complexes would be required to coordinate the position of nucleoids, the inner and outer mitochondrial membranes and the apposing ER, as well as the fission machinery. In yeast, the ER–mitochondrial tethering complex (and potential lipid transfer complex) ERMES also colocalizes with nucleoids at ER-marked constriction and fission sites, suggesting that it may have a role in this process[16] (FIG. 5a). In animal cells, the ER–mitochondrial tether responsible for regulating contact at constriction and fission sites has not yet been discovered.

ER–endosome

ER contact sites also mark the positions at which early and late endosomes undergo fission during cargo sorting[13] (FIG. 5b). Live-cell imaging of multiple fluorescently tagged components has revealed the order of events during endosome sorting and fission. First, endosomal cargoes are sorted between vacuolar and budding domains. Then, the ER–endosome MCS is assembled at the base of the budding domain, where it colocalizes with puncta of FAM21 (REF. 13) (FIG. 5b). FAM21 is a component of the actin nucleation-promoting WASH complex and interacts with VPS35, a subunit of the retromer cargo-sorting complex, which could potentially recruit the ER to endosome fission sites. Within seconds of ER recruitment, an ER tubule rearranges around the base of the bud, and this rearrangement is accompanied by bud fission (FIG. 5b). When fission is inhibited, ER tubules form stable contacts with stalled constrictions on tubular endosomes[13]. Thus, the ER seems to have a similar role in constriction and fission of both endosomes and mitochondria.

How do ER contact sites regulate the constriction and fission of two very different organelles? First, MCSs may provide a general platform for the recruitment of cytoskeletal proteins, which then mediate constriction. In mammalian cells, actin–myosin complexes are recruited to ER–mitochondria contact sites by an ER-localized protein, inverted formin 2 (INF2)[107,108]. Mitochondria are elongated in cells depleted of INF2 and are shorter in the presence of dominant-active INF2 (REF. 107). Consequently, it has been proposed that the assembly of actin–myosin complexes mediated by INF2 drives the initial constriction of the mitochondrial membrane at ER MCSs[107,108] (FIG. 5a). ER MCSs could also provide a platform for the recruitment of lipid-modifying enzymes that would work with LTPs to transfer lipids, promoting the acquisition of high membrane curvature at the constriction site. Once formed, such ER-associated constriction sites would recruit the fission machinery, which could be facilitated by the specific association of additional adaptor proteins at these constricted sites. For instance, in mitochondria, mitochondrial fission factor (MFF), which is an adaptor protein for DRP1, colocalizes with the ER-associated constrictions[9] and is required for DRP1 recruitment[9,109–113]. However, what targets such adaptor proteins to the ER MCSs is so far elusive. Furthermore, ER Ca^{2+} release could have an additional, regulatory role in triggering the completion of the fission process. Currently, these are only speculations, and further studies are needed to better understand the factors and the mechanism that regulate the assembly of ER-marked constriction and fission sites on mitochondria and endosomes.

As well as being involved in fission, ER–endosome MCSs are implicated in regulating sorting and degradation of the EGFR. Endocytosis of ligand-bound EGFR targets it for degradation by the lysosome and thus regulates EGF signalling. Following endocytosis, EGFR is internalized into intra-luminal vesicles (ILVs) that will be degraded upon fusion with the lysosome. EGFR is dephosphorylated on the cytosolic surface of the endosome by the ER-localized protein-Tyr phosphatase 1B (PTP1B)[11], and this dephosphorylation event is required for EGFR internalization into ILVs[11]. Immuno-electron microscopy shows that PTP1B and EGFR colocalize at ER–endosome MCSs[11] and, on the basis of the co-immunoprecipitation experiments, it can be suggested that the two proteins may in fact interact directly to bridge the MCS (although they are not required to maintain contact)[11].

Interestingly, when visualized with the use of fluorescently labelled EGF, EGFR–EGF can be observed to localize to punctate structures along the endosomal membrane, and these puncta colocalize with the positions of ER–endosome contacts[13]. These MCSs only partially overlap with the sites at which endosome fission events take place, reinforcing the initially postulated idea that the ER is able to form multiple discrete contacts with other organelles and that these discrete contacts are probably not all functionally redundant.

Conclusions

The expansive ER network extends throughout the cell interior to make stable contacts with multiple organelles that are ensnared like flies in a spider's web. The continuity of the ER and the extensive contacts that it makes with other organelles indicate that this is probably a mechanism that allows various signals to be propagated throughout the ER network, thereby rapidly reaching several contacting organelles and subsequently coordinating a widespread cellular response to a particular cue. In this way, a signal originating from the extracellular space and passing through ER–plasma membrane contact sites could be delivered to contacting organelles and routed back to the plasma membrane in rapid succession, promoting efficient intra- or intercellular signalling. The mitochondrial field has already begun to elucidate the role of cellular signalling throughout the ER with respect to coordinating the apoptotic signalling cascade through the timely release of Ca^{2+}. Unveiling the importance of ER MCSs for other signalling pathways within the cell will provide crucial insight into how the cell coordinates signalling events that require a network response spanning the entire cell. Future work will be aimed at identifying the composition of the MCS machinery, as well as the functional impact of each of the MCSs. This will provide important insight into the role of these complex multimembrane interactions in maintaining cellular health and homeostasis.

