Inter-organelle membrane contact sites are zones where heterologous membranes, usually the endoplasmic reticulum plus a partner organelle, come into close apposition. These sites are very poorly understood because so few of their components have been identified; however, it is clear that they are specialised for traffic of material and information between the two membranes. There have been recent advances in the study of lipid transfer proteins, such as ceramide transfer protein (CERT) and homologues of oxysterol binding protein (OSBP). Not only can these proteins carry lipids across the cytoplasm, but they have been found to target both the endoplasmic reticulum and a partnering organelle, and in some cases have been localised to membrane contact sites. Further work will be needed to test whether these lipid transfer proteins act when anchored at inter-organelle contact sites.

**Addresses**

1 Division of Cell Biology, UCL Institute of Ophthalmology, Bath St, London EC1V 9EL, UK
2 Department of Cellular and Physiological Science and the Brain Research Centre, Life Sciences Institute, 2350 Health Sciences Mall, University of British Columbia, Vancouver, BC, Canada, V6T 1Z3

Corresponding author: Levine, Tim (tim.levine@ucl.ac.uk)

**Introduction**

Several organelles make close contacts with each other at zones of apposition called membrane contact sites, which provide an alternative means of communication to membrane-bound carriers. Membrane contact sites are found in all organisms, from simple prokaryotes with two membranes (and hence also between the two mitochondrial membranes) to eukaryotes, where one of the partnering membranes (and hence also between the two mitochondrial membranes) to eukaryotes, where one of the partnering organelles is always the endoplasmic reticulum (ER). Since the ER network spreads into all corners of a cell, including highly asymmetric cellular projections such as neuronal dendrites, axons and synapses [1], it is not surprising that strands of the ER should be found near other organelles. For this reason, the significance of zones of very close apposition (~10 nm) is still open to question.

However, where studied, membrane contact sites are relatively stable [2] and the portion of ER involved is biochemically distinct from the bulk of the ER [3,4]. Instead of being random, membrane contact sites are specialised for communication, in particular the efficient traffic of small molecules such as Ca²⁺ ions and lipids, as well as enzyme–substrate interactions occurring in trans (Figure 1a). The permanence of membrane contact sites indicates that they are structured by bridging complexes; however, only a single example demonstrates how these bridges are constructed (Figure 1b) [5].

Although no new structural components have been discovered recently, an increasing number of proteins are now known to function on two contacting organelles. This review will focus on lipid traffic, since this is often independent of NSF/Sec18-mediated vesicular traffic [6]. The hydrophobicity of lipids and the existence of lipid transfer proteins (LTPs), which reversibly bind selected lipids with 1:1 stoichiometry in hydrophobic pockets, suggest that non-vesicular transport is mediated by LTPs. While it is possible that LTPs act by diffusing to-and-fro across relatively large cytoplasmic gaps, an alternative hypothesis is that LTPs are firmly attached to a membrane and yet efficiently transport lipids [7], implying that they function at a membrane contact site. Here an LTP might provide an almost static hydrophobic crossing point. Data supporting this hypothesis has been accumulating over the past few years (Figure 1c), as will be discussed below.

