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Organelle remodeling at membrane contact sites

W. Mike Henne

Department of Cell Biology, UT Southwestern Medical Center, 6000 Harry Hines Blvd, Dallas, TX 75390, USA

Abstract

Cellular organelles must execute sophisticated biological processes to persist, and often communicate with one another to exchange metabolites and information. Recent studies suggest inter-organelle membrane contact sites (MCSs) are hubs for this cellular cross-talk. MCSs also govern membrane remodeling, thus controlling aspects of organelle shape, identity, and function. Here, we summarize three emerging phenomena that MCSs appear to govern: 1) organelle identity via the non-vesicular exchange of lipids, 2) mitochondrial shape and division, and 3) endosomal migration in response to sterol trafficking. We also discuss the role for ER-endolysosomal contact sites in cholesterol metabolism, and the potential biomedical importance this holds. Indeed, the emerging field inter-organelle cross-talk promises substantial advances in the fields of lipid metabolism and cell signaling.

Keywords

Membrane contact site (MCS); Plasma membrane (PM); Endoplasmic Reticulum (ER); Phospholipid; Endosome; Mitochondria

1. Introduction

Eukaryotic cells harbor numerous membrane bound organelles, and must cope with significant spatial constraints to properly contain and compartmentalize organelles efficiently. As a result, organelles are often in very close proximity. Even in early electron micrographs from Keith Porter and George Palade, the ability of organelles like the Endoplasmic Reticulum (ER) to physically touch mitochondria or the plasma membrane (PM) was noted, but these phenomena were generally ignored (Porter and Palade, 1957; Porter and Machado, 1960). More recently, elegant microscopy, genetic, and biochemical studies have revealed numerous proteins and protein complexes specifically tasked with tethering organelles together. Moreover, these proteins function in numerous cellular pathways, including lipid synthesis and trafficking, Ca^{2+} signaling, and organelle movement and identity. These functions are also intimately connected to organelle shape and function.

The purpose of this short review is to highlight recent findings that illustrate the importance of membrane contact sites on organelle reshaping, and how inter-organelle communication contributes to organelle structure and function. Particular focus will be placed on contacts

formed between the ER and mitochondria, as well as with the endomembrane system. In addition, we will examine the state of the membrane contact site field, and discuss exciting future directions that should be investigated. As Membrane Contact Sites (MCSs) are emerging as important sites of inter-organelle nutrient exchange, we also postulate upon the function of organelle contacts in metabolic decision-making.

2. ER-PM contact sites in organelle identity and membrane remodeling

The lipid composition of an organelle contributes significantly to its identity and function. At the plasma membrane (PM) of both yeast and mammals, the phosphoinositide phospholipid PtdIns4P is generated as a precursor to PtdIns(4,5)P₂ (also known simply as PIP₂). PIP₂ serves as a biomarker of PM identity, and coordinates numerous cellular responses at the cell surface, including clathrin-mediated endocytosis and actin turnover (Jost et al., 1998; Miki et al., 1996). PtdIns4P itself accumulates at the Golgi as well as the cell surface, and thus PtdIns4P levels at the PM must be carefully regulated to ensure proper organelle distinction.

How are PtdIns4P levels regulated? A series of recent studies using both yeast and mammalian systems suggest ER-PM contact sites serve as hubs for the regulation of PM-localized PtdIns4P, and thus “hotspots” for membrane remodeling at the PM. Initial studies identified numerous proteins that physically tether the ER to the PM (Manford et al., 2012; Toulmay and Prinz, 2012). To date, at least three ER-resident protein families—the Tricalbins (E-Syts in mammals), Scs2/22 (VAPs in mammals) and Ist2 (Tmem16 in mammals)—contribute to ER-PM tethering. All three were found to co-purify with Sac1, the ER-resident PtdIns4P phosphatase, indicating that PtdIns4P turnover is coupled to ER-PM contacts. Consistent with this, deletion of these tethers causes PM-localized PtdIns4P levels to increase substantially (Manford et al., 2012).

