Membrane contact sites (MCS) are close appositions between two organelles that facilitate both signaling and the passage of ions and lipids from one cellular compartment to another. Despite the fact that MCS have been observed for over 50 years now, we still know very little about the molecular machinery required to create them or their structure, function and regulation. In this review, we focus on the three best-characterized contact sites to date: the nucleus–vacuole junction and mitochondria–ER and plasma membrane–ER contact sites. In addition, we discuss principles arising from recent research and highlight several unanswered questions in the field.

Membrane contact sites: from visualization to a molecular understanding

One of the hallmarks of eukaryotic cells is the presence of membrane-bound organelles. For many years the study of organelles focused on the advantages of the creation of distinct optimized environments best suited for promoting the various chemical reactions required to sustain life. However, for the entire cell to function as a unit, coordination and cooperation between specialized organelles must take place.

One way in which organelles communicate and coordinate cellular functions is through interorganellar or membrane contact sites (MCS), whereby two organelles come into close apposition. Such zones of close proximity have been visualized using electron microscopy (EM) techniques since the late 1950s, when an association between the endoplasmic reticulum (ER) and mitochondria was first described [1]. Since then, EM-based techniques have enabled the description of contact sites in yeast, plants and animal cells between the ER and: Golgi; mitochondria; chloroplasts; vacuoles (lysosomes in animal cells); endosomes; and plasma membrane (PM; Figure 1) [2,3]. Additional contact sites have since been suggested. For example, associations between peroxisomes and several other organelles such as the ER, mitochondria and lipid particles have been postulated based on calculations from 3D EM images of yeast cells [4,5]. Moreover, an association between the Golgi and mitochondria was proposed based on the proximity observed by confocal microscopy in pancreatic acinar cells [6].

Despite the decades that have passed since MCS were first observed, we still know very little about their biogenesis, structure, function and regulation. Most of our current knowledge regarding MCS architecture comes from high-resolution EM and suggests that in all documented cases, one organelle comes near to, but does not fuse with, the opposing organelle. Given that the original studies were performed without molecular markers, it is often unclear whether bona fide contact sites were investigated, or if the observed membranes happened to be juxtaposed. To overcome the problem of a lack of molecular markers, most studies refer to a measured distance of up to ~30 nm between two heterologous membranes as an association, because it is in the order of the size of a protein complex (10–30 nm) [7,8]. Accordingly, we still do not have a good 3D model of how an MCS might look. In fact, there are many different ways in which two membranes can come into close apposition (Figure 2) and more work will be required to resolve which is utilized in each MCS. The lack of molecular markers also meant that for many years biochemical techniques such as cellular subfractionation and measurement of enzyme activity and protein and lipid composition served as the only means to functionally characterize MCS. However, in recent years there has been a renaissance of interest in these sites with the identification of new molecular determinants required to create contact sites and the discovery of proteins that reside in these special structures. These discoveries are providing new tools in the study of MCS biology. This review focuses on the three most intensely studied MCS: the nucleus–vacuole junction, mitochondria–ER contact sites and PM–ER contact sites. Using these three MCS as a knowledge base, we try to infer several basic rules regarding these cellular phenomena.

The nucleus–vacuole junction

Nucleus–vacuole junctions (NVJs) are contact points studied in the yeast Saccharomyces cerevisiae that occur between the nucleus and the vacuole, and thus bridge three membranes: the vacuolar membrane, the ER membrane (continuous with the outer nuclear membrane) and the inner nuclear membrane. NVJs are the best-characterized contact sites to date.

Cellular function

There have been, to date, two roles suggested for the NVJ.

Microautophagy

The NVJ is the site of piecemeal microautophagy of the nucleus (PMN), a microautophagic process that targets portions of the yeast nucleus for degradation in the vacuole lumen [9]. PMN is upregulated upon carbon or nitrogen starvation through the target of rapamycin (TOR) nutrient
response pathway [9] and requires autophagic components shared between the selective cytoplasm to vacuole (CVT) autophagy pathway and unselective macroautophagy [10,11], as well as the electrochemical gradient across the vacuolar membrane [12].