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Glossary

Peripheral ER	The ER network that spans from the perinuclear region of the cell to the cell periphery
ER sliding	ER tubules attach to a motor protein on a stable microtubule. The motor protein then pulls the ER tubule along the microtubule
Early endosomes	Endosomes that have been recently internalized into cells and labelled with RAB5 GTPase, have a relatively low pH, and have not further internalized cargo, such as signalling receptors, from the plasma membrane into intraluminal vesicles

Late endosomes	Mature endosomes that have not yet fused with the lysosome. These endosomes are labelled with RAB7 GTPase, have a relatively high pH, and have abundant intraluminal vesicles internalized into the lumen for easier degradation of cargo when the late endosome fuses with the lysosome
Cortical ER	Peripheral ER that is found directly underneath and tethered to the plasma membrane
Microsomes	ER vesicles resulting from the breakage of the ER network as the ER is isolated from cells
Nucleoid	Mitochondrial DNA associated with proteins that compact into one region of the mitochondrion

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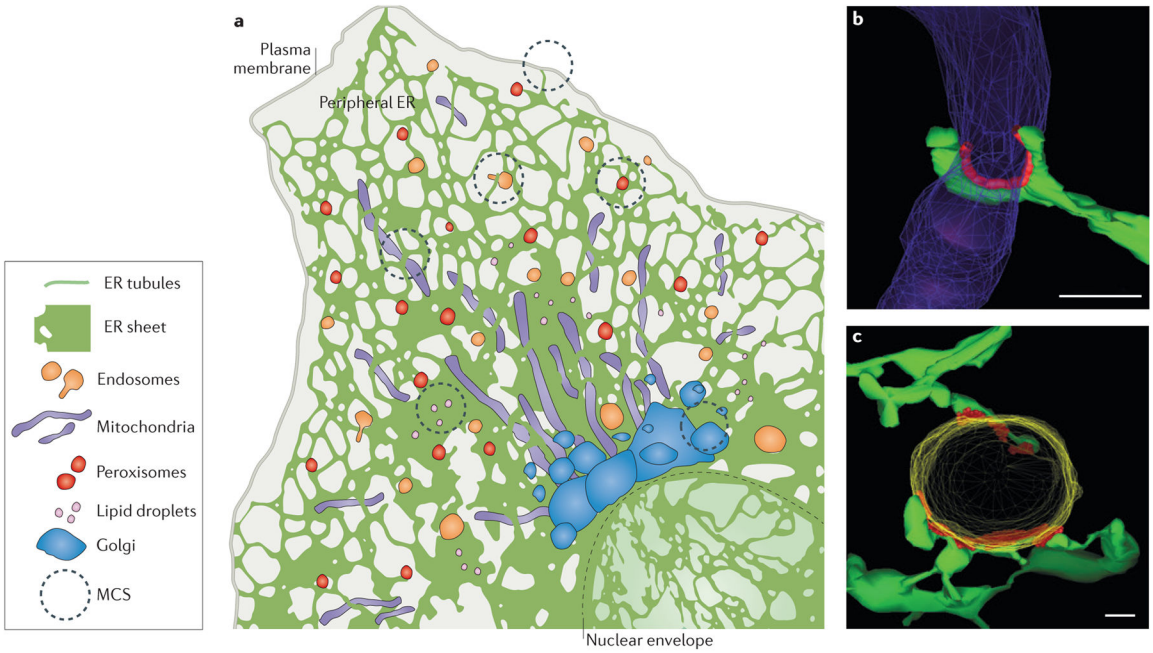

Figure 1. Structure of endoplasmic reticulum (ER) membrane-contact sites (MCSs)

a | The ER consists of the nuclear envelope (outlined with a dashed line) and the peripheral ER, which spreads into the cytosol as a network of sheets and tubules. The peripheral ER forms MCSs with the plasma membrane, mitochondria, endosomes, peroxisomes, lipid droplets and the Golgi. **b, c** | Electron tomography reveals the three-dimensional structure of MCSs (coloured red) between ER tubules (green) and mitochondria (purple) in a yeast cell (**b**) or an endosome (yellow) in an animal cell (**c**). Scale bars represent 200 nm in parts **b–c**. Image in parts **b** reproduced with permission from REF. 9, AAAS. Image in part **c** republished with permission of the American Society for Cell Biology, from Endoplasmic reticulum-endosome contact increases as endosomes traffic and mature. Friedman, J. R., Dibenedetto, J. R., West, M., Rowland, A. A. & Voeltz, G. K. *Mol. Biol. Cell* **24**, 1030–1040 (2013); permission conveyed through Copyright Clearance Center, Inc.