**Plasma membrane–ER contacts**

One organisational feature of the ER is its relationship in the cell periphery with the plasma membrane (PM). The interaction of the two organelles has been studied biochemically in only one instance, which showed the portion of ER adherent to the PM in yeast is enriched in synthases for phosphatidylserine (PS) and phosphatidylinositol (PI), lipids that are needed in the PM [4]. Several complexes bridge between the ER and the PM, although all are likely to be transient, in other words not structural [8–10], implying that the critical bridging components have yet to be found. Myocytes have enormously enlarged PM and ER contact sites called triad junctions, and a family of ER membrane proteins called junctophilins may be structural components [11], but their binding partners have yet to be identified. Another reported PM–ER interaction is between SNAREs that might mediate fusion of ER cisternae with large phagocytic cups [12]. Although the importance of this process has been called into question [13], findings in divergent systems suggest...
Components of membrane contact sites (MCSs). (a) Generic composition of a membrane contact site. In addition to a relatively stable bridging complex, unstable interactions fall into one of three categories: (1) channels connecting two organelles (for example, TOM and TIM23 for protein import into mitochondria, ANT/mtCK/porin for creatine phosphate production by mitochondria, DHPR and RyR for excitation of striated muscle, or InsP3R and TRP-C for store operated calcium entry) [46,47,64]; (2) lipid transfer proteins binding to both membranes (for example OSBP homologues, CERT and RdgBα) [38,43,49]; (3) enzyme–substrate pairs (for example PTP-1B and either EGF receptor on endosomes or Src on the plasma membrane) [45,58]). (b) Components of the nucleus vacuole junction (NVJ) in yeast. The electron micrograph shows the typical apposition of the nucleus and vacuole in S. cerevisiae, with a gap as small as 10 nm. The bridging complex consists of Vac8 bound to the limiting membrane of the vacuole and Nvj1 integral to the outer nuclear envelope, the amount of which determines the extent of the NVJ [5]. Other proteins recruited to the NVJ by binding to Nvj1 are Tsc13 (a lipid synthase subunit) and Osh1 (an OSBP homologue that also binds yeast VAP, short for vesicle-associated membrane protein [VAMP]-associated protein) [9,54,55]. Image courtesy of Anjana Roy (UCL Institute of Ophthalmology). Scale bar = 500 nm. (c) Detailed mechanism by which lipid transfer proteins might act at membrane contact sites. OSBP homologues (left) and CERT (ceramide transfer protein, right) have a similar domain structure, with targeting domains for two membranes: an N-terminal pleckstrin homology (PH) domain typically binds both a PIP lipid and a protein factor (ARF1 for OSBP PH, not yet determined for CERT PH) to specify targeting within the late secretory pathway [34,76]; a central FFAT motif binds VAP on the ER [9]. The C-terminal lipid transfer domain transfers sterol [29,30] or ceramide [49] respectively. Osh4, the OSBP homologue studied in greatest detail, is a short form consisting of the sterol transfer domain alone, which interacts with PIP2, possibly on both donor and acceptor membranes [30]. In addition, functions of the two LTPs are linked, as OSBP recruited to the TGN acts (either as a sterol sensor [28], or as a sterol transporter, or as a modulator of the contact site) to recruit and activate CERT [38]. Double headed arrows indicate the non-directionality of the transport mechanism, which is driven by gradient of free lipid [23,24].
temporarily a fusion of PM and ER as a possible explanation for non-vesicular traffic of specific polytopic membrane proteins [14,15] and membrane lipids [16].

The formation of cortical ER is best understood in new yeast buds [17]. One of the latest proteins to be implicated in this process is Ice2, a polytopic ER protein found only in yeast, which regulates cortical ER morphology as well as its inheritance into buds [18]. In addition, reticulons have been shown to regulate ER morphology by tubulating membranes and switching the ER from cisternal to reticular morphology [19], and these conserved proteins bind the exocyst at the plasma membrane, suggesting a possible role in PM–ER bridging [20].

**Sterol traffic and oxysterol binding proteins**

Sterols are the single most common PM lipid, and are critical for PM function. Sterol traffic is of great medical importance as aberrant handling of excess sterols by macrophages underlies atherosclerosis [21,22]. Recent studies in yeast have confirmed earlier findings in mammalian cells that sterol traffic is non-vesicular [23,24]. The proposed mechanism for this traffic is passive diffusion down a concentration gradient facilitated by LTPs. A passive mechanism that might concentrate sterols in the PM over the ER (observed PM concentration ≥10x that of ER) is mediated by sphingolipids in the exofacial leaflet of the PM forming complexes with sterol [25], although this is at odds with old data suggesting that the bulk of cholesterol is in the cytofacial leaflet [26]. The advantage of yeast studies is the relative ease with which genetic experiments can be carried out, for instance demonstrating the importance of the lipid environment in regulating sterol traffic to the PM [23,24].