**Lipid biosynthesis**

Although two proteins, Osh1 and Tsc13, which are involved in lipid biosynthesis and trafficking, reside in the NVJ, no clear role for the NVJ in regulation of lipid biosynthesis has been established [13–16]. Osh1 belongs
to the yeast oxysterol-binding proteins homology (Osh) family, whose members are suggested to function as lipid transfer proteins or lipid sensors, and is the only one of the seven Osh family members that localizes to NVJs [17]. Osh1 localizes to both the Golgi network and NVJ, but localization to the NVJ becomes exclusive during stationary phase [13]. However, OSH1 itself is not required for the formation of NVJs or for PMN function in starved cells [15].

The second protein shown to reside in the NVJ is the enoyl-CoA reductase Tsc13, an essential ER membrane protein that catalyzes the terminal step in the biosynthesis of very-long-chain fatty acids (VLCFAs) [14], that are known to influence the structure and fluidity of membranes. Tsc13 function is required for efficient PMN, and a decrease in the size of PMN blebs and vesicles is observed in its absence [16].

One of the characteristics of the NVJ is that nuclear pore complexes are completely excluded from the nuclear side of the interface [18,19], whereas the V-ATPase is excluded from the vacuolar membrane sections that form the junction [12]. This implies the existence of a diffusion barrier that probably depends on a specialized lipid composition in the NVJ [12] and serves as a first hint to the possible roles of NVJ-localized expression of Osh1 and Tsc13. However, the reason why two lipid-modulating enzymes would be specifically recruited to these contact sites and whether additional proteins reside in the NVJ are still far from understood.

**Structural components**

The NVJ is created by direct association of the vacuolar protein Vac8 and the ER-localized protein Nvj1. Vac8 is a soluble protein that is post-translationally anchored to the vacuolar membrane by N-terminal myristate and palmi- tate moieties. Nvj1 is an ER single-pass membrane protein (Figure 1, inset) [19]. Deletion of either one of these genes causes complete loss of the MCS and dissociation of the two organelles [19]. Both Tsc13 and Osh1 are recruited to NVJs through specific interactions with Nvj1 independently of Vac8 [15,16].

It is not yet clear whether this MCS is conserved in higher eukaryotes in terms of its structure (bridging the nucleus and vacuole) and function in PMN. However, contacts between the ER and the endosome/lysosome (an organelle that is the higher eukaryote counterpart of the yeast vacuole) have been described [20]. Apparently, the late endosome (LE)-localized oxysterol-binding protein-related protein 1L (ORP1L) functions as a cholesterol sensor and adopts different conformational states that reflect the LE cholesterol content. Under conditions of low cholesterol, ORP1L undergoes a conformational change that induces the formation of LE–ER contacts, whereas under high cholesterol, LE–ER contact is prevented and association with motor proteins is promoted. Thus, cholesterol levels are translated into LE intracellular positioning [20].

In summary, despite the fact that the molecular tether forming the NVJ has been identified, there are many open questions to be answered regarding this intriguing contact site. What other proteins reside in it? Does it have additional undiscovered functions? Does it have relevance to mammalian cell biology?

**The mitochondria–ER contact site**

Proximity between the outer mitochondrial membranes (OMM) and those of the ER has been observed by EM in various organisms [1,7,21]. Narrow particles, spanning distances of 10–30 nm, have been observed via electron tomography of isolated mitochondria, rat liver cells and DT40 cells [8]. Based on calculations, it is thought that 80–110 such contact points exist between the ER and mitochondria per yeast cell [7].

**Cellular function**

Although the molecular identity of the tethering factor(s) that mediates this MCS remained elusive until recently, extensive research spanning the past several decades has been performed on the mitochondria-associated membrane (MAM) fraction, the fraction of ER that co-purifies with mitochondria. The immense wealth of existing functional and biochemical data arising from these studies [22–24] has led to two hypotheses on the major roles of mitochondria–ER contact sites.