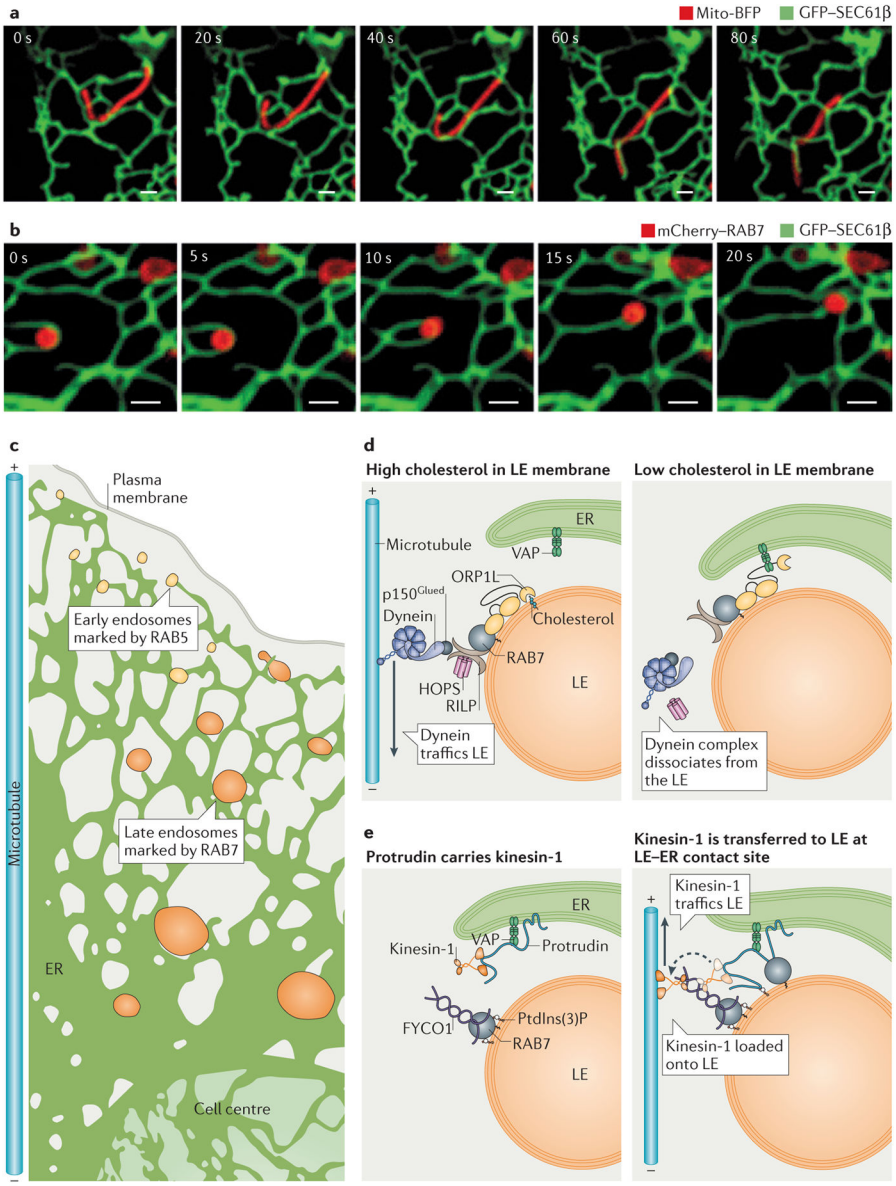

Figure 2. Dynamics of endoplasmic reticulum (ER) membrane contact sites (MCSs)

a,b | Endosomes and mitochondria are tightly tethered to ER tubules even as they traffic. Time-lapse fluorescence images of ER and organelle dynamics in live Cos-7 cells expressing GFP–SEC61β (labelling ER in green) and (**a**) mito-BFP (labelling mitochondria in red) or (**b**) mCherry–RAB7 (labelling late endosomes (LEs) in red). Note how the contact sites are maintained as the apposing organelles move. Scale bars represent 1 μm. **c** | Endosomes mature as they traffic from the cell periphery along microtubules to the cell centre. ER–endosome contact increases as endosomes mature, with 53% of early endosomes (EEs; marked by RAB5) and 99% of LEs (marked by RAB7) remaining in contact with the ER during trafficking. **d** | Model of how cholesterol levels regulate the composition of ER–LEs MCSs and LE trafficking. When the LE contains high cholesterol levels (left panel), oxysterol-binding-related protein 1L (ORP1L) can bind to cholesterol on the LE membrane

and does not associate with ER VAPs (VAMP-associated proteins). In addition, ORP1L interacts with RAB7 GTPase, which stimulates minus-end-directed LE trafficking through a complex that includes RILP (RAB-interacting lysosomal protein), the HOPS (homotypic fusion and vacuole protein sorting) complex, dynactin ($p150^{Glued}$) and the motor protein dynein. At low cholesterol levels (right panel), ORP1L is not bound to cholesterol and instead interacts with ER VAPs. The ORP1L–VAP interaction displaces dynein from the endosome. **e** | Protrudin is an ER integral membrane protein that interacts with VAP and kinesin-1 (left panel). Protrudin binds to RAB7 and phosphatidylinositol-3-phosphate (PtdIns(3)P) on the LE membrane. Protrudin can bind to and transfer kinesin-1 to the LE protein FYCO1 (FYVE and coiled-coil domain-containing protein 1), and this promotes plus-end-directed microtubule trafficking of LEs (right panel).

