

1 The Endoplasmic Reticulum–Mitochondria 2 Encounter Structure

3 Coordinating lipid metabolism across membranes

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Abstract

Endosymbiosis, the beginning of a collaboration between an Archaeon and a Bacterium and founding step in the evolution of Eukaryotes, owes its success to the establishment of communication routes between the host and the symbiont to allow the exchange of metabolites. As far as lipids are concerned, it is the host that has learnt the symbiont's language, as eukaryote lipids appear to have been borrowed from the bacterial symbiont. Mitochondria exchange lipids with the rest of the cell at membrane contact sites. In fungi, the Endoplasmic Reticulum-Mitochondria Encounter Structure (ERMES) is one of the best understood membrane tethering complexes. Its discovery has yielded crucial insight into the mechanisms of intracellular lipid trafficking. Despite a wealth of data, our understanding of ERMES formation and its exact role(s) remains incomplete. Here, I endeavour to summarize our knowledge on the ERMES complex and to identify lingering gaps.

Introduction: the evolutionary origin of lipid exchange

One hallmark of the eukaryotic cell is the partitioning of biochemistry into separate organelles. While membrane-less organelles have recently received a lot of attention, it is membrane-bound organelles that still occupy centre stage as means to tailor the environment for specialized and sometimes incompatible biochemical reactions. The evolutionary origin of membrane-bound organelles is diverse and fascinating. While the evidence for the endosymbiotic origin of genome-containing organelles, like mitochondria and chloroplasts, is overwhelming (Gray and Doolittle, 1982; Sagan, 1967), how a simple archaeal cell could evolve a complex endomembrane is a matter of conjecture. Among several hypotheses (Martin et al., 2015), an interesting one, termed the “inside-out origin of the eukaryotes” posits that the plasma membrane (PM) and endomembrane structures have evolved from protrusions of the original archaeal cell (Figure 1A, top), which sealed off, defining a cytoplasm; the original cell itself (Figure 1A, bottom) topologically giving rise to the nucleus (Baum and Baum, 2014). Such a model explains how vesicular transport may have evolved stepwise from open channels connecting the nuclear envelope to the external milieu; by progressive closing of these channels until they became vesicular transport routes. This theory also explains how endosymbionts might have been trapped within the protrusions, giving rise to today's mitochondria and chloroplasts. The question of the origin of organelles might soon

be tremendously advanced by the discovery of Asgard archaea, “missing links” between archaea and eukarya (Imachi et al., 2020; Zaremba-Niedzwiedzka et al., 2017). The only modern Asgard archaeon isolated to date morphologically resemble the archaeon in Figure 1A (top), and Asgard genomes bears signatures of eukaryotic-specific membrane remodelling genes, such as proto-coatomers and proto-ESCRT complexes. It will thus be enlightening to figure their function in Asgard archaea, and imagine evolutionary scenarios.

A potentially more abrupt stage in eukaryogenesis was achieved when eukaryotes hijacked the bacterial lipid biosynthesis process and abandoned the archaeal one (Lombard et al., 2012). Indeed, membrane lipids in archaea are different from bacteria and eukarya. While in the latter,