**Lipid biosynthesis**

Early work demonstrated that the MAM fraction contains enzymes involved in phospholipid biosynthesis, and that the ER and mitochondria must collaborate to produce certain lipids [25–27]. This is exemplified by the biosynthetic pathway for phosphatidylcholine (PC). The substrate for this
pathway, phosphatidylserine (PS), is synthesized in the ER and then imported to the intermembrane space of the mitochondria, where the inner mitochondrial membrane enzyme Psd1 catalyzes a decarboxylation reaction that yields phosphatidylethanolamine (PE). PE is then shuttled back to the ER, where it is further processed to yield PC [28,29]. Several lines of evidence that support lipid delivery via a direct contact between the ER and mitochondrial membranes, and the observation that newly synthesized PS accumulates in the MAM fraction, but not in bulk ER, on blocking of PS translocation to mitochondria, suggested that close contacts are required for lipids to traverse from the ER to the mitochondria [30]. Another example of the functional crosstalk between ER and mitochondria is provided by the biosynthesis of steroids in vertebrates that depends on shuttling of cholesterol to the intermembrane space of the mitochondria, where it is converted to the steroid precursor pregnenolone by the cytochrome P450 side-chain cleavage enzyme. Pregnenolone is then transported to the ER for further processing of steroid hormones [31].

Ca\(^{2+}\) homeostasis

The second major role assigned to ER–mitochondria contact sites is the transmission of Ca\(^{2+}\) [32]. Maintenance of mitochondrial Ca\(^{2+}\) homeostasis is crucial. On one hand, ATP production rate in mitochondria is controlled by Ca\(^{2+}\) through activation of several key metabolic enzymes localized in the matrix (pyruvate, \(\alpha\)-ketoglutarate and isocitrate dehydrogenases). Ca\(^{2+}\) overload, on the other hand, induces cell death [33]. Selective measurement of Ca\(^{2+}\) concentrations in mitochondria ([Ca\(^{2+}\)]\(_{m}\)) of living cells revealed that following cell stimulation, a rapid [Ca\(^{2+}\)]\(_{m}\) peak surges, reaching values well above those of the bulk cytosol. Given that mitochondria harbor a low-affinity uptake system for Ca\(^{2+}\), these surprising measurements led to the proposal that on cell stimulation, mitochondria are exposed to [Ca\(^{2+}\)]\(_{m}\) concentrations much higher than those in the cytosol owing to their proximity to the micro-environments of open ER Ca\(^{2+}\)-release channels (inositol 1,4,5-trisphosphate receptor; IP\(_3\)R). It was suggested that mitochondria–ER MCS enable the positioning of mitochondria next to microdomains of high local Ca\(^{2+}\) concentrations, which results in efficient signaling [33].

Structural components

Experiments in which limited proteolysis affected both lipid transport [7] and transfer of released Ca\(^{2+}\) to mitochondria [8] pointed to the existence of a proteinaceous tether that couples both organelles. Moreover, the existence of such a tether is supported by electron micrographs of mitochondria–ER contact sites [8]. Efforts to reveal the identity of the physical tether between mitochondria and the ER has yielded several candidates to date (Figure 1, inset).

The first candidate tether was identified in HeLa and rat liver cells. These experiments demonstrated that the voltage-dependent anion channel (VDAC1) on the outer mitochondrial membrane physically binds the ER Ca\(^{2+}\)-release channel IP\(_3\)R through the molecular chaperone glucose-regulated protein 75 (GRP75) [34]. However, in cells lacking IP\(_3\)R, ER–mitochondria associations were not affected, which suggests the possibility that an IP\(_3\)R-independent linkage exists between ER and mitochondria [8]. Interestingly, the yeast ortholog of VDAC1, Porin1, physically associates with a large ER membrane complex (EMC) [35]; the functional significance of this interaction, however, remains unknown.