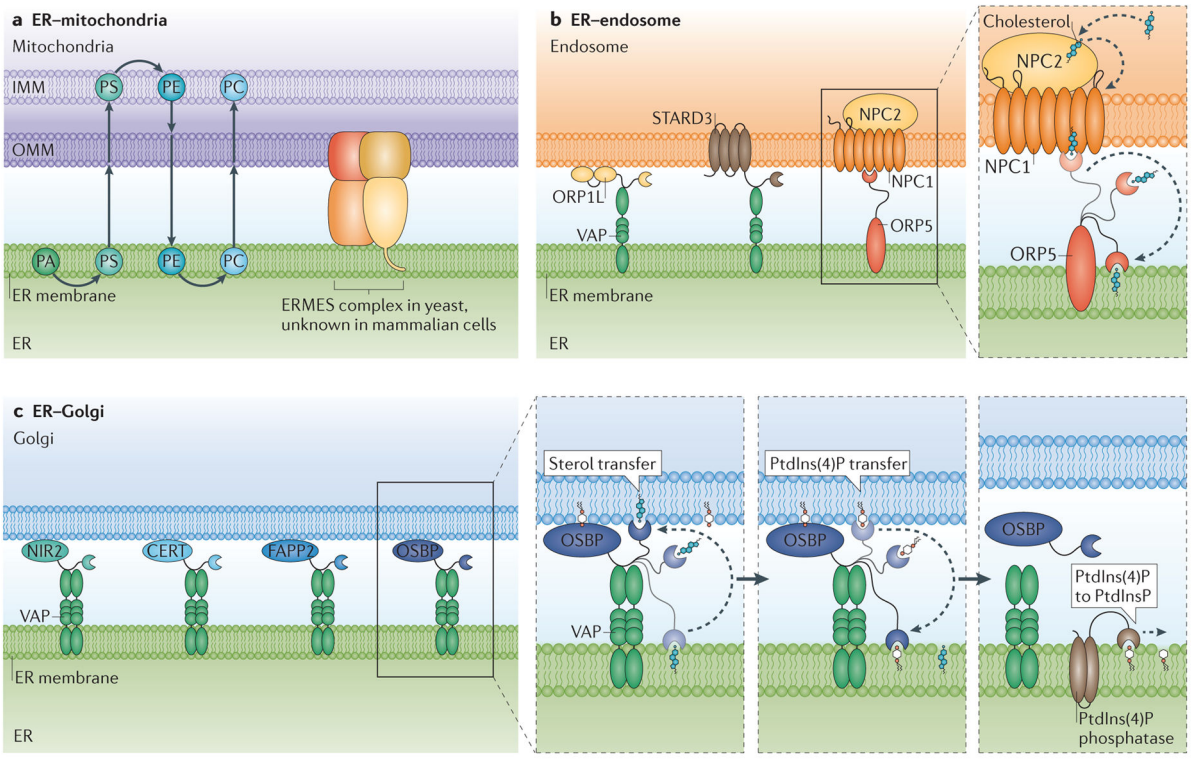

Figure 3. Endoplasmic reticulum (ER) membrane contact sites (MCSs) function in lipid biosynthesis and exchange

a | ER– mitochondria MCSs are rich in lipids and lipid-synthesis enzymes. Lipids are transferred between organelles at MCSs. In one pathway, phosphatidic acid (PA) is converted to phosphatidylserine (PS) at the ER. PS is transferred to the inner mitochondria membrane (IMM) where it is converted to phosphatidylethanolamine (PE). PE is transferred back to the ER, where it is converted to phosphatidylcholine (PC). PC is likely to also be transported back to mitochondria. ER–mitochondria membrane-tethering proteins (such as the ER–mitochondrial encounter structure (ERMES) in yeast) may aid this process; however, the exact mechanism of their action is currently elusive, and a mammalian counterpart has not been identified. **b** | Various complexes sense, modify or potentially transfer lipids at ER–endosome MCSs. Oxysterol-binding-related protein 1L (ORP1L) and START domain-containing protein 3 (STARD3) on the endosome have both been shown to interact with ER-resident VAMP-associated proteins (VAPs), but how they aid the exchange of lipids at ER–endosome MCSs is unclear. Niemann–Pick type C2 protein (NPC2) resides in the endosome lumen and interacts with endosome membrane protein NPC1. NPC1 interacts with the ER protein ORP5. The interactions between NPC2, NPC1 and ORP5 provide a potential mechanism for cholesterol transfer between the endosome lumen and the ER (blow-up). In this model, NPC2 transfers cholesterol from the endosome lumen to NPC1 on the endosome membrane. ER-resident ORP5 then accepts cholesterol from NPC1 and may transfer the cholesterol to the ER for redistribution. **c** | Multiple potential lipid-transfer proteins localize to the Golgi membrane and interact with ER VAPs. These include the phosphatidylinositol-transfer protein NIR2 (PYK2 N-terminal domain-interacting receptor 2), the ceramide-transfer protein (CERT), the glycosylceramide-transfer protein Golgi-

