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Piecing together the structural organisation of lipid exchange at membrane contact sites

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Abstract

Membrane contact sites (MCSs) are areas of close proximity between organelles, implicated in transport of small molecules and in organelle biogenesis. Lipid transfer proteins at MCSs facilitate the distribution of lipid species between organelle membranes. Such exchange processes rely on the apposition of two different membranes delimiting distinct compartments and a cytosolic intermembrane space. Maintaining organelle identity while transferring molecules therefore implies control over MCS architecture both on the ultrastructural and molecular levels. Factors including intermembrane distance, density of resident proteins, and contact surface area fine-tune MCS function. Furthermore, the structural arrangement of lipid transfer proteins and associated proteins underpins the molecular mechanisms of lipid fluxes at MCSs. Thus, the architecture of MCSs emerges as an essential aspect of their function.

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Introduction

Many biochemical processes in eukaryotic cells are organised in organelles with distinct proteomes, membrane lipidomes, and chemical environments. While such compartmentalisation facilitates regulation of metabolic functions, it also necessitates crosstalk between organelles to coordinate cell physiology. Key elements for interorganelle exchange are membrane contact sites (MCSs). MCSs can be defined as microcompartments where the membranes of two organelles are in close physical proximity and the interaction is established by specifically localising proteins. While some of these proteins might act as stable tethers, others can be temporarily recruited to MCSs [1–3]. MCSs mediate direct exchange of ions and small molecules such as lipids between interacting organelles and are involved in organelle fission and biogenesis. The diversity of MCS functions is covered by many reviews, for example [4–6]. Notably, MCSs permit exchange whilst maintaining membrane identity, thus segregating most of the membrane content. This distinctive functionality is provided by specific proteins connecting the organelles. Lipid transfer proteins (LTPs) are particularly enriched at MCSs and enable nonvesicular trafficking of lipids between interacting membranes [7]. Thereby, MCSs contribute to the specific lipid compositions underlying organelle membrane identities and deliver lipids for organelle biogenesis [8, 9]. A prevalent argument suggests that MCSs provide specificity by compartmentalising lipid transfer, rather than solely reducing the distance between organelles [10]. The structural assembly formed of MCS proteins and two differing membranes is thus a key principle of interorganelle exchange and separation. Accordingly, regulation of MCS architecture is a determinant of optimal MCS function. Here, we discuss emerging concepts and open questions on MCS ultrastructure including the roles of membrane shape, interorganelle distance, and MCS size. We further zoom into distribution, dynamics, and organisation of proteins assembling between two bilayer structures. Finally, we discuss recent advances on the structures of MCS proteins, with a particular focus on MCS-resident LTPs.

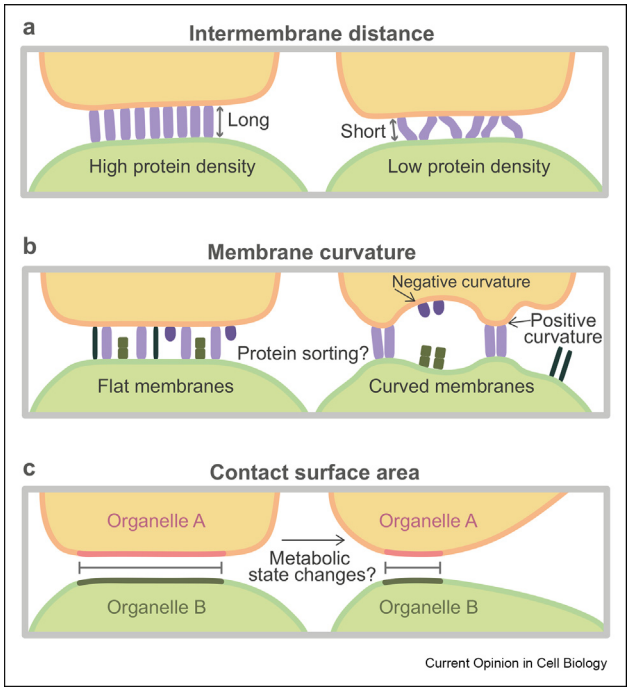
Ultrastructure of membrane contact sites