The second proposed tether is mitofusin 2 (MFN2). MFN2 is one of the two isoforms of the mitochondrial outer membrane dynamin-like GTPases (MFN1 and MFN2) that mediate mitochondrial fusion. Mammalian cells lacking MFN2, but not cells lacking Mfn1, display defects in ER morphology. MFN2 enrichment in the MAM, together with a reduction in the mitochondria–ER interface in Mfn2-deficient cells, suggested a role for MFN2 in interorganellar tethering [36]. However, it has been noted that ER–mitochondria contact zones are still formed (albeit to a lesser extent) in Mfn2-deficient cells, which implies that additional tethering factors might exist or that MFN2 plays an indirect role in the tethering process. There has not yet been an examination of MAM formation in cells lacking both Mfn2 and Vdac; such a finding could help to determine whether these two proteins provide redundant functions for tethering.

The third proposed tether was identified using a synthetic biology approach in yeast. It was assumed that a defect in the contact-site tether would cause mitochondrial dysfunction and prevent yeast from growing on a non-fermentable carbon source (a condition under which mitochondrial function becomes essential in yeast). Therefore, the authors screened for mutants that cannot grow on such a medium, but in which this growth defect can be suppressed by a synthetic linker tethering the ER and the outer mitochondrial membrane. The screen and follow-up analysis revealed a four-protein complex, termed ER–mitochondria encounter structure (ERMES), comprising the cytosolic protein Mdm12, two mitochondrial outer-membrane proteins (Mdm10 and Mdm34), and an ER membrane protein (Mmm1) [37]. Consistent with the notion that the ERMES complex mediates contacts between the ER and mitochondria, mutants in which one of the ERMES-encoding genes was deleted displayed two- to five-fold reductions in the PS-to-PC conversion rate [37]. Interestingly, analysis using bioinformatic tools suggested that three out of the four proteins comprising the ERMES complex contain the yet uncharacterized SMP (synaptotagmin-like, mitochondrial and lipid-binding proteins) domain, which is also present in a number of other eukaryotic proteins that bind lipids or hydrophobic ligands [38]. This prediction suggests a putative role in promoting lipid transport. However, given that an artificial linker could rescue cells in which ERMES components were knocked out, it is probable that the ERMES complex plays mainly a structural role. In ERMES mutants, lipid shuttling between the ER and mitochondria is not abolished, so it is reasonable to think that additional tethering elements exist in yeast too. In support of the idea that ERMES is not the sole tether is the observation that none of the four ERMES components has an identifiable homolog in mammalian cells. However, there is one highly conserved mitochondrial GTPase, Gem1, which was recently shown to co-localize to ERMES foci in yeast and regulate their size...
[39]. Interestingly, its mammalian homolog, MIRO, can also be found on sites of ER–mitochondria proximity in mammalian cell culture. However, whether its regulatory function is conserved is still unknown. Given differences in the number, size and function of mitochondria between yeast and mammalian cells, completely different mechanisms might be required to tether them to ER membranes and to control their function and architecture.

A few other proteins have been implicated as operating at the mitochondria–ER interface. Mitochondrial fission protein 1 (FIS1) conveys apoptotic signals from mitochondria to the ER by physically interacting with the ER integral membrane protein BAP31 [40]. PACS2 (phosphofurin acidic cluster sorting protein 2), a protein localized to both mitochondria and ER, regulates mitochondria–ER communication, presumably also through BAP31 [41]. Other proteins suggested as modulators of this MCS are: trichloplein/mitostatin [42], which modulates mitochondria–ER juxtaposition in an MFN2-dependent manner; presenilin 2, overexpression of which increases mitochondria–ER tethering [43]; and sigma-1 receptor, which stabilizes IP3 receptors at the MAM [32].

The next steps in the study of mitochondria–ER contact sites will be to identify the relationships between the various complexes that are thought to maintain contact between both organelles and the degree of conservation between distant eukaryotes. Furthermore, future research should aim to pinpoint additional proteins that are localized to these MCS, as well as the proteins required for their formation, function and regulation.

PM–ER contact sites
It has long been noted that areas of proximity between the ER and the PM exist in various cell types [44–48]. EM analysis in yeast indicated that as many as ~1100 contact sites might exist per cell [47], and the distance between the ER and PM in human Jurkat T cells varies across the range 10–25 nm [48]. Similar to mitochondria–ER junctions, PM–ER contact sites have been linked to cellular Ca2+ homeostasis and to lipid synthesis and trafficking.