Exciting developments in this field centre on the interactions of the likely sterol-specific LTPs. Oxysterol binding protein (OSBP) was originally identified as the cytoplasmic receptor for 5-hydroxycholesterol, a relatively water-soluble oxysterol, and OSBP was thought not to bind cholesterol itself [27]. However, a study on the regulation of extracellular-signal-regulated kinase (ERK) by sterols showed that OSBP in a complex with ERK phosphatases solubilises unmodified cholesterol [28].

This has now been followed by a structural analysis of an OSBP homologue, both empty and complexed to four different (oxy-)sterols, which indicates that most OSBPs can act as sterol transfer proteins [29]. The same team has also shown that sterol traffic in vivo is in part mediated by OSBP homologues [30], extending their earlier study on retrograde traffic of sterols in yeast [25]. As a result of this work, these proteins have been tested for a role in anterograde ergosterol traffic, which was reduced five-fold upon inactivation of OSBP homologues [31], a partial effect that does not clarify whether their involvement is direct or indirect.

Importantly, this identification of OSBPs as putative sterol transfer proteins leads to testable predictions. First, OSBP homologues are recruited to membranes by phosphoinositides such as PI(4)P and PI(4,5)P2 [32–34], and so these signalling lipids should be important for sterol traffic. This prediction has now been borne out experimentally [30], although the precise role of individual phosphoinositides is not yet clear (Figure 1c). Second, OSBP homologues contain domains that target both plasma membrane and ER, which places them at membrane contact sites [9,32,34,35]. Given the profusion of OSBP homologues in a single cell [32], they may be able to pass sterols between different membranes of the late secretory pathway, for example from the PM to recycling endosomes [22], via a two-step process using the ER as conduit [36]. Other advances in our knowledge of OSBP homologues have included the demonstration that their dissociation from membranes is mediated by AAA-ATPases [37], and that OSBP itself is a sterol sensor [28,38].

**Other PM–ER communication**

Phospholipids also traffic between the ER and plasma membrane; for example, during PI(4,5)P2 production, phosphatidylinositol (PI) is transferred from the ER to the PM rapidly and independently of carrier vesicles [7,39]. This requires proteins that can transport PI, which fall into two families that share no sequence homology: those related to mammalian PI transfer protein (PITP), and those related to yeast Sec14. The striking functional convergence between these families was confirmed recently by the demonstration that a mammalian homologue of RdgBα (a PITP family member, also known as Nir2) regulates the critical lipid diacylglycerol in Golgi membranes by the same mechanism used by Sec14 in yeast [40]. Similarly, while mammalian PI(4,5)P2 production at the PM relies on PITP family members [7], plant root hairs and budding yeast use Sec14 homologues for the same purpose [41,42]. The possibility of non-vesicular PI transport across PM–ER membrane contact sites, suggested by old morphological studies of *Drosophila* RdgBα [43], has been supported by biochemical studies of mammalian PITP-α [7], as well as being envisaged for the yeast Sec14-homologue Sfh5 [39].

Another type of link between the PM and the ER involves the interaction of enzymes across membrane contact sites. The only clearly described case is the interaction between PTP-1B, a tyrosine phosphatase embedded in the ER, which has substrates on the PM including insulin receptor [44], and multiple components of focal adhesions. These have now been studied in living cells using a substrate-trapping mutant form of the phosphatase [45]. ER tubules grow out on microtubules to attach to a sub-population of cell–matrix adhesions, and these PM structures are stabilised as a result of the enzymatic action of PTP-1B on the ER. This suggests
that proximity to the ER may define sub-domains of the PM. This idea is also envisaged for zones of calcium signalling, where channels on the PM and ER form complexes and activate each other, particularly in muscle cells during excitation [46,47].