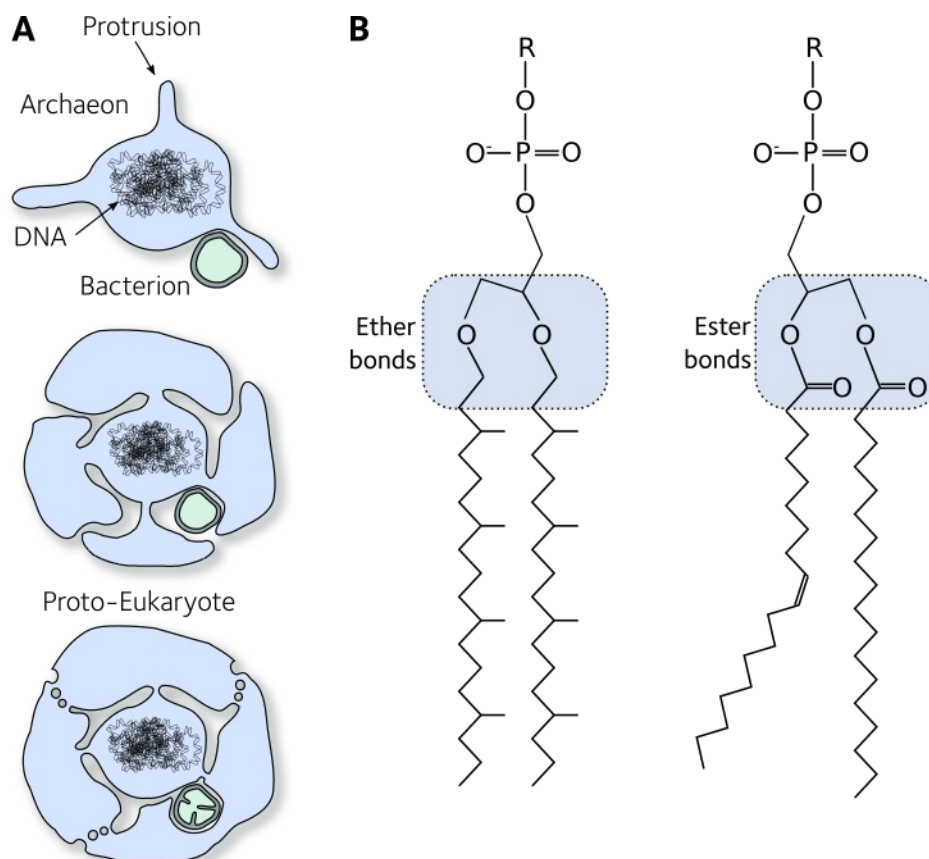


Figure 1: A) the “inside-out” theory of eukaryotic evolution. An archeon extends protrusions. These protrusions extend and engulf bacteria symbionts (green, future mitochondria). A proto-eukaryotic cell is formed by sealing off communication between the proto-nuclear envelope and the proto-plasma-membrane. Proto-mitochondria must still escape into the cytoplasm. B) Comparison of ether-based archaeal membrane lipids (left) and ester-based lipids that form the majority of lipids in eukaryotic and bacterial membranes.

lipids are mostly constituted of linear fatty acids esterified to a glycerol backbone, they are, in the former, made of branched fatty alcohols linked to glycerol by an ether bond. This means that a switch in lipid composition must have happened, likely by the incorporation of bacterial phospholipids from proto-mitochondria into the proto-eukaryotic host membrane. This suggests that, for a very long time, endosymbiotic organelles and their hosts have been exchanging

metabolites, in particular lipid molecules, from one membrane to another and for their mutual benefit.

The discovery of contact sites

For decades, membranes have been considered as separate entities, secluded islands that are only connected by shuttles, such as trafficking vesicles. The first evidence that the biochemistry of two membranes could be coupled comes from the pioneering work of Walter Neupert showing that translocating mitochondrial proteins could simultaneously traverse the outer- and inner-mitochondrial membranes (Schleyer and Neupert, 1985). *In vitro* translated proteins containing mitochondria targeting sequences (MTS) can be imported into isolated mitochondria. In the matrix the MTS is cleaved by the Matrix Processing Peptidase. If import is slowed or inhibited, intermediates can be observed where most of the protein is still outside mitochondria, but the MTS is cleaved, indicating that the import through the outer- and inner-membrane happens at once, at contacts sites between both membranes. This first evidence of functional contacts between two otherwise separate membranes opened the way to the idea that membranes are generally biochemically coupled. This idea provided a basis to start answering a lingering question in the field; where do mitochondria get their lipids from and how do they exchange them?

ER-mitochondria contacts

One consequence of the separation and specialization of membranes within eukaryotic cells was the necessity to evolve exchange routes between them. Most lipid molecules, which are the main building blocks of membranes, are made in the Endoplasmic Reticulum (ER) and are then distributed to other organelles and to the PM. Because the ER constantly exchanges vesicles with the Golgi to send proteins through the secretory and endocytic pathways, and because these vesicles have a lipid shell, it was long assumed that lipid exchange was a by-product of vesicular traffic. While this might have been true in proto-eukaryotic cells, it is unlikely to be the mechanism by which mitochondrial lipids have taken over eukaryotic life. In fact, mitochondria do not receive lipids nor anything else from vesicular transport, even though they have lost their ability to synthesize lipids. This non-reliance on vesicular transport appears to be the rule in the cell rather than a mitochondrial exception. In a seminal paper, Jean Vance presented an assay following the delivery of newly synthesized lipids to the PM (Vance et al., 1991). Radiolabeled ethanolamine is incorporated into phosphatidylethanolamine (PE) by an ER-localized enzyme. After transport to the PM, a membrane-impermeable compound is used to modify the pool of PE accessible on the outer leaflet of the PM; the amount of modified radiolabelled lipid can thus be used as a proxy for ER-to-

PM transport. These experiments show that vesicular transport inhibitors that inhibit protein secretion have no effect on ER-to-PM lipid transport.