Although the reason for this PtdIns4P increase requires additional study, it may be explained by recent models proposed by De Camilli, Drin, and colleagues (Chung et al., 2015; Moser von Filseck et al., 2014, 2015). Using both yeast and mammalian model systems, they demonstrate that members of the Oxysterol-binding Protein (OSBP)-related family (ORP) are able to efficiently exchange PtdIns4P between the PM and ER at contact sites. In mammals, this required ORP5 and ORP8. This mechanism requires a dual-switch model, where PtdIns4P is initially extracted from the PM by the ORP protein, shuttled across the cytoplasm, and deposited in the ER membrane. Here, the phosphatase Sac1 can efficiently hydrolyze PtdIns4P to phosphoinositol (PtdIns). In exchange for releasing PtdIns4P, the ORP protein retrieves phosphatidylserine (PtdSer), which is normally synthesized at the ER, but accumulates at the PM. PtdSer is subsequently shuttled to the PM via the ORP, thus completing the 1:1 lipid exchange cycle (Fig. 1). In this way, both lipids are efficiently exchanged between organelles in a timely manner, allowing for localized membrane remodeling. A similar mechanism using OSBP has been proposed for the cycling of sterols with PtdIns4P at the ER-Golgi interface (Mesmin et al., 2013).

Although this lipid exchange cycle elegantly explains the bidirectional shuttling of lipids, several questions remain. How are sites of lipid exchange chosen and demarcated? How is

this exchange coupled to the synthesis of new PtdIns4P and PtdSer? Earlier studies of phospholipid synthesis indicate that synthesis enzymes can be coupled to organelle-organelle junctions, suggesting regulatory pathways exist that couple synthesis with shuttling (Stone and Vance, 2000).

3. ER-mitochondrial contact sites in mitochondrial shape and division

As they are not connected to the endomembrane system, mitochondria require non-vesicular mechanisms of obtaining essential phospholipids in order to function and replicate. The ER appears to supply these lipids in certain circumstances, and uses specific ER-mitochondrial contact sites mediated by protein complexes like ERMES (ER-mitochondrial Encounter Structures) in yeast to achieve this lipid exchange (Kornmann et al., 2009; Lang et al., 2015; Flis and Daum, 2013). Loss of ERMES components leads to mitochondrial dysfunction and the inability to grow on non-fermentable carbon sources, suggesting that ER-mitochondrial contacts are essential for proper mitochondrial metabolism (Kornmann et al., 2009).

In addition to mediating lipid exchange, ER-mitochondrial contact sites appear to regulate mitochondrial shape and division, and thus govern mitochondrial remodeling. Using dynamic live-cell microscopy, Voeltz and colleagues recently demonstrated that ER tubules demarcate sites of assembly for the Dynamin-like GTPase Drp1 (Dnm1 in yeast), which drives mitochondrial division (Smirnova et al., 2001; Friedman et al., 2011) (Fig. 1). How this is coordinated is unclear, but it is tempting to speculate that ER-mitochondrial contact sites exhibit unique lipid compositions that promote Drp1 assembly and membrane constriction (Friedman et al., 2011; Rambold and Lippincott-Schwartz, 2011). Similarly, the large GTPase Mitofusion2 (Mfn2), which governs mitochondria fusion in mammals, has recently been implicated in governing ER-mitochondria contact sites, as mammals do not seem to express an ERMES complex like yeast (de Brito and Scorrano, 2008).

How ER-mitochondria contact sites coordinate membrane fission machinery, and how this relates to general mitochondrial lipid exchange with other organelles, still remain outstanding questions. It is also unclear whether the ER could mediate the fission (or fusion) of other organelles. One intriguing possibility is that the ER may serve as a general regulator of organelle dynamics by demarcating regions prone to membrane fission, which may contain unique lipid compositions. The ER itself may be a source of these lipids, such as phosphatidic acid (PA), which is thought to enrich at ER-lipid droplet contact sites prior to the fission of lipid droplets from the ER surface (Han et al., 2015). Whether the ER serves to regulate the fission of other organelles beyond mitochondria remains to be seen, but it is likely to regulate the fission of lipid droplets and autophagosomes that bud from it.