associated four-phosphate adaptor protein (FAPP2) and the cholesterol and phosphatidylinositol-4-phosphate (PtdIns(4)P)-transfer protein oxysterol-binding protein (OSBP). Studies specifically on OSBP (right panels) show that it associates with the Golgi membrane through PtdIns(4)P binding. The OSBP oxysterol-binding-related domain (ORD domain) can bind and transfer sterol from the ER to the Golgi and PtdIns(4)P from the Golgi to the ER. When PtdIns(4)P levels are depleted at the Golgi, OSBP dissociates from the Golgi membranes. PtdIns(4)P at the ER is converted back to PtdInsP by ER-associated PtdIns(4)P phosphatase. OMM, outer mitochondria membrane.

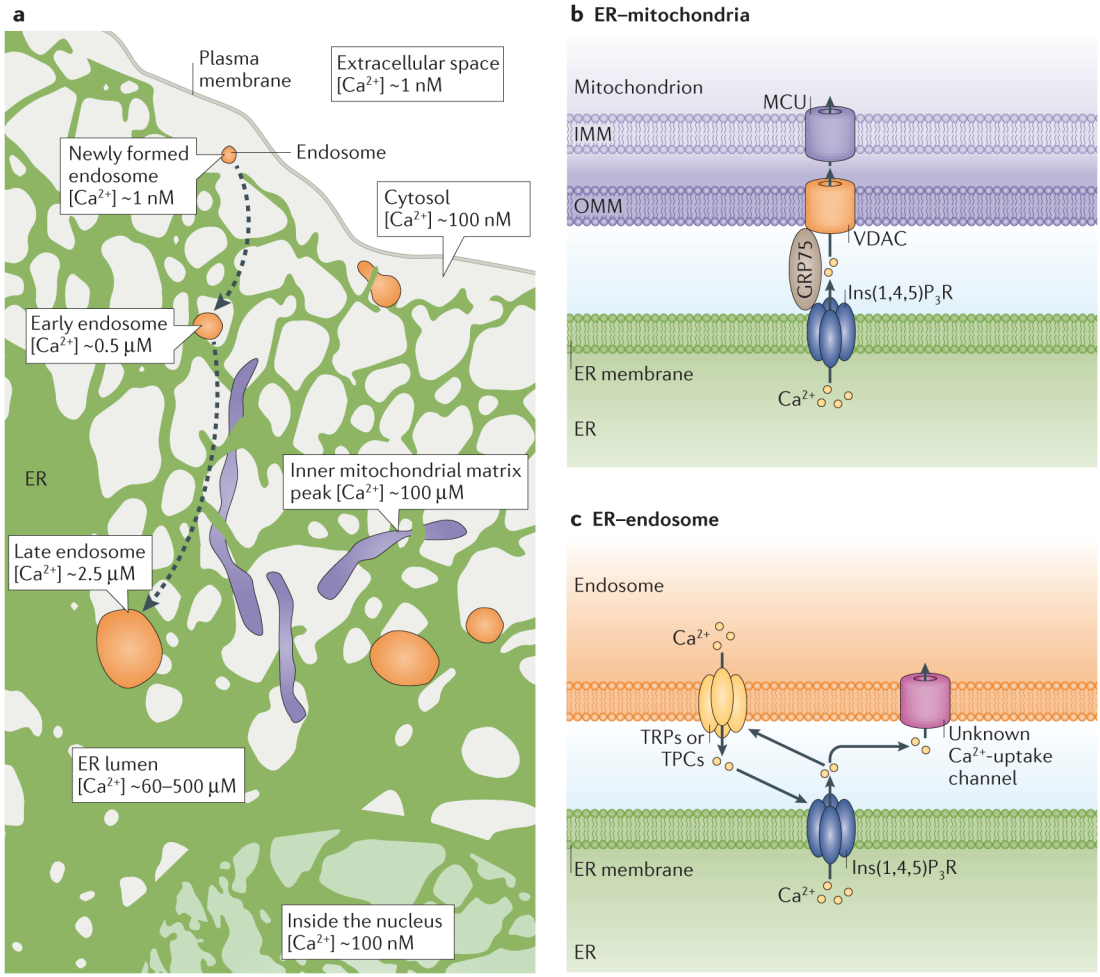

Figure 4. Calcium (Ca^{2+}) exchange at endoplasmic reticulum (ER) membrane contact sites (MCSs)