If not vesicular transport, then what mechanism does allow lipid exchange between two membranes? One idea is that proteins might be able to extract lipids from one membrane and deliver it to another. A plethora of these Lipid Transport Proteins (LTPs) have now been discovered (Balla et al., 2019). They are characterized by the presence of a hydrophobic pocket that shelters lipid's hydrophobic moieties, thus allowing them to transit through an aqueous phase. Another idea is that lipids might exchange at sites where two organelles come into close proximity. Such sites have long been observed by electron microscopy (Robertson, 1960) and their involvement in lipid transport has first been suggested for mitochondria. During a classical mitochondria isolation procedure, a fraction of membrane must be separated from mitochondria by isopycnic centrifugation. These "mitochondria associated membranes" (MAMs) appear to be ER-derived but, compared to bulk ER, are enriched in lipid biosynthesis enzymes, leading to the idea that contacts between the ER and mitochondria serve as platforms to directly exchange lipids from one organelle to the other (Vance, 1990). Of course, the LTP and contact sites model are not mutually exclusive, and indeed many LTPs, if not most, function at contact sites.

The discovery of ERMES

To discover factors involved in tethering the ER to mitochondria, we took a genetics approach whereby we assumed that (a) protein complex(es) might be essential to attach the ER to mitochondria and promote lipid exchange. Using a clever design originally published by the Hajnocsky group (Csordás et al., 2006), we expressed an artificial ER-mitochondria tether in yeast cells. We then asked whether mutants of a presumed natural tether would survive, now that an artificial one was effectively there to do the tethering job. What we found was a known protein complex made of both membrane and soluble proteins (see below) (Kornmann et al., 2009). This protein complex, found widely in the eukaryotic lineage, but lost in plants and metazoans (Wideman et al., 2013), had been identified before because of its role in mitochondrial distribution and morphology (Berger et al., 1997; Boldogh et al., 2003; Burgess et al., 1994; Sogo and Yaffe, 1994). While wild-type yeast sport elongated, tubular and branched mitochondria, mutants of any component of the complex have swollen, ball-like organelles. This complex was then renamed ER-Mitochondria Encounter Structure (ERMES).

Architecture of the ERMES complex

How does such a complex tether two organelles? The core components of the complex are Mdm10, a β -barrel protein of the outer mitochondrial membrane (OMM) (Flinner et al., 2013; Sogo and Yaffe, 1994), Mdm12 and Mdm34, two cytosolic proteins (Berger et al., 1997; Ellenrieder et al., 2016) and Mmm1, a protein harbouring a clear transmembrane domain. However, because GFP-tagged Mmm1 could always be seen on mitochondrial membranes, it was assumed to be a mitochondrial protein. In fact, different lines of evidence situated Mmm1 at contact sites between the Outer and Inner Mitochondrial membranes, which, like translocating proteins, Mmm1 was thought to span (Kondo-Okamoto et al., 2003). We could show that Mmm1 was in fact inserted in the ER membrane, with its N-terminus modified by N-linked glycosylation, and only appeared on the mitochondria owing to its interaction with mitochondrial components of the complex (Kornmann et al., 2009; Stroud et al., 2011). Indeed, loss of any of the other ERMES components sent Mmm1 freely diffusing along ER membranes (Figure 2A, B).

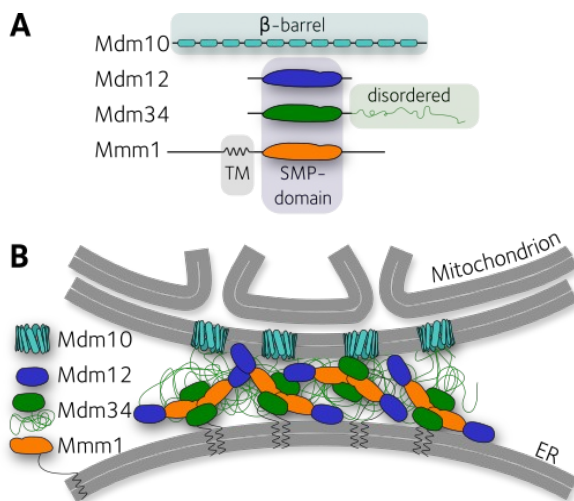
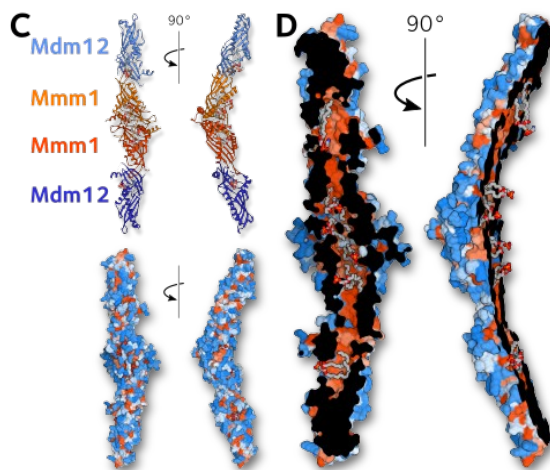