Intermembrane distance

The first notices of physical interactions between organelles came with the early use of electron microscopy to describe cellular ultrastructure. In 1956, Bernhard and Rouiller visualised close interfaces between mitochondria and the endoplasmic reticulum (ER) [11]. Their electron micrographs showed that in areas of contact, the distance between the membranes is less than 50 nm, suggesting a physiological link between organelles [11]. More recently, three-dimensional imaging by electron tomography (ET) enabled a systematic assessment of intermembrane distances, revealing ranges of 5 to 40 nm with high variability even within an

individual MCS [12–16]. Whether proximity itself is sufficient to identify a bona fide functional MCS is not clear, as random collisions between organelles could also result in proximity without the presence of LTPs or other MCS proteins conveying functionality. Furthermore, although the narrow spacing is considered a defining feature of MCS, it is unclear how it is maintained and what the importance of its length is, notably for the transfer of lipids. MCS-mediating proteins differ in the domains that span the space between the organelles [17], but domain lengths do not seem to strictly encode intermembrane distance. At least in the case of ER-plasma membrane MCS in yeast, the range of intermembrane distances is independent of the tethering protein [13]. Thus, intermembrane distance must be determined by different mechanisms. One possible mechanism was described for vesicle associated membrane protein (VAMP)-associated protein A (VAP-A), an MCS hub protein coordinating interactions [18], and its partner LTP Oxysterol-binding protein (OSBP) [19]: The more densely packed VAP-A molecules are, the larger is the resulting intermembrane distance (Figure 1a). This mechanism is attributed to the flexibility of VAP-A, which is in a straighter and more rigid conformation when protein density is higher [19]. But how precisely must intermembrane distance be maintained? Do LTPs require a locally fine-tuned spacing between the membranes? Do other MCS functions such as organelle fission depend on precise intermembrane distances? These questions are largely unanswered at present, although it is clear that *in vitro* lipid transfer efficiency can depend on intermembrane distance [20].

Figure 1



Possible regulatory mechanisms involving membrane contact site (MCS) ultrastructure. **a:** The intermembrane distances vary and can influence or be influenced by the density of MCS proteins [19]. **b:** Membrane curvature within the MCS might play a role in accumulation and segregation of protein components, in facilitating lipid transfer function, or initiation of MCS formation [19,23]. **c:** The surface area of a particular MCS type can depend on the metabolic state of the cell [30].

Membrane curvature

Does the curvature of organelle membranes differ within and outside the MCS, and if yes, why? This question is particularly interesting for the ER, which displays high plasticity in shape and is involved in MCS with almost all other organelles [21]. Accordingly, both planar and tubular ER were observed contacting mitochondria in human cells [22]. Conversely, proteins resident at yeast ER-plasma membrane MCS have different ER membrane curvature preferences. For instance, the extended synaptotagmin (E-Syt) orthologues tricalbins preferably localise to high-curvature ER due to their reticulon-like transmembrane domain [13]. Furthermore, the occurrence of very high-curvature membrane peaks decreases upon lack of tricalbins [14]. High membrane curvature favours extraction or insertion of lipid molecules from or into the bilayer [23]. Consistent with this, high curvature is associated with regulation of lipid transfer by E-Syts *in vitro* [24]. Interestingly, the LTP complex formed by the yeast ER-mitochondria encounter structure (ERMES) [25, 26] localises to ER of highly diverse curvature [27], but the relevance of this observation is unclear. Perhaps curvature relates to the efficiency of ERMES: tubular ER could associate with active ERMES, while planar ER regions might contain less active ERMES. On the other hand, perhaps extraction and insertion of lipid molecules by ERMES do not depend on membrane curvature. Instead, curvature could introduce membrane asymmetry to influence which lipid species are available to ERMES. Furthermore, membrane curvature could dictate the distribution of biosynthetic enzymes [28], such as their accumulation near sites of lipid transfer.