a | The ER lumen is the major Ca^{2+} store in the cell, with a Ca^{2+} concentration ($[Ca^{2+}]$) of ~60–500 µM). In the extracellular space, $[Ca^{2+}]$ is high (~1mM) compared to the intracellular cytosol (~100nM). Newly formed endosomes have taken up Ca^{2+} from the extracellular space, so the luminal $[Ca^{2+}]$ is close to the same as that of the extracellular space (~1 mM). Luminal Ca^{2+} is then released so that early endosomes have $[Ca^{2+}]$ ~0.5 µM and late endosomes have $[Ca^{2+}]$ ~2.5 µM. The ER–endosome MCS is a site of dynamic Ca^{2+} crosstalk. Endosomes may be able to sequester Ca^{2+} released from the ER. The ER transfers Ca^{2+} to mitochondria, with peak mitochondrial Ca^{2+} concentrations reaching 100 µM. **b** | ER Ca^{2+} released from the ER through inositol-1,4,5- trisphosphate receptors ($Ins(1,4,5)P_3Rs$) provides a concentrated Ca^{2+} spike that can be taken up through the outer mitochondrial membrane (OMM) by VDACs (voltage dependent anion channels) and then through the inner mitochondrial membrane (IMM) by the mitochondrial Ca^{2+} uniporter (MCU) ion transporter into the mitochondrial matrix. The 75 kDa glucose-regulated protein (GRP75) functions as a chaperone, coupling $Ins(1,4,5)P_3R$ to the VDACs. **c** | Endosomes are capable of releasing Ca^{2+} though transient receptor potential channels (TRPs) or two-pore channels (TPCs). ER Ca^{2+} released from ER via $Ins(1,4,5)P_3Rs$ could be taken up into

endosomes through unknown endosome Ca^{2+}-uptake channels. Ca^{2+} release from endosomes can also stimulate Ca^{2+} release from the ER through Ins(1,4,5)P_3Rs and vice versa.

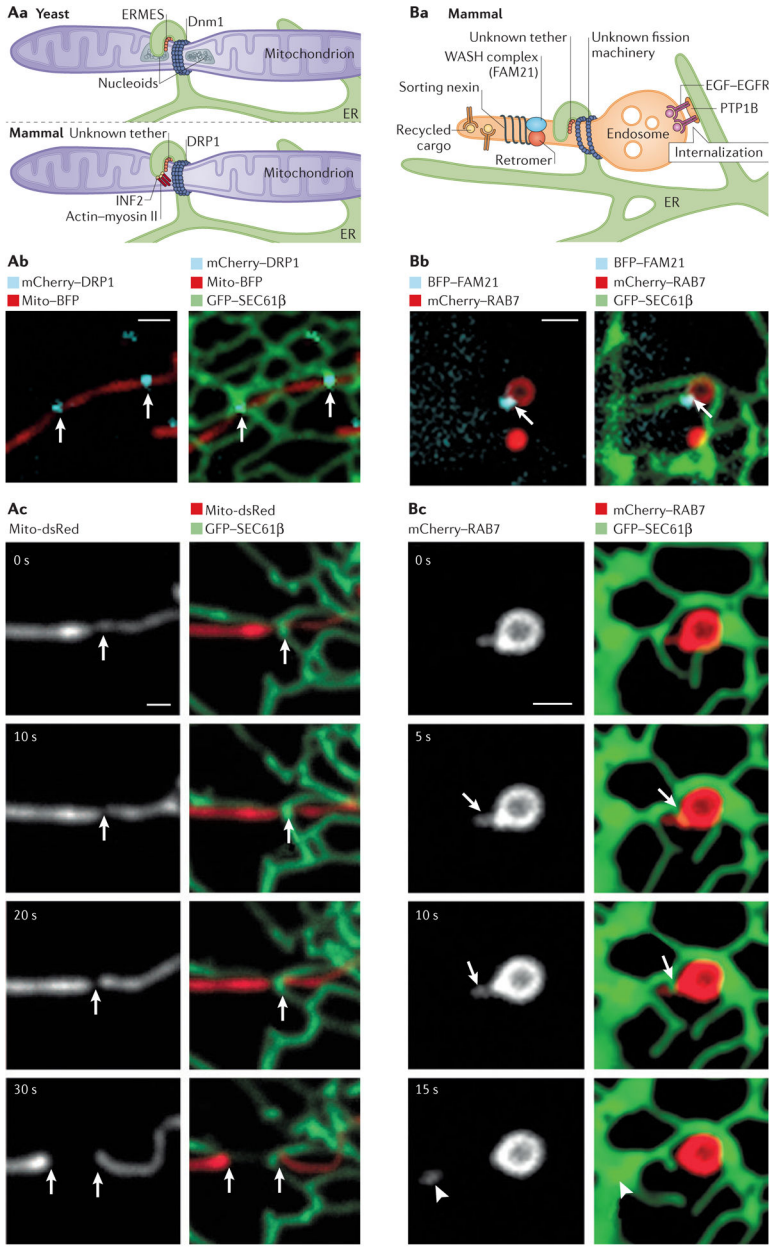

Figure 5. Endoplasmic reticulum (ER) membrane contact sites (MCSs) define the timing and position of both mitochondrial and endosome fission