Cellular function
Recent exciting publications have shed new light on the biological processes taking place in PM–ER MCS. Notably, although a role for PM–ER MCS in regulating Ca2+ homeostasis was appreciated for many years, the mechanisms by which this is achieved were only recently uncovered.

Ca2+ homeostasis
In excitable cells, such as cardiomyocytes, coupling between PM depolarization and ER calcium release is essential for generation of a robust global calcium signal. This is facilitated at PM–ER/sarcoplasmic reticulum (SR) junctions. Specifically, a depolarization signal at the cell membrane opens the PM voltage-dependent Ca2+ channel CaV1.2. The resulting Ca2+ influx induces amplification of the signal by triggering Ca2+-induced Ca2+ release from the SRryanodine receptor channels (RyR) [49].

The PM–ER junction is not restricted to excitable cells. In non-excitable cells this contact site promotes the maintenance of ER Ca2+ levels. Indeed, regulation of Ca2+ levels is necessary for proper protein synthesis and folding, as well as for signaling. Therefore, Ca2+ levels in the ER must be tightly controlled in all cell types. Accordingly, non-excitable cells regulate the extent of Ca2+ influx through PM Ca2+ channels by sensing luminal ER Ca2+ levels (hence, this was termed store-operated or capacitative Ca2+ entry) [50]. Genome-wide RNAi screens revealed the molecular constituents of store-operated Ca2+ entry signaling. Stromal interacting molecule 1 (STIM1) was identified as the ER Ca2+ store sensor in both S2 [51] and HeLa cells [52]. Soon after that, calcium release-activated calcium modulator 1 (ORAI1) was identified as the PM Ca2+-release-activated Ca2+ current (I_{CRAC}) mediating channel responsible for Ca2+ entry following calcium store depletion [53–55]. Since then, it has been shown that STIM1 redistributes in the ER upon ER Ca2+ depletion and accumulates in discrete regions of the ER that are located in close proximity (10–25 nm) to the PM. ORAI1 accumulates at sites in the PM directly opposite STIM1. This enables the local activation of CRAC channels by direct binding of STIM1 to ORAI1 [48,56]. Interestingly, it seems that store depletion promotes the formation of new ER–PM contact sites and causes STIM1 to accumulate at these sites, as well as in pre-existing regions of proximity (Figure 1, inset) [48]. Recent work demonstrated that STIM1 also interacts and inhibits Ca2,1.2 voltage-gated Ca2+ channels, and thus regulates ORAI1 and Ca2,1.2 reciprocally and ensures that only one type of Ca2+ channel will be active at any given time [57,58].

Lipid biosynthesis
It has been suggested that the PM–ER contact site is a site of non-vesicular lipid trafficking [59]. For example, sterols traffic between the ER, where they are synthesized, to the PM, where they account for ~30% of lipid content, in a non-vesicular pathway [60–62]. Because lipids are insoluble in water, they must be shielded by a protein during transit. Several families of lipid transfer proteins (LTPs) that can shield proteins during membrane transfer have been identified. The first identified, the oxysterol-binding protein (OSBP) related proteins (ORPs), are a large family of LTPs conserved from yeast to man. ORPs bind sterols and are believed to function collectively in non-vesicular sterol transfer between the ER and PM [17]. In support of this view, the solved structure of Osh4, both in the apo and sterol-bound states, revealed a soluble β-barrel protein with a hydrophilic exterior and a hydrophobic tunnel that can accommodate a single sterol molecule [63]. Indeed, movement of exogenous sterol from the PM to the ER occurs more slowly in yeast harboring a temperature-sensitive osh4 allele (at the non-permissive temperature) [64]. Recent data, however, argue that Osh proteins regulate organization of sterols at the PM, rather than their movement between the ER and PM [65]. Interestingly, Osh proteins have been implicated in binding phospholipids, as well as sterols. A new role for Osh3 in regulating phosphatidylinositol 4-phosphate (PI(4)P) metabolism has recently emerged and seems to function through the Sac1 phosphoinositide (PI) phosphatase at the PM–ER contact site [66]. The data suggest that high PM PI(4)P levels at PM–ER contact sites recruit Osh3 through its pleckstrin homology (PH)
domain. Interactions between Osh3 and the ER resident VAP proteins Scs2/Sc22, mediated by the FFAT motif of Osh3, activate ER-localized Sac1, which can then act on PI4P [66]. This work demonstrates how a phosphatase located in the ER can regulate PI at various sites in the cell through interorganellar contact sites.