Figure 2: A) Domain structure of ERMES core components. B) Topology of components in the ERMES complex. C) Crystal structure of the Mmm1-Mdm12 heterodimer, viewed as ribbon (top) or as surface (bottom), where red and blue indicate hydrophobic and hydrophilic residues respectively. The crystal structure of the tetramer here is that published in (Jeong et al., 2017) (pdb: 5Yk7), however, the lipid molecules bound to Mmm1 are arranged as in the Mmm1 dimer (pdb: 5YK6) while these bound to Mdm12 are arranged as in (Kawano et al., 2018), (top Mdm12 subunit, pdb: 5H5A) and (Jeong et al., 2016), (bottom subunit, pdb: 5GYD), because lipids are not resolved in the tetramer structure. D) Slice through the Mmm1-Mdm12 heterotetramer exposing the lipid binding groove running along the length of the complex.



Therefore, the ERMES complex is made of both ER and mitochondrial proteins stably interacting at ER-mitochondria contact sites. What do these proteins look like? Mdm10, as said above displays all the sequence signatures of β -barrel membrane proteins (Figure 2A, B). Although its structure is unknown, primary sequence analysis suggests that Mdm10 is constituted of 19 β -strands, like the structurally characterized mitochondrial β -barrels VDAC and Tom40 (Flinner et al., 2013).

Mdm12, Mdm34 and Mmm1 share a common protein domain, the SMP domain (found in Synaptotagmin-like, Mitochondrial, and lipid-binding Proteins Figure 2A, B) (Lee and Hong, 2006), which is part of a larger family of protein domains that harbour a common Tubular Lipid-binding (tulip) domain (Kopeck et al., 2010), an elongated hydrophobic cavity that binds membrane lipids by their hydrophobic tails and shelter them from the aqueous environment. The SMP domain constitutes almost all of Mdm12, and almost all of the cytosolic moiety of Mmm1 (while the ER-luminal moiety is quite long in *S. cerevisiae*, it is reduced to a few amino acids in many other fungi, suggesting that it might not be central for function). Mdm34's SMP domain is at its N-terminus while the C-terminal part of the protein appears to be a long unfolded domain, which is important for function (Michel et al., 2017) (Figure 2A, B).

ERMES complexes assemble into large supercomplexes, visualized as 1-5 foci per yeast cell in standard conditions, each foci containing a complement of ~50-100 copies of each of the four core ERMES components as well as substoichiometric levels of regulatory components, such as Gem1 and Tom7 (Kornmann et al., 2011; Stroud et al., 2011; Yamano et al., 2010). It is entirely unknown how ERMES core four subunits assemble into supercomplexes. One possibility is that, because ERMES complexes can only localize to pre-existing ER-mitochondria contact sites, they may tend to assemble in the vicinity of pre-existing ERMES complexes and may not be able to diffuse away. Another possibility is that ERMES complexes interact *in trans* to form larger complexes. This possibility is reinforced by the fact that other ER-mitochondria contacts that lack ERMES foci can be found (Elbaz-Alon et al., 2015; Murley et al., 2015), indicating that ERMES-independent contacts exist, and that the formation of more foci is not purely limited by the availability of contact sites. One possibility is that the disordered region in Mdm34 provides a platform to promote such an interaction *in trans*.

The elusive function of ERMES

Crystal structures of Mmm1 and Mdm12 SMP domains in isolation and together indicate that these proteins assemble as elongated banana-shaped complexes (AhYoung et al., 2015; Jeong et al., 2017). ERMES components Mmm1 and Mdm12 bind lipids within their hydrophobic groove, and

can transport them from one liposome to another *in vitro* (AhYoung et al., 2017; Jeong et al., 2016, 2017; Kawano et al., 2018) (Figure 2C). The polar head of the lipids make little contact with the protein, and indeed ERMES components bind phospholipids with little specificity (AhYoung et al., 2015; Kawano et al., 2018). These lipid-transporting and membrane-tethering activities of the ERMES complex make it an ideal candidate for importing ER lipid into mitochondria.