Aa. In yeast (top panel), mitochondrial ER-marked constriction and fission sites contain the ER–mitochondrial tethering complex (ERMES), mitochondrial nucleoid DNA and the fission-machinery protein dynamin-related protein 1 (Dnm1). In mammalian cells (bottom panel), an ER-localized inverted formin (INF2), actin and myosin II are candidates for driving ER-associated constriction of mitochondria. Then, the fission-machinery protein dynamin-related protein 1 (DRP1) is recruited by adaptor proteins to ER-marked constrictions, where it drives fission. **Ab** | Live confocal fluorescence microscopy images of a Cos7 cell expressing mito-BFP (mitochondria in red) and mCherry–DRP1 (in cyan), merged with GFP–SEC61 β (ER in green) in the right panel. ER tubules contact two

mitochondrial constrictions labelled with DRP1, as marked by the white arrows. **Ac.** Live fluorescence microscopy, as in **Ab**, of a cell expressing mito-dsRed (mitochondria in grey in left panels, red in right panels) and GFP-–SEC61β (ER in green). Note that the ER tubule circumscribes the position of constriction and fission (white arrows) (t=30s). **Ba.** In ER-associated endosome fission in animal cells, cargo is sorted into tubules marked by the retromer, sorting nexins and WASH complex protein FAM21. ER tubules are recruited to these sorting domains by an unidentified tether, and fission is rapid following ER recruitment. Note that another ER–endosome MCS regulates dephosphorylation and internalization of epidermal growth factor receptor (EGFR) by ER-localized protein-Tyr phosphatase 1B (PTP1B). **Bb.** Live confocal fluorescence microscopy images of a Cos7 cell expressing mCherry–RAB7 (late endosome in red) and BFP–FAM21 (late endosome cargo-sorting domain in cyan), merged in the right panel with GFP–SEC61β (ER in green). The arrow marks a MCS between the tip of an ER tubule and the FAM21-labelled sorting domain on the late endosome. **Bc.** Time-lapsed images of a cell expressing mCherry– RAB7 (late endosome shown in grey in the left panels and red in the right panels) and GFP–SEC61β (ER in green) show ER tubule recruitment to the neck of the late endosome bud (t=5 s, arrow at the constriction), followed by fission (arrow, between t=10 s and t=15 s; bud marked by arrowhead, t=15 s). Scale bars in **Ab, Ac** and **Bb, Bc** represent 1 μm. Images in **Ab** courtesy of Jason Lee, University of Colorado Boulder, USA. Images in part **Ac** were adapted with permission from REF. 9, AAAS. Images in parts **Bb** and **Bc** were adapted with permission from REF. 13, Elsevier.

Table 1

Location, characteristics and proposed functions of mammalian and yeast ER–organelle MCS proteins

MCS proteins	Characteristics	Proposed function	Refs
Mitochondria–ER			
MFN2–MFN2 or MFN1–MFN2	• MFN2, both ER and mitochondria localized by FM data • MFN1, localized to mitochondria	Calcium (Ca^{2+}) transfer at ER–mitochondrial contact sites	15
VDAC–GRP75–Ins(1,4,5)P_3R	• VDAC, outer mitochondrial membrane Ca^{2+}-uptake channel • GRP75, soluble protein • Ins(1,4,5)P_3R, ER integral membrane protein and ER Ca^{2+}-release channel	Ins(1,4,5)P_3R is the Ca^{2+}-release channel on the ER, when stimulated Ca^{2+} can be transferred to mitochondria at MCSs and taken up into the mitochondria through VDACs. The GRP75 chaperone couples the Ins(1,4,5)P_3R and the VDAC	73, 81, 84
PTPIP51–VAPs	• PTPIP51, outer mitochondrial membrane protein with PtdInsP-transfer domain • VAP, ER integral membrane protein	Lipid transfer between ER and mitochondria may be facilitated by the PtdInsP-transfer domain of PTPIP51	114
FIS1–BAP31	• FIS1, outer mitochondrial membrane protein • BAP31, ER integral membrane protein	FIS1–BAP31 interaction allows for transmission of apoptotic signals from the mitochondria to the ER	115
Mmr1 *	Localizes to yeast cortical ER–mitochondria contact sites	Important for mitochondrial inheritance into yeast bud	12
ERMES complex *	Contains both ER- and mitochondria-localized proteins	In yeast, tethers ER and mitochondria. ERMES components contain SMP domains that are potentially capable of transferring lipids	14,44, 46,47, 50
Endosome–ER			
ORP1L–VAP-A	• ORP1L associates with the late endosome membrane through RAB7 • VAP, ER membrane protein	Senses sterol levels and regulates endosome positioning. Under low cholesterol concentrations, ORP1L negatively regulates late endosome association with dynein. Dynein no longer translocates late endosomes to the cell centre	28
STARD3–VAP-A	• STARD3, integral membrane protein in late endosomes	Possible role in sterol sensing and endosome positioning	7, 59