Structural components
Although it has been demonstrated that several proteins either reside in or are recruited to PM–ER junctions on specific signals, the molecular identity of the physical tether that mediates PM–ER contact sites remains unclear (Figure 1, inset). The only strong candidates to date have been suggested in metazoan excitable cell types and are a class of highly conserved proteins termed junctophilins. Junctophilins most probably contribute to stabilization of the junctions by anchoring the ER/SR to the PM, and would thus provide a structural basis for physiological coupling between PM and ER/SR Ca$^{2+}$ channels [67–69].

In summary, despite the major importance of PM–ER contacts sites for Ca$^{2+}$ and lipid signaling, the identity of tether proteins that mediate this contact is still unknown. Further study is needed to uncover the molecular machinery for the formation and regulation of PM–ER contact sites.

Concluding remarks
Although the established view of MCS function is as a site for trafficking of lipids and ions [59], recent findings highlight the functional significance of MCS in signaling and effective transmission of metabolic cues within the cell [66]. Moreover, recent work suggests that MCS function in organellar inheritance control mechanisms [70]. To this end, it is obvious that as molecular components of such MCSs are uncovered, the next years should provide major breakthroughs and a better understanding of the extent of their cellular roles.

One striking feature in the literature on MCS is their dynamic nature: Ca$^{2+}$ store depletion promotes the formation of new PM–ER contact sites [48] and the size of the NVJ patch seems to expand on entry into stationary phase [19], conditions under which Osh1 relocates exclusively to the NVJ [13]. Moreover, to accomplish a role in signaling, functional complexes operating in the MCS must be sensitive to changes in ion and lipid concentrations. Another example of the potential dynamic nature of contact formation is the recently published proposal that in response to increased cholesterol levels at the limiting membrane of the late endosome/lysosome (LE/LY), the ER sterol carrier ORP5 interacts with the endosomal sterol sensor NPC1 to form transient functional contacts allowing for the efficient removal of endosomal cholesterol. It is assumed that the interactions between ORP5 and NPC1 are enabled in areas of proximity that are LE/LY–ER MCS [71]. Such dynamic behavior points to the need to study MCS under a wide variety of conditions in order to allow a full understanding of their function and regulation. Moreover, their dynamics might explain why some MCS functions have remained elusive despite the many efforts to uncover them. More generally, this suggests that potentially less-visualized and condition-specific MCS between cellular organelles are yet to be discovered.

An additional aspect that seems to be regulated is the range of proximity. Electron tomography analysis of yeast ER organization revealed that the distance between the PM and the ER is of variable size ranging from 15.7 to 58.9 nm (with a mean spacing of 33.0 nm) [72]. It is plausible that this variation in distance is used to regulate MCS function. Indeed, it was demonstrated that the degree of proximity between ER and mitochondrial membranes in mitochondria–ER MCS affects Ca$^{2+}$ signaling. On tightening of the physical link, mitochondria become susceptible to Ca$^{2+}$ overload [8] and cells become more prone to apoptosis [8]. Thus, the degree of proximity is probably regulated and being too close, as well as too far, will have physiological implications.

Finally, an important feature of MCS highlighted in the literature is redundancy. For example, several complexes have been suggested to play a role in tethering between the ER and mitochondria. Indeed, no single deletion completely abolishes functional interactions between the two organelles. Thus, it seems likely either that there is more than one complex that mediates the physical connections between two organelles or that some studies have focused on indirect players. It is tempting to speculate that the MCS contain both physical elements that are stable or constant tethers and thus allow the interorganellar communication that is vital for housekeeping processes, alongside transient or fast-forming and fast-collapsing connections that permit signaling and regulation.