Could membrane shape be an initiator of MCS formation? In mammalian ER-mitochondria MCS, local negative curvature “entraps” the VAP-A homologue VAPB within the MCS by inducing a slowdown of its diffusion [29]. Curvature-based sorting of proteins [28] might facilitate accumulation of tethering proteins to induce MCS formation, but this is a speculation at present (Figure 1b). At least in the case of the highly variable ER curvature associated with ERMES, if MCS formation was curvature-induced, reshaping after formation would have to be possible. Whether such dynamic rearrangements occur is unknown.

An interesting aspect is the interplay between curvature and intermembrane distance. VAP-A molecules in high-curvature proteoliposomes form MCSs with few interacting molecules, resulting in low VAP-A density at the MCS and consequently a shorter intermembrane distance than when the membrane is flat and VAP-A density high [19].

Membrane surface area engaged in the MCS

The extent to which different organelles engage in contacts is likely important for the physiological impact of MCS functions. There are large variations in relative and absolute contact area depending on the investigated cell type, organelle, intactness of the cytoskeleton, as well as nutritional state [22,30] (Figure 1c). Recent technical advances allow quantitative, systematic assessments of contact areas [12,22] (Table 1), although they can be difficult to directly compare. The total surface area of ER-mitochondria MCS in human cells was estimated by focused ion beam-scanning electron microscopy imaging as 80-120 μm^2 [22]. By cryo-ET, the surface area in contact with the ER per imaged mitochondrion was 0.02-0.4 μm^2 [12], but this estimate does not pertain to whole mitochondria, because in this case cryo-ET visualised sections through cells which rarely contain complete mitochondria. Similarly, in cryo-ET of yeast cells, the median surface area of individual ER-mitochondria MCSs was approximately 0.02 μm^2, with large variability [27]. But is individual MCS surface area a relevant measure? Are MCSs discrete entities at all, or are they dynamically rearranging, interconnected structures? The latter might be true for dynamic ER-mitochondria MCS networks in mammalian cells [29].

A peculiar case of changing contact area are interfaces between lipid droplets (LDs), which serve the transfer of neutral lipids from one LD core to the other [31]. Strictly speaking, these unique homotypic contacts are not MCSs as they occur between two monolayers. The intermonolayer distance is shorter than in other MCSs [32]. Remarkably, the lipid transfer process proceeds until the smaller of the two LDs is entirely consumed [33]. As the process completes, the size of the interface area must be

Table 1

Emerging technologies for new insights into the membrane contact site architecture.

Method	Typical sample	Description	What new aspects of MCS or lipid transfer can be studied?	Example results in the literature
Focused ion beam–scanning electron microscopy (FIB-SEM)	Resin-embedded cells.	3D imaging of whole cellular volumes at isotropic voxel sizes of <5 nm. The resulting volumes can be segmented, and quantitative parameters can be extracted.	Ultrastructure of MCS on whole cell level, and quantitative comparisons thereof between cell types or experimental conditions.	MCS surface area and curvature of the ER at the MCS with mitochondria compared between different human cells [22].
Electron cryo-tomography (cryo-ET), optionally combined with cryo-fluorescence microscopy (cryo-FM) into correlative approaches	Vitrified cells expressing proteins of interest, optionally with fluorescent label for identification.	High-resolution imaging of cellular structures preserved in a near-native state. Can be label-free.	Structural visualisation of protein-membrane assemblies in their native cellular environment.	Architecture of yeast ERMES complex [27] and of human VPS13C [52].
Single-particle tracking-photoactivation localization microscopy (sptPALM)	Living cells expressing fluorescently labelled component of interest.	Speed and trajectories of the movements of single molecules within their native environment.	The dynamic behaviour of individual proteins at the MCS, quantification of their lateral movement, and correlation with ultrastructure.	The dynamic movement of human VAPB which changes upon a disease-relevant mutation [29].
Integrative computational modelling	Low-resolution structures, amino acid sequences.	Structure prediction by AlphaFold [59] or RoseTTaFold [60] combined with MD simulations and MD-based flexible fitting.	Interpretation of low-resolution structures of LTP complexes, e.g., those obtained by cryo-ET. Investigating protein–lipid interactions. See also[61] in this issue.	Understanding complex LTP assemblies such as ERMES and VPS13 [27,51,52].