However, this simple model is not entirely consistent with the following observations. First, the complex was identified from the fact that its phenotype could be rescued by an artificial protein, which tethered the ER to mitochondria but did not bear lipid transport activity (Kornmann et al., 2009). The extent of this rescue is largely dependent on which ERMES component has been inactivated. The rescue works best for mutants of *MDM12* and *MDM34* and double mutants thereof, and poorly for mutants of *MDM10* and *MMM1*. That Mdm12 and Mdm34 can be replaced by an artificial tether indicates either that ERMES's lipid transport function is not important for cell growth (e.g. can be carried out by some other LTP), or that the remaining lipid-transport domain in Mmm1 is able to perform all of the lipid transport activity by itself, provided that the ER and the mitochondria are firmly attached together. Second, the rate of lipid transport by Mdm12-Mmm1 dimers *in vitro* is in the order of 3 molecule per minute per protein (Kawano et al., 2018). Counting 500 ERMES complexes per cell (Ho et al., 2018), it would require about two months to double the size of the mitochondrial membranes, hardly compatible with the ~2h doubling time of yeast (Petrungaro and Kornmann, 2019). This can only be reconciled with a significant role of ERMES in lipid transport, if the *in vitro* lipid exchange rates are off several by orders of magnitude (see perspective). Three, the absence of functional ERMES does not prevent lipids from reaching mitochondria. Indeed, mutants of ERMES components are viable, and while they grow slowly and display aberrantly shaped mitochondria, these mitochondria still have lipids in their membranes (Burgess et al., 1994; Kornmann et al., 2009).

ERMES bypass, suppressors and compensatory mechanisms

Because ERMES mutant mitochondria still receive lipids made in the ER, several groups launched genetic approaches to identify potential redundant pathways. The Dimmer/Rapaport and Tamura/Endo groups took a gain-of-function approach to identify genes which could suppress the phenotypes of ERMES mutants when overexpressed (Kojima et al., 2016; Tan et al., 2013). They identified a handful of factors, one of which – Mcp1 – is on the outer mitochondrial membrane, and hence well situated to be part of a redundant exchange pathway with the endomembrane. The Schuldiner lab screened for mutants that affect the number and extant or ERMES foci. They identified a plethora of factors affecting ERMES number and many of them showing synthetic

lethality with ERMES mutants (Elbaz-Alon et al., 2014). Interestingly, most of these genes encoded endosomal/vacuolar components. The Schuldiner and Ungermann groups showed that the overexpression of Vps39, one of the factor with strongest genetic interaction with ERMES, led to the formation of large patches of contact between the mitochondria and the vacuole (Elbaz-Alon et al., 2014; Hönscher et al., 2014). The Ungermann group subsequently showed that this was due to the interaction of Vps39 on the vacuole with Tom40 on the mitochondrial membrane (González Montoro et al., 2018). Thus, an interesting idea was that these vacuole-And-Mitochondria Patches (vCLAMPs) could serve as a second exchange platform to provide lipids to mitochondria. This idea was however soon disproved by the Ungermann group who showed that mutants of Vps39 that could not bind to Tom40 (and hence could not form vCLAMPs), were not synthetic lethal with ERMES mutants (González Montoro et al., 2018). Instead, it was shown that the synthetic lethality was associated with another known function of Vps39 within the HOPS complex, that promotes endosome and vacuole fusion. Thus, it remains to clarify what the function of vCLAMPs is and why inhibiting endosome and vacuole fusion is synthetic lethal with ERMES. An answer to the second question may possibly come from the discovery of Vps13's involvement with Mcp1 in an ERMES bypass pathway. Both the Neiman group and our lab have discovered that dominant point mutations in the endosomal protein Vps13 rescued ERMES mutant phenotype (Lang et al., 2015; Park et al., 2016). By contrast, *VPS13* deletion is synthetic lethal with ERMES. Vps13 partitions between various organelle contact sites by binding to different organelle-specific adaptors, which interact with Vps13 in a competitive fashion (Bean et al., 2018). One of these adaptors is the mitochondrial Mcp1. Mcp1-Vps13 interaction allows mitochondria to contact the vacuole and endosomes (John Peter et al., 2017). Mcp1 overexpression exaggerates the recruitment of Vps13 to mitochondria, and presumably the contacts they make with endosomes and vacuoles. A recent crystal structure of a Vps13 fragment shows that Vps13, like ERMES components, harbours a lipid-transport domain, which shelters lipid molecules in a hydrophobic cavity, without much headgroup specificity (Kumar et al., 2018). Unlike SMP domains however, the hydrophobic cavity in Vps13 is large enough to accommodate several lipid molecules (Figure 3, right). The configuration of lipids within Vps13 pocket is unknown but can be approximated by that of PE within the pocket of a structural homolog – Atg2, which is likely to provide lipids to the growing autophagosome membrane (Figure 3, left and middle) (Osawa et al., 2019; Valverde et al., 2019). Note that the structures of both Atg2 and Vps13 are of small fragments of two very large proteins. EM analysis of full length Atg2 and Vps13 suggest more binding domains (Chowdhury et al., 2018; De et al., 2017; Otomo et al., 2018; Valverde et al., 2019; Zheng et al., 2017). Thus, Vps13, like ERMES, is able to exchange lipids between mitochondria and other organelles at membrane contact sites.