Many questions remain open. One main issue is the regulation of MCS formation and architecture. How are the actual points of contact arranged to obtain maximum efficacy of ion and lipid shuttling? Do the opposing membranes adopt specific curvatures (Figure 2a–e) at the site of contact? If so, how is this structure maintained and regulated? How many contact points are formed between the ER and each organelle, and what is the mechanism for counting or regulating the total area of contact? In the absence of the ER tubule-shaping proteins reticulons (Rtns) and Yop1 in yeast, the amount of PM-associated ER increases dramatically, so it was speculated that Rtns/Yop1 and ER membrane curvature might have a role in regulating the abundance of PM-associated ER. However, why and how this regulation is achieved is yet another open question [72]. In addition, we lack structural information about any of the tethering molecules and complexes suggested to date. Moreover, a mechanistic and functional understanding of MCS tethers will require their purification, reconstitution into membranes, and characterization.

Interestingly, for some of the components that were suggested to act as MCS-tethers, other functions have already been demonstrated. For example Vac8, which forms the NVJ through association with Nvj1, plays multiple independent roles in vacuole inheritance, fusion and morphology [73]. In addition, Mdm10, which is part of the ERMES complex, has an important role as part of the sorting and assembly machinery (SAM) complex that inserts β-barrel proteins into the MOM [74]. Mfn1/2, localized to mitochondria–ER contact zones, is also part of the machinery responsible for mitochondrial fusion and dynamics [75]. These dual roles, and the fact that these proteins participate in more than one complex, might represent an
essential part of their regulation and yet another layer of complexity in understanding their function.

In summary, the molecular era of MCS is now upon us. This has been facilitated by the identification of tethering molecules between opposing membranes and functional complexes that reside in these areas. These discoveries have opened up an enormous number of new questions. As these questions are answered, a detailed understanding of contact site formation and regulation will emerge.

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References
18 Severs, N.J. et al. (1976) Nuclear pore absence from areas of close association between nucleus and vacuole in synchronous yeast cultures. J. Ultrastruct. Res. 54, 374–387
19 Pan, X. et al. (2000) Nucleus–vacuole junctions in Saccharomyces cerevisiae are formed through the direct interaction of Vac8p with Nvj1p. Mol. Biol. Cell 11, 2445–2457
20 Rocha, N. et al. (2009) Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning. J. Cell Biol. 185, 1209–1225
21 Morre, D.J. et al. (1971) Connections between mitochondria and endoplasmic reticulum in rat liver and onion stem. Protoplasma 73, 43–49
38 Kopec, R.O. et al. (2010) Homology of SMP domains to the TULIP superfamily of lipid-binding proteins provides a structural basis for lipid exchange between ER and mitochondria. Bioinformatics 26, 2192–2197
40 Iwasawa, R. et al. (2011) Fis1 and Bap31 bridge the mitochondria-ER interface to establish a platform for apoptosis induction. EMBO J. 30, 558–568
41 Simmen, T. et al. (2005) PACS-2 controls endoplasmic reticulum-mitochondria communication and Bid-mediated apoptosis. EMBO J. 24, 717–729


52 Liu, J. et al. (2005) STIM is a Ca\textsuperscript{2+} sensor essential for Ca\textsuperscript{2+}-store-depletion-triggered Ca\textsuperscript{2+} influx. Curr. Biol. 15, 1235–1241


54 Vig, M. et al. (2006) CRACM1 is a plasma membrane protein essential for store-operated Ca\textsuperscript{2+} entry. Science 312, 1220–1223


63 Im, Y.J. et al. (2005) Structural mechanism for sterol sensing and transport by OSBP-related proteins. Nature 437, 154–158


65 Georgiev, A.G. et al. (2011) Osh proteins regulate membrane sterol organization but are not required for sterol movement between the ER and PM. Traffic DOI: 10.1111/j.1600-0854.2011.01234.x