**Golgi–ER contacts**

Ultrastructural evidence shows some ER cisternae in very close contact with elements of the trans side of Golgi stacks [48], but the nature of the link is unclear. Non-vesicular lipid traffic from the ER direct to the trans-Golgi network (TGN) is documented for ceramide, being mediated by ceramide transfer protein (CERT), an LTP with targeting domains to both TGN and ER [49], similar to OSBP [32]. It has now been shown that binding to both membranes is important for the function of CERT [49] and OSBP [38**]. There is also a functional link, as OSBP acts as sterol sensor, and recruits CERT, providing a means by which sterol metabolism post-translationally modulates sphingolipid synthesis [38**]. The precise origin within the ER of lipids trafficked to Golgi membranes is still open to question. It might be ER outside the Golgi region [38**]. Alternatively, it may be the intra-Golgi ER, as both OSBP and CERT could bridge across Golgi–ER membrane contact sites [32] (Figure 1c). This model, in which LTPs would function very efficiently without needing to diffuse between donor and acceptor, has yet to be proven. However, it has been shown that full-length OSBP homologues in yeast are recruited to membrane contact sites by their interaction with VAP on the ER (Figure 1c) [9].

Such a static model has also been postulated for the yeast Sec14-homologue Sfh4, which is required for non-vesicular PS traffic in the late secretory pathway, although Sfh4 does not itself bind PS [50]. Interestingly, this lipid traffic step can be reconstituted in vitro, and requires bridging from the acceptor to the donor by the very enzyme that utilises the lipid [51**], suggesting that both the enzyme and Sfh4 act at Golgi–ER contacts [39], although these contacts have yet to be demonstrated in yeast. Another good candidate for a component of Golgi–ER contacts is RdgB/Nir2, which interacts with both the Golgi and the ER [43,52].

**Endosome/lysosome–ER contacts**

Membrane contact sites between the ER and organelles of the endocytic pathway lie at the extremes of how clearly these structures have been defined. In budding yeast, the nucleus–vacuole junction is a membrane contact site between a portion of the ER (on the outer nuclear envelope) and the vacuole (equivalent to the lysosome). This is the sole membrane contact site whose components are known: single proteins embedded in each of the opposing membranes bind each other directly, forming velcro-like patches [5] (Figure 1b). NVJ size depends merely on the quantity of component proteins available, in particular Nvj1 on the ER. As the first membrane contact site to be defined at the molecular level, the NVJ forms the basis for our ideas of how other membrane contact sites may be constructed by a simple protein bridge.

The only function so far ascribed to the NVJ is that it acts as a zone through which nuclear material buds to reach the vacuole for autophagic recycling, and this is affected by the interaction of Nvj1 both with the lipid synthase Tsc13 and with Osh1, an OSBP homologue [53–55]. Interestingly, ORP1, the closest mammalian homologue of Osh1, binds to Rab7 on multivesicular late endosomes [56] and has a domain that targets the ER [9]. Thus ORP1 might target mammalian ER–endosome contacts, which contrast with their yeast counterparts by being very poorly documented, although clearly detectable under some circumstances [57,58].

**Mitochondrial–ER contacts**

Organelles derived from endosymbiotic prokaryotes are not connected to the secretory pathway by vesicular traffic, and so mitochondria [50] and chloroplasts [59] acquire a large proportion of their lipids from the ER by non-vesicular routes. For mitochondria, there is considerable evidence that a specialised sub-domain of the ER, called the mitochondrial-associated membrane (MAM), adheres to mitochondria. Importantly, MAM is enriched in synthases of lipids required by mitochondria [3,60], indicating specialisation for non-vesicular traffic. Another role for ER–mitochondrial membrane contact sites is in calcium traffic [2,46,47]. Because gross manipulation of these contacts does not necessarily affect calcium traffic from the ER to mitochondria [61], an alternative view is that calcium entry into mitochondria is determined by proximity to the PM. However, this does not necessarily imply a direct PM–mitochondrial membrane contact site, as ER elements are often found interposed between the PM and the mitochondrion [46]. In addition to the traffic of small molecules, a number of proteins translocate from the ER to mitochondria, one of which modulates the strength of ER–mitochondrial contacts [62**].