In conclusion, it appears likely that ERMES and the Vps13-Mcp1 complex fulfil a partially redundant role in providing lipids to mitochondria, explaining why mutants of ERMES components are viable and harbour lipid-containing mitochondria.

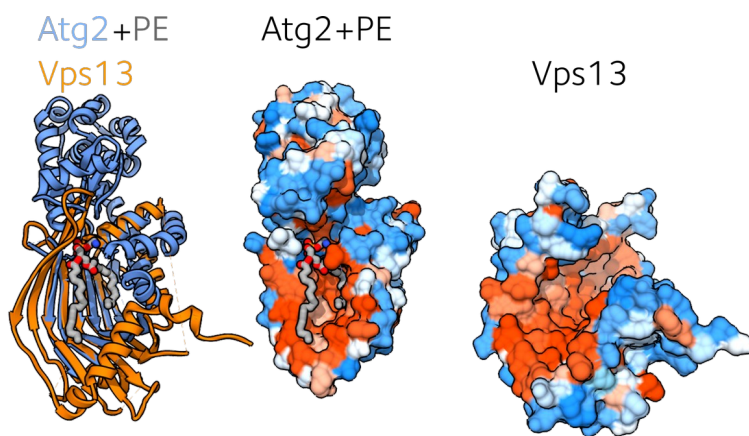


Figure 3: Structure of the N-termini of Vps13 and Atg2. Left, structural comparison of Atg2 (with bound PE, blue, from (Osawa et al., 2019), pdb: 6A9J) and Vps13 (orange, from (Kumar et al., 2018), pdb: 6CBC). Middle and left, surface representation of both proteins with hydrophobic residues coloured in red and hydrophilic coloured in blue.

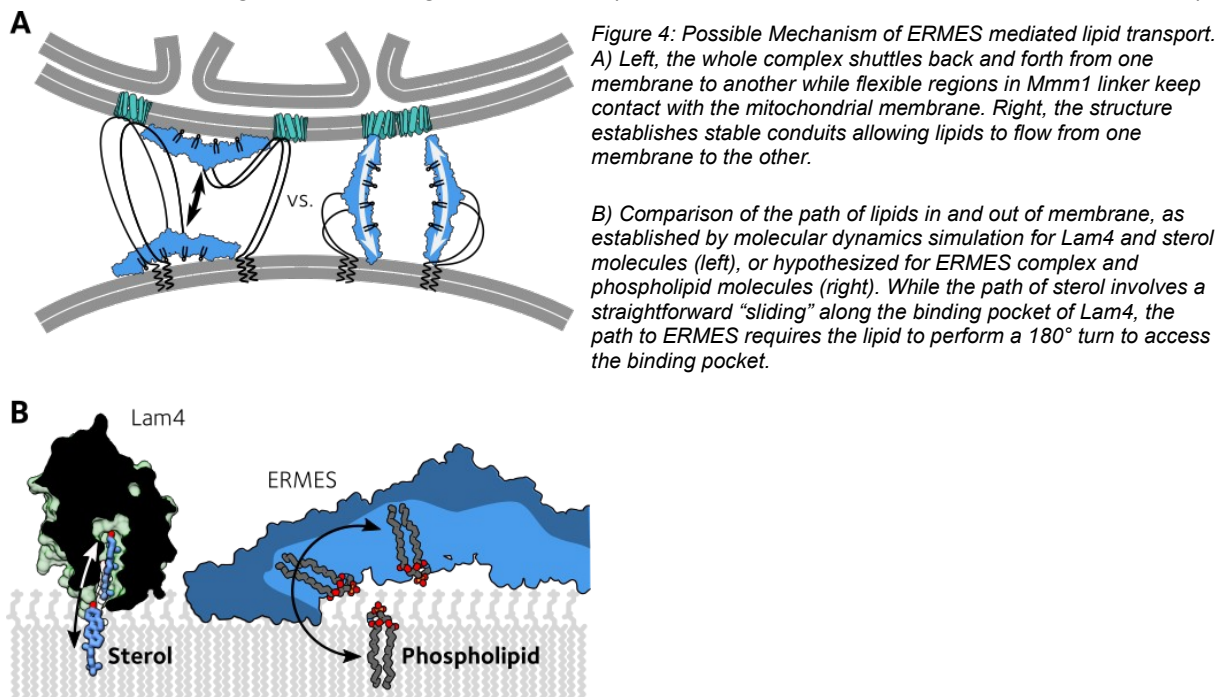
An explosion of contacts and lipid transport routes