4. ER-endosome/lysosome contact sites in endosome migration and cholesterol metabolism

Endosomes, late endosomes (LEs), and lysosomes also make extensive contact with the ER, and numerous studies implicate ER-endolysosomal cross-talk in organelle reshaping and migration. Several studies suggest the ER dictates LE and lysosome positioning within the cell cytoplasm, and this positioning is both dependent upon intracellular sterols, and in turn

influences sterol metabolism. In mammals, the cholesterol-binding ORP1L localizes to LEs, where it regulates the formation of ER-LE contact sites based on LE cholesterol levels. When cholesterol levels are low, the Rab7-interacting protein ORP1L promotes ER-LE contact, presumably to regulate sterol efflux out of endosomes (Johansson et al., 2005; Rocha et al., 2009). To achieve this, the ER-resident protein VAP interacts with LE-resident Rab7-RILP. This interaction displaces p150^{Glued} and microtubule motors, and LEs migrate to the cell periphery. When LE cholesterol levels increase, this migration is prevented, and LEs accumulate near the microtubule minus ends at the perinuclear region in a dynein-dependent manner. Consistent with this, Niemann Pick Type C patients exhibit high endomembrane cholesterol levels, and LEs and lysosomes accumulate in the peri-nuclear region, underscoring the relationship between cholesterol metabolism and endomembrane transport (Sokol et al., 1988).

Despite these observations, the molecular mechanisms that govern sterol-sensing at LEs and lysosomes remain unclear. Furthermore, the established model for ORP1L-mediated endosomal migration, and how it relates to LE cholesterol efflux, is also somewhat counterintuitive. The model posits that ER-LE contact increases with *decreasing* LE cholesterol levels at the cell periphery (and thereby, cholesterol-laden LEs may actually have *less* contact with the ER). Since it is commonly thought that ER-endolysosomal contact sites may serve as sites for cholesterol efflux out of the endomembrane pathway, why ER-LE contact sites would *decrease* as LE cholesterol levels *increase* is perplexing. Furthermore, more recent studies in living cells suggest that the ER makes extensive contact with both early endosomes and LEs, with LEs making more extensive contact with the ER as they mature and move inward away from the cell surface (Friedman et al., 2013).

One explanation for these collective phenomena may be that ER-LE MCSs regulate LE maturation and fusion with lysosomes, and thereby control the timing of cholesterol delivery to fully mature lysosomes. This may regulate dietary cholesterol turnover, and prevent premature sterol efflux from pre-lysosomal compartments. This model is supported by recent observations that ORP1L regulates the Rab7-HOPS complex interaction, which governs LE-lysosome fusion (van der Kant et al., 2013). The mechanism by which ORP1L senses LE cholesterol levels, and whether this directly contributes to NPC1/2-mediated cholesterol efflux out of lysosomes also remains to be elucidated.

More recently, other proteins that facilitate ER-endolysosomal contact have been implicated in coordinating endosomal positioning. Protrudin, an integral ER-anchored protein with a FYVE domain that binds endosomal PtdIns3P, was found to enrich at ER-endosome contact sites through binding both lipids and Rab7 (Raiborg et al., 2015). Here, ER-endosome contact facilitates endosome migration to the cell periphery that is dependent on the kinesin-1 and the motor adaptor FYCO1. How this relates to ORP1L-mediated endosome migration remains to be reconciled.