MD: molecular dynamics; 3D: three-dimensional; MCS: membrane contact site; ER: endoplasmic reticulum; LTP: lipid-transfer protein; ERMES: endoplasmic reticulum–mitochondria encounter structure

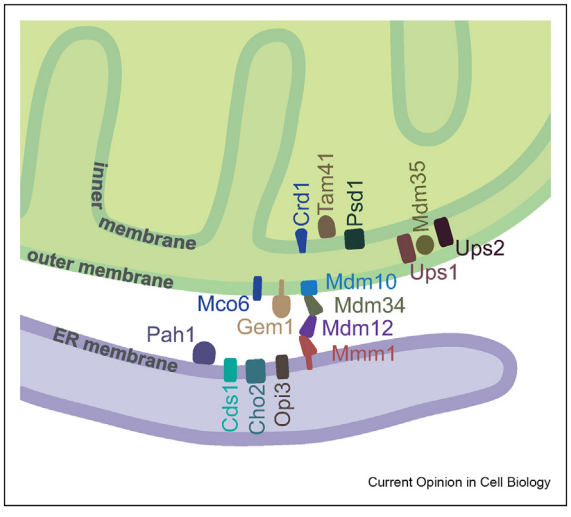
decreasing until it disappears [32]. Thus, part of the contact site function is its own remodelling.

Distribution and dynamics of proteins at the MCS

What factors regulate protein distribution and dynamics within the MCS? An emerging concept is that intrinsically disordered regions (IDRs) play a role in the dispersal and movement of MCS proteins. For instance, IDRs contribute to the scattering of OSBP molecules within the MCS by increasing their hydrodynamic radius [34]. Furthermore, the IDRs of VAP-A are decisive for its localisation to either short-lived ER-Golgi or stable ER-mitochondria MCS [35].

What is the density of LTPs per MCS membrane area, and how is it regulated? Such information would help estimating local efficiency of LTPs and global cellular lipid fluxes. Work on reconstituted VAP-A shows that protein density can have an impact on intermembrane distance [19] and thereby potentially on the efficiency of transfer reactions. For ERMES *in situ*, there are on average 7 LTP complexes per 10,000 nm^2 of membrane, with an upper limit of 12 [27]. At this concentration, ERMES is not overcrowded, raising the question of what constrains the density of particular MCS proteins. A possibility is that the accumulation of other functional partners, for example, lipid biosynthetic enzymes or regulatory proteins [36,37], on either of the interacting organelles generates crowding within the MCS (Figure 2).

Figure 2



Potential protein composition of a paradigm MCS. The yeast endoplasmic reticulum-mitochondria encounter structure (ERMES) is present at a density of no more than 12 bridge structures per 10,000 nm^2 surface area. Could the presence of other protein components, in particular lipid biosynthetic enzymes, limit the space available for ERMES bridges? Here, some of the potential coinhabitants of ERMES membrane contact site are depicted at their annotated locations [25,36,37,57,58].

Another largely open question is how static or dynamic the localisation of MCS proteins within the contact area is. Single-molecule tracking showed high mobility of VAPB molecules into and out of the ER-mitochondria MCS [29] (Table 1). Within an MCS, VAPB slowed down [29]. The behaviour of VAPs might however not be representative for all MCS proteins. VAPs are versatile interaction partners of LTPs at many types of MCSs, without themselves associating with both membranes [18]. Therefore, VAPs might require particular versatility and dynamic behaviour.

Initiation and positioning of new MCSs are open topics as well. Are MCSs initiated by an organised machinery as for lysosomal repair [2]? Do MCSs form at predefined places? Can MCSs result from stochastic organelle collisions? The movement of organelles due to their cytoskeleton attachment or due to cytoplasmic streaming could lead to collisions and induce a haphazard proximity between membranes. As a result, the spatial arrangement of proteins could change, possibly leading to formation or reorganisation of MCSs.