The discovery of the ERMES complex has opened the way to the characterization of several other organelle tethering complexes. In addition to vCLAMPs (the role of which still needs to be clarified), and Vps13-Mcp1, the analysis of other proteins harbouring SMP domains revealed that all of them were present at contact sites (Toulmay and Prinz, 2012). The family of the extended-synaptotagmin (or tricalbins) are anchored in the ER by transmembrane domains and contact the PM by calcium-regulated C₂ domains. Interestingly, like in the case of Mmm1, these proteins were originally thought to be inserted in the PM, because they constantly colocalized with it (Creutz et al., 2004). Extended synaptotagmins serve an important role to bring diacylglycerol formed by the hydrolysis of PIP₂ back to the ER (Saheki et al., 2016). Their role in yeast is less clear.

The protein Nvj2 is traversing the ER membrane, and contacts the vacuole and the Golgi via a PH domain (Toulmay and Prinz, 2012). Nvj2 might transfer ceramide molecules between these membranes (Liu et al., 2017).

Finally, one more SMP-domain containing protein can be found in metazoan genomes but is absent in fungi (Lee and Hong, 2006; Wideman et al., 2018). PDZD8 inactivation causes a drastic reduction in ER-mitochondria contact, as assessed by 3D Electron microscopy, suggesting it could be an important tether between these two organelles (Hirabayashi et al., 2017). However, GFP-tagged PDZD8 accumulates at ER-endosome contacts and the C-terminus of the protein interacts with Rab7 at the endosome (Guillén-Samander et al., 2019). Clearly, more data are necessary to understand the precise location and function of this protein.

In parallel, structural homology searches using Vps13 as a bait, identified Atg2 as a homolog and indeed, further work shows that Atg2 is found to exchange lipids at contact sites between the ER and the autophagosome isolation membrane (Osawa et al., 2019; Otomo et al., 2018; Valverde et al., 2019). Thus, quite indirectly, the discovery of ERMES might help solving the long standing question of the origin of autophagosome lipids (Mari et al., 2011; Tooze and Yoshimori, 2010).



Perspective

Several burning questions remain regarding the ERMES complex. What is its contribution to lipid delivery to mitochondria? This question requires technical advances to be able to measure lipid fluxes in and out of mitochondria and the influence ERMES defects have on them. Thus far, the classical assay was to rely on the presence of the phosphatidylserine (PS) synthase in the ER and of the PS decarboxylase in the mitochondria (Daum and Vance, 1997). Labelling with radioactive serine and monitoring the decarboxylation of PS was used as a proxy for assaying the efficiency of ER-mitochondria lipid exchange. With this assay, ERMES mutation had a small but detectable effect on decarboxylation kinetics (Kornmann et al., 2009). However, it has since then been found that a fraction of the PS decarboxylase is present and active on the ER (Friedman et al., 2018). This assay might therefore not be suited to monitor ER-mitochondria lipid exchange.

What is the mechanism of ERMES-mediated interorganelle lipid exchange? EM and crystal structures of Mmm1 and Mdm12 SMP domains show that they form long tubular structures (AhYoung et al., 2015; Jeong et al., 2017). How do they transport lipid? Is it by shuttling their SMP

domains from one membrane to another or is it by creating a conduit between the two membranes (Figure 4A)? The Mmm1-Mdm12 heterotetramer spans 20nm and could thus be sufficient to bridge the cytosolic gap at ER-mitochondria contact sites. While no evidence exists for ERMES, overexpression of a single extended synaptotagmin in yeast leads to the appearance of elongated electron-dense structures of ~20nm at ER-PM contacts (Hoffmann et al., 2019). Unlike ERMES proteins, extended synaptotagmin only dimerize their SMP domains, forming a tube of only ~10 nm, thus insufficient to explain the span of the electron density. Yet, it is tempting to speculate that these elongated structures might serve as hydrophobic tunnels rather than shuttles. Perhaps, Calcium-regulated tightening of the contact sites might connect the two openings of the tunnel to the ER and plasma membranes.