Despite a relatively large amount being known about ER specialisation in mitochondrial–ER membrane contact sites, no bridging components between ER and mitochondria are known. A ubiquitin ligase integral to mammalian ER membranes localises to MAM [63], and studies in yeast have demonstrated the involvement of ubiquitination in the normal functioning of both MAM and mitochondria [64,65], but the mechanism is still unclear [50].

**Intra-mitochondrial contacts**

Contacts between the outer mitochondrial membrane (OMM) and the inner mitochondrial membrane (IMM)
parallel similar contacts between the two membranes of many bacteria, and have the full range of activities envisaged to occur at membrane contact sites between heterologous membranes (Figure 1a) [46,66]. During import of cytoplasmic proteins into the mitochondrial matrix, TOM complexes on the OMM pass polypeptides directly to TIM23 complexes on the IMM. A new, conserved subunit of the TIM23 complex, Tim21 is embedded in the IMM and directly links the two complexes by binding Tom22 [67,68]. Despite interest in the TOM–TIM23 interaction, it is unlikely to be responsible for the adhesion of OMM and IMM [64]. Firstly, the TOM–TIM23 interaction is dynamic, only being detected when arrested protein intermediates are present. Also, when intra-mitochondrion contacts are purified biochemically, they have a unique protein profile that does not include either TOM or TIM complexes.

So what molecular mechanism brings about OMM–IMM contacts 15–20 nm in diameter, occupying ~5% of the mitochondrial surface? Importantly, the thickness of these adhesion sites is almost uniformly reported to be equal to the sum of the thicknesses of the OMM and IMM together [64]. This suggests that there is no hemifusion of OMM and IMM, and also that the adhering proteins do not have large domains in the intermembrane space (IMS). One possibility is that Tim23 alone forms contacts, as it spans both membranes [69]. However, Tim23 is not enriched in contacts either biochemically or morphologically, and the importance of its interaction with the OMM is called into question by studies that show this is not essential for protein import [70].

OMM–IMM contacts facilitate traffic of both large polypeptides and small metabolites between the mitochondrial matrix and the cytoplasm. In addition to inward traffic of calcium ions [46], OMM–IMM contacts are the site for production of the energy storage molecule phosphocreatine. A complex forms from the adenine nucleotide translocase in the IMM (which provides ATP from the matrix), octameric mitochondrial creatine kinase (mtCK) in the IMS, and porin in the OMM (porin is also known as voltage-dependent anion channel, VDAC, which shuttles [phospho]-creatine to the cytoplasm). Recent work has shown that over-expression of mtCK in a tissue where it is normally absent (mouse liver) increases more OMM–IMM contacts and makes the mitochondria more resistant to disruption by detergents [71*]. This is similar to the effect of over-expressing a protein that arrests during mitochondrial import, increasing TOM–TIM23 supercomplex formation [72]. Both cases show that a single set of bridging proteins can ‘zipper up’ OMM and IMM, and so it is tempting to speculate that these proteins have structural roles in the membrane contact site [71*] and might affect other functions such as phospholipid traffic [50]. But neither complex is likely to be the glue that forms the mitochondrial contact site. For mtCK, its very absence from wild-type liver indicates that it is unlikely to form constitutive contacts. Furthermore, the large size of octameric mtCK is incompatible with the absence of any gap between OMM and IMM at contacts.

Conclusions

The seeds of our current understanding of vesicular traffic were sown with ground-breaking genetic and cell-free reconstitution studies that identified key components in the trafficking machine. For membrane contact sites, such discoveries are still to be made, and we remain in an earlier phase where we are still defining the very nature of the non-vesicular traffic. This article documents the increasing numbers of proteins that localise to membrane contact sites, providing candidates that might modulate membrane contact site structure and function. However, the Holy Grail still remains: to identify components that constitutively form membrane contact sites. When these are known, it will be possible to perform the critical experiments, both in vivo and in cell-free reconstitution systems, where membrane contact sites are taken apart and put back together in order to properly dissect their functions.