5. The biomedical potential of ER-endolysosomal MCSs

Although metabolic cross-talk between the ER and the endolysosomal system appears to be of major biomedical importance in the understanding of diseases like Niemann-Pick Type C,

ER-endolysosomal cross-talk remain among the least understood forms of inter-organelle communication. A general perception, however, is that ER-lysosome MCSs facilitate the efflux of metabolites generated by lysosomes (a catabolic organelle) to the ER, where they may be sensed or utilized in anabolic metabolism. Understanding this lysosomal efflux, and how specific contact sites between the ER and lysosomes contribute to it, is thus a major goal of the MCS field. Indeed, more than 50 distinct genetic diseases, collectively known as lysosomal storage diseases (LSDs), can be attributed to defects in lysosomal efflux and homeostasis, resulting from the pathological accumulation of cellular debris in lysosomes and autophagosomes. Altogether this makes LSDs a leading cause of childhood genetic disease, affecting perhaps 1:7700 children in the U.S. each year (Ratko, 2013). Whether lysosomal efflux requires direct ER-lysosome contact is unclear, but it is intriguing that contact between the ER and LE/lysosomes appear to be governed by cholesterol, and sterols modulate LE/lysosome migration that appears perturbed in these diseases.

In yeast, the ER-lysosome (the vacuole in yeast) MCS is much better defined, and forms a patch-like strip called the Nuclear ER-Vacuole Junction (NVJ) Pan et al., 2000. NVJs are created by direct interaction of two proteins: the ER-resident protein Nvj1, which engages the vacuolar protein Vac8 (Pan et al., 2000). The role of the NVJ is not entirely clear to date, but serves as a site for piecemeal autophagy of the nucleus (PMN), as well as an organization site for the synthesis of long-chain fatty acids. As such, numerous metabolic proteins localize to NVJs including Tsc13 and the SMP-domain protein Nvj2 (Toulmay and Prinz, 2012; Kvam et al., 2005).

Whether the yeast NVJ may serve as a model for the study of mammalian ER-lysosome MCSs remains to be determined. However, this is supported by recent studies revealing NVJ-resident proteins with clear metazoan homologs that appear to play roles in lysosomal homeostasis and metabolism. Yeast Mdm1 and Nvj3 both localize the NVJ (Henne et al., 2015). Both contain PX-Associated (PXA) domains that are unique to this protein family, and are thus denoted members of the PXA domain-containing proteins. Mdm1 is ER-localized, yet functions as an ER-vacuole tether through binding vacuolar PtdIns3P via its Phox homology (PX) domain. Four mammalian Mdm1 homologs (Snx13, -14, -19, and -25) have been identified, and one homolog—Snx14—was recently implicated in pediatric cerebellar ataxia and intellectual disability (Thomas et al., 2014; Akizu et al., 2015). The cellular basis of this disease involves a loss of lysosome homeostasis, although the reason for this pathology is unclear. Intriguingly, cells biopsied from *SNX14*-deficient patients exhibited granular aggregates in their cytoplasm, consistent with defects in lysosomal and autophagosomal clearance. It is therefore tempting to speculate that Snx14 and its homologs function in lysosome efflux through inter-organelle communication. In *Drosophila*, the Mdm1 ortholog Snazarus is linked to fat metabolism and lifespan extension, further implicating this protein family in lipid metabolism and disease (Suh et al., 2008).

6. Perspectives and questions remaining

In many ways, the field of inter-organelle communication today mirrors the field of vesicle trafficking 30 years ago. Elaborate protein machinery that mediate organelle-organelle contacts continue to be revealed, providing fundamental insights into the molecular

mechanisms governing inter-organelle communication. Just as pioneering work on vesicle trafficking dissected how key *proteins* are trafficked between organelles, innovative work on MCSs may now reveal how essential *lipids* shuttle between organelles. However, just as in the golden era of vesicle trafficking, many key questions remain to be solved. Here, we briefly outline just a few major questions the MCS field face:

1. *How do we image spatially and temporally dynamic MCSs in living cells?*

Whereas organelle shape and identity are easily discernible with conventional light microscopy using fluorescent probes, or through TEM, sites of inter-organelle contact are proving difficult to image. This is particularly true in mammalian cells, where MCSs appear to be less prominent (yeast ER-PM MCSs cover ~40% of the cell surface, but cover <5% of a mammalian cell surface). Yeast MCSs are generally easier to image, perhaps because they also appear more stable over longer periods of time. ER-PM MCSs in yeast are stable for minutes, and the yeast NVJ is omnipresent throughout the life of the cell. Indeed, the ephemeral nature of some metazoan MCSs (eg. ER-mitochondrial and peroxisome-lysosome, (Friedman et al., 2011; Chu et al., 2015) may indicate that MCSs are under selective pressure to become highly dynamic and transient in nature. As inter-organelle metabolic exchange may occur quickly, there would be minimal need for long-lasting bridge-like “tethering” in a dynamic cell. Other organelles like the PM and ER may wish to maintain more stable associations, albeit at specific sites in that serve as centers for lipid exchange. What dictates these sites, and how the cell maintain control of ER-PM MCS formation, remains a mystery.