MCS proteins	Characteristics	Proposed function	Refs
	• VAP, ER membrane protein		
STARD3NL–VAP-A	• STARD3NL, integral membrane protein in late endosomes • VAP, ER membrane protein	Possible role in sterol sensing and endosome tabulation	7
NPC1–ORP5	• NPC1, integral membrane protein in late endosomes • ORP5, ER integral membrane protein	Proposed mechanism for cholesterol transfer through late endosome–ER MCSs by the ORD domain of ORP5	58
• RAB7–GTP, PI3P • Protrudin, VAP-A	• RAB7–GTP and PI3P are inserted in the late endosome membrane • Protrudin, ER membrane protein with FFAT domain that interacts with VAP-A	Regulates endosome positioning. Protrudin transfers kinesin-1 from the ER to late endosomes. Kinesin facilitates late endosome translocation to the cell periphery	34
EGFR–PTP1B	• EGFR, receptor protein in the endosome membrane • PTP1B, ER-localized phosphatase	PTP1B dephosphorylates receptors to regulate EGFR signalling	11
G-CSFR–PTP1B	G-CSFR, receptor protein in the endosome membrane PTP1B, ER-localized phosphatase	PTP1B dephosphorylates receptors to regulate G-CSFR signalling	116
Golgi–ER			
OSBP–VAP	OSBP associates with the Golgi membrane through PtdIns4P binding and contains a FFAT domain capable of interacting with ER VAPs	OSBP regulates PtdIns(4)P levels in the Golgi by transferring PtdIns(4)P from Golgi to the ER. OSBP transfers sterol in the opposite direction, from ER to Golgi	60, 64
CERT–VAP	CERT associates with the Golgi membrane through PtdIns(4)P binding and contains a FFAT domain capable of interacting with ER VAPs	CERT has a role in ceramide transfer at ER–Golgi MCSs	60, 61
FAPP2–VAP	FAPP2 associates with the Golgi membrane through PtdIns(4)P binding and contains a FFAT domain capable of interacting with ER VAPs	FAPP2 has a role in glucosylceramide transfer at ER–Golgi MCSs	62
NIR2–VAP	NIR2 associates with the Golgi membrane and contains a FFAT motif capable of interacting with ER VAPs	NIR2 plays a part in maintaining diacylglycerol levels in the Golgi	60, 63, 60
Lipid droplet–ER			
DGAT2–FATP1	DGAT2 localizes to lipid droplets FATP1 localizes to the ER	DGAT2 and FATP1 coordinate lipid droplet expansion at lipid droplet–ER MCSs	117
Peroxisome–ER			

MCS proteins	Characteristics	Proposed function	Refs
Pex3–Inp1–Pex3 *	Pex3, integral membrane protein localized to both peroxisomes and ER Inp1 cytosolic factor	In yeast, Inp3 binds to Pex3 and regulates tethering of peroxisomes to ER	118

CERT, ceramide-transfer protein; DGAT2, diacylglycerol *O*-acyltransferase 2; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERMES, ER–mitochondrial encounter structure; FAPP2, four-phosphate adaptor protein 2; FATP1, fatty acid transport protein 1; FFAT, diphenylalanine in an acidic tract; FM, fluorescence microscopy; G-CSFR, granulocyte–macrophage colony-stimulating receptor; GRP75, glucose-regulated protein 75; Inp, inheritance of peroxisomes; Ins(1,4,5)P_3R, inositol-1,4,5-trisphosphate receptor; LE, late endosome; MCS, membrane contact site; MFN, mitofusin; Mmr1, mitochondrial Myo2p receptor-related 1; NIR2, PYK2 N-terminal domain-interacting receptor 2; NPC1, Niemann–Pick C1 protein; ORD, oxysterol-binding-related domain; ORP, oxysterol-binding-related protein; Pex3, peroxin 3; PtdInsP, phosphatidylinositol phosphate; PTP1B, protein-Tyr phosphatase 1B; STARD3, START domain-containing protein 3; STARD3NL, STARD3 N-terminal-like protein; VAP, VAMP-associated protein; VDAC, voltage-dependent anion channel.

* indicates yeast proteins, all other proteins are of mammalian origin.