An equally important and related question is how lipids are desorbed from their membranes. While crystal structures of ERMES components present a snapshot in which a lipid snugly fits into a hydrophobic pocket, we have no idea how it got there. This question is lingering for virtually all lipid transport proteins. Only recently, a possible path from a membrane into a lipid binding pocket was identified by molecular dynamics simulation of the sterol transporter Lam4 (Khelashvili et al., 2019). However, desorption of sterols from membranes is energetically less hindered than that of glycerophospholipids (Jones and Thompson, 1990). Moreover, while the path of sterols towards Lam4 binding pocket is relatively straightforward, involving just “sliding” of the sterol along the lipid-binding pocket (Figure 4B, left), binding of a glycerophospholipid into an SMP binding pocket requires a ~180° rotation of the lipid, so that the lipid head remains at the mouth of the pocket, while the extracted acyl chains bury themselves into the pocket (AhYoung et al., 2017; Jeong et al., 2016, 2017; Kawano et al., 2018; Schauder et al., 2014)(Figure 4B, right). How this manoeuvre is executed is a complete mystery, although it likely holds the key to effective lipid transport. Indeed, *in vitro* work indicates that desorption is the limiting step in lipid exchange (Dittman and Menon, 2017). It is therefore highly likely that the poor transport rates observed for ERMES *in vitro* are due to defective desorption. Is it possible that cellular factors, absent from *in vitro* reconstitutions, catalyse lipid desorption? The role of Mdm10, the only non-SMP core ERMES component, might need better scrutiny. Thus far, Mdm10 has been assumed to be a passive tether recruiting the rest of the ERMES complex to mitochondria, maybe orienting the SMP domains of other proteins. It is possible however that it plays a more important role in lipid desorption. Akin to Tom40, Mdm10 is a β -barrel protein. The recent cryoEM structures of the TOM complex show that β -barrel proteins can bind phospholipids and bend the membrane (Araiso et al., 2019; Bausewein et al., 2017; Tucker and Park, 2019), both might facilitate lipid desorption and subsequent handover to a cytosolic lipid transport protein. Of note, other SMP-domain proteins – the extended synaptotagmins – were shown to bend the ER membrane, which might be important for their role in ER-PM lipid exchange

(Collado et al., 2019; Hoffmann et al., 2019). Such a role might also be played by Mcp1 in the Mcp1-Vps13 complex. Mcp1 is integral to the Outer Mitochondrial Membrane and resembles a bacterial pyruvate dehydrogenase subunit. Mcp1 sports two heme-binding sites within its trans-membrane segments. Mutating them does not alter Mcp1 localization, nor its ability to recruit Vps13 to mitochondria, but almost abrogates the ability of Mcp1 to function as ERMES suppressor (John Peter et al., 2017). Thus, Mcp1 is not a mere Vps13 recruiter. Intramembrane portions of the protein perform important functions, perhaps participating in lipid loading in Vps13 pocket. Incorporating such potential lipid desorbers in *in vitro* experiments might prove difficult but may be key to reach exchange rates compatible with the demand of cells.

Is ERMES performing more than lipid exchange? For instance, in several distant fungi, components of ERMES appear to moonlight in a complex involved in protein import (Meisinger et al., 2004), a clearly separable function (Ellenrieder et al., 2016). What is the role of such a connection between lipid and protein import and why has it been conserved for hundreds of millions of years (Wideman et al., 2010)?

A last burning question is the conservation of ERMES and of its function in other clades. Paradoxically, while ERMES components are seemingly the most important SMP-domain containing proteins in yeast (i.e. the mutants of which have the most deleterious phenotypes), they are also the least conserved, being lost in several lineages including plants and metazoans. It has been proposed that PDZD8 might be a constituent of a mammalian ERMES (Hirabayashi et al., 2017). Yet, both the sequence conservation (Wideman et al., 2018), and the reported localization of PDZD8 at ER-endosome contacts (Guillén-Samander et al., 2019) argue against such an idea.

In conclusion, the discovery of the ERMES complex paves the way to understand the mechanisms by which hydrophobic lipids can cross an aqueous phase to shuttle between membranes. While much still needs to be done, it is an exciting time for the field. Many players have been identified, providing the basis to understand the biology of contact sites with respect to their role in lipid transport, their redundancy, and their other potential roles.

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