Faced with substantial microscopy challenges, advances in super-resolution microscopy in living cells will be invaluable to monitoring the dynamics of MCSs. 3-D cryo-electron tomography using fast-freeze methodologies, which preserve fragile MCSs, will also be instrumental in the understanding MCS architecture. Finally, simultaneous multi-color imaging in living cells will undoubtedly prove invaluable to understanding the inter-play between multiple organelles, and will reveal how these organelles contact one another during various cellular processes.

2. *How are different organelle contact site networks coordinated?* Several studies indicate that different MCSs work in overlapping metabolic pathways, and can even substitute for one another in times of nutrient stress. For example, yeast devoid of ERMES-mediated ER-mitochondrial contacts compensate by increasing their mitochondrial-vacuole contacts via vCLAMP (vacuole and mitochondrial patch), presumably to provide mitochondria with essential lipids they normally receive from the ER (Honscher et al., 2014; Elbaz-Alon et al., 2014). How this multi-organelle cross-talk is coordinated is unclear, but suggests organelles contain elaborate mechanisms of sensing their lipid nutrient status. In the ER, cholesterol levels are monitored continually by the SREBP pathway, which also functions in transcription to respond to cholesterol nutrient status (Brown and Goldstein, 1997). How other organelles like the mitochondria and lysosome/ vacuole sense their own lipid status is less understood, but will

undoubtedly play a significant role in how these organelles maintain homeostasis, as well as mediate contact with other organelles. How these sensing mechanisms play into metabolic decision-making may be one of the greatest questions in the field.

3. *How are organelle contact sites maintained as distinct microenvironments, yet prohibit inter-organelle fusion?* Electron microscopy suggests ER-PM MCSs are maintained with <15 nm spacing between organelles, and to date no evidence suggests that these organelles fuse at these MCSs. How is this achieved? Furthermore, how is the micro-environment of MCSs distinct from the rest of the cytoplasm, and does this contribute to the biological processes which occur at these sites? An emerging feature of multiple MCSs is the exchange of lipids. How these lipids are extracted from the donor organelle is unclear, but it is tempting to speculate that unique lipid and/or ion concentrations at MCSs may promote lipid extraction and exchange. As such, local concentrations of ions such as Ca^{2+} may influence lipid dynamics, and affect the energetic penalties of lipid extraction. MCSs may also serve as “metabolic crucibles” for the synthesis of new lipids. This is supported by the presence of numerous biosynthetic enzymes at MCSs, as well as early studies from Vance and colleagues indicating that MCSs involving mitochondria and other membranes, called MAMs, are rich with lipid biosynthetic enzymes, such as phosphatidylserine synthases (Stone and Vance, 2000).

7. Closing

In conclusion, the field of inter-organelle communication is at an exciting time. The continued discovery of new proteins and complexes which mediate organelle cross-talk open the field to mechanistic understanding. At the same time, new functions and pathways that utilize inter-organelle contact sites continue to be identified. The result is a *Renaissance* of Cell Biology, which has traditionally focused on understanding organelles as discrete and separable units for much of the 20th century. In the 21st century, an emerging paradigm suggests all organelles are interconnected in elaborate networks that govern cellular pathways, and use contact sites as their communication super highways.

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Fig. 1.
An example of three biological processes mediated by inter-organelle contact sites: 1) organelle-organelle lipid exchange (and thus organelle identity), 2) mitochondrial division, and 3) sterol trafficking and its role in late endosomal positioning within cells.