Molecular structures

How do MCS proteins arrange between two membranes? What is the molecular structure of MCS proteins, LTPs, and adaptors when they assemble into functional units? Which LTPs form shuttles, which form continuous lipid conduits? Do interactors affect LTP conformations?

Small lipid-binding proteins

Available structures of soluble LTPs often contain lipid binding sites in specific domains. Examples are OSBP-like proteins [38–41], steroidogenic acute regulatory transfer (StART)-like domains [42], and synaptotagmin-like mitochondrial-lipid-binding protein (SMP) domains [43–47]. While these structures reveal the chemical environment for harbouring lipid molecules within the proteins, how LTPs arrange in the MCS remained elusive until recently. For OSBP-like proteins, it is widely assumed that they shuttle between membranes through diffusion while being anchored in the MCS via long linkers and interaction partners such as VAPs. Recent reconstitution experiments indicate that also the interacting VAP-A displays flexibility, possibly contributing to the shuttling function [19].

There are also insights on how the SMP domain containing LTPs arrange between membranes. The E-Syts/tricalbins dimerise via their SMP domains [47], oriented roughly perpendicularly to the membranes with which they interact via their charged tips [13,24]. As the intermembrane distance is not entirely bridged by the SMP dimer [13], E-Syts might shuttle between the membranes in a perpendicular manner [20,24],

possibly enabled by long linkers. In contrast to E-Syts, ERMES contains three SMP domain proteins that bridge the entire intermembrane space, likely forming a continuous tunnel between membranes [27]. An elegant assay showed that ERMES indeed transfers phospholipids *in vivo* [26]. Is this lipid conduit regulated? Are the interfaces between subunits, where the tunnel is most constrained [27], points of regulation or selectivity? What role do interactors such as the GTPase Gem1 [37] or Emr1/Mco6 [36] play in regulating ERMES lipid transfer and organisation?

Large bulk lipid-binding proteins

While ERMES is formed of multiple subunits, members of the Vps13 family form lipid tunnels as single protein molecules and likely mediate bulk lipid transfer [48]. These proteins, occurring at various MCSs, are very large [49]. Initial structural data on fragments suggested a lipid slide on a large hydrophobic groove [50]. The advent of structure prediction allowed to model full yeast Vps13 [51] and to interpret a low-resolution cryo-ET *in situ* structure of mammalian VPS13C, which revealed that VPS13C spans 30 nm between two membranes [52] (Table 1). Similar structures are predicted for other Vps13-like proteins, including Fmp27, Csf1, and Atg2 [,48,53,54]. How is Vps13-mediated bulk transfer regulated? Is it always "on"? Would that not be detrimental to organelle identity? A potential clue is the various interaction partners of Vps13 on different membranes, which might play regulatory roles [55]. In yeast, the mitochondrial protein Mcp1 interfaces with Vps13 [51,55,56] and could regulate Vps13-based lipid transfer by providing lipid substrate through its scrambling activity [26,51]. In fact, in the system that allowed visualisation of overexpressed VPS13C *in situ*, such interactions might potentially explain an observed discontinuity between the ER membrane and rod-shaped VPS13C [52].

In summary, it emerges that many MCS proteins, including LTPs, form functional heterooligomeric complexes anchored within MCSs. High-resolution structures of these complexes and their relation to the membranes are needed to understand mechanisms of lipid extraction, insertion, transport, regulation, and specificity.

Conclusions

Recent advances indicate that investigating the spatial organisation within MCSs bears immense potential for mechanistic insights. We are only beginning to understand how the molecular architecture of MCSs might contribute to functions such as lipid transfer. Moreover, many related questions await answers. For example, how is the formation of an MCS initiated? Which architectural parameters must be stringently maintained for MCS function? How are the dynamics of MCS components regulated, and how do they in turn regulate processes at MCSs? Recent technical development in molecular-imaging methods, combined with computational, biochemical, and cell biological investigations, promise an exciting era in MCS research.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data were used for the research described in the article.

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