The problems faced in this field are exemplified by a thorough study reconstituting phospholipid traffic between MAM and mitochondria in vitro [73]. Here, all current data predict that a protein bridge mediates efficient non-vesicular lipid traffic [74,75]; however, lipid traffic was only slightly reduced by protease treatment of both membranes. This suggests that the purification of the two organelles has disrupted bridging complexes. Indeed, any purification to homogeneity of an organelle that normally adheres firmly to another will disrupt bridges, possibly irreversibly by proteolysis. For many years, we have had only a partial knowledge of membrane contact sites, and I suggest that if we shall know fully how they function, we will first need to identify the structural bridging components and then manipulate their activity to see these fundamental, conserved structures clearly.

Acknowledgements

We thank Anjana Roy for the use of the electron micrograph image, and we apologize to our colleagues whose work we have not been able to include because of space constraints.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


29. Dick Anderson and co-workers discover that OSBP forms a complex with two phosphatases that both target ERK. The finding that free cholesterol is needed to inactivate ERK by stabilising the complex leads to the first identification of OSBP as a sterol binding protein. Interestingly, oxysterol has the opposite action to sterol, which might be explained by subtle structural effects in the protein’s structure depending on the bound sterol (see [29]).


31. Hurley and colleagues solve the structure of an oxysterol binding protein, showing that they all share a hydrophobic pocket that accommodates a single sterol (with little specificity for any particular [oxy-]sterol), suggesting that they are all capable of acting as sterol transfer proteins, and pointing to a transfer mechanism, whereby the lig above the pocket binds to anionic phospholipids before opening to allow sterol exchange.


33. Prinz and co-workers combine their earlier work on non-vesicular transport of sterols from plasma membrane to ER [23] and on OSBP [29] to show that yeast OSBP homologues transfer sterols in vivo (Osh3 and Osh5 being the most likely candidates) and in vitro. Also, a detailed study of one homologue (Osh4/Kes1) confirms predictions that this lipid traffic is inversely correlated with the ability of sterols to form complexes with sphingolipids in the PM.


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44. Arregui and co-workers co-localise Src and FAK in focal adhesions with a substrate-trapping mutant of PTP-1B on the ER in the puncta that form at the tips of ER tubules that are pulled out of the central ER network on microtubules.


52. Ambudkar IS: Poburko D, Kuo KH, Dai J, Lee CH, van Breemen C: Microtubules.


57. Perry and Ridgway provide a molecular explanation for a previous finding that oxysterols stimulate sphingolipid production. They show that when changes in sterol metabolism induce the translocation of OSBP to the TGN, the ceramide transporter (CERT) also translocates to Golgi membranes, transporting more ceramide to sphingomyelin synthase at that site, as predicted by Hanada [49].


63. Arregui and co-workers co-localise Src and FAK in focal adhesions with a substrate-trapping mutant of PTP-1B on the ER in the puncta that form at the tips of ER tubules that are pulled out of the central ER network on microtubules.


Work from the Pfanner lab showing that Tim21 in the IMM links the TIM23 complex to Tom22 in the TOM complex, and that two forms of TIM23 complex can be defined by the presence/absence of Tim21, switching from a state that captures polypeptide from TOM, to a state that passes incoming proteins to the matrix.


Ectopic expression of octameric mtCK changes mitochondrial morphology, probably as a result of increased bridges being formed by mtCK (binding to the IMM) and porin/VDAC in the OMM, resulting in both zipperong of OMM and IMM and resistance of mitochondria to lysis. While bridges such as these and those involving other complexes [67], may modulate general OMM–IMM inter-relatedness, they are not the glue that holds the two membranes together.


