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Making the Connection: How Membrane Contact Sites Have Changed Our View of Organelle Biology

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Review

Making the connection: How membrane contact sites have changed our view of organelle biology

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SUMMARY

The view of organelles and how they operate together has changed dramatically over the last two decades. The textbook view of organelles was that they operated largely independently and were connected by vesicular trafficking and the diffusion of signals through the cytoplasm. We now know that all organelles make functional close contacts with one another, often called membrane contact sites. The study of these sites has moved to center stage in cell biology as it has become clear that they play critical roles in healthy and developing cells and during cell stress and disease states. Contact sites have important roles in intracellular signaling, lipid metabolism, motor-protein-mediated membrane dynamics, organelle division, and organelle biogenesis. Here, we summarize the major conceptual changes that have occurred in cell biology as we have come to appreciate how contact sites integrate the activities of organelles.

INTRODUCTION

We wrote our first review together on organelle structure 16 years ago. This review was titled "Sheets, ribbons, and tubules—how organelles get their shape"[1] and summarized what was known about how membrane-shaping proteins, tethers, lipids, and the cytoskeleton all contribute to the shape of complex organelles. There was not a word in the review about membrane contact sites (MCSs), regions of close contact between organelles. This might seem like a major oversight, given the present central role of contact sites in cell biology (Figure 1). Back then, however, we saw organelles as independent cytoplasmic entities and considered all aspects of their shapes and functions in an autonomous way. The only exceptions to this rule, at the time, were signaling at specialized contacts between the plasma membrane (PM) and the endoplasmic reticulum (ER) in muscle cells and the nascent idea that contacts between the ER and mitochondria might play a role in lipid and Ca^{2+} transport between them.[2–7] We and others also ignored the wealth of electron microscopy showing close contacts between organelles (Figure 1),[8–10] though it was not clear whether these contacts had functions. Nevertheless, the idea that organelles functioned independently was roughly where organelle biology stood in 2007, a view of the cell reflected by drawings of cells on the covers of nearly every cell biology textbook up until just the past few years. The players shaping organelles have not really changed. They are still membrane-shaping proteins, tethers, lipids, and cytoskeletal components, but what has changed is that we now know that these factors are guided, in many cases, to locations where organelles converge to form MCSs. We also now understand that many cellular functions previously thought to require vesicular trafficking or diffusion of signals or small molecules through the cytoplasm do not; they occur at MCSs.

MCSs are locations where two organelles are close enough together to be tethered by molecular machineries (typically within 30–50 nm, but sometimes more). The organelles at MCS do not fuse. Protein complexes that hold organelles together at MCSs can insert into the organelles at an MCS or bind to proteins or lipids in the apposing membranes. These complexes, often called tethers, usually have additional functions that occur at these sites beyond physical tethering. This review will describe how the study of contact sites has changed our view of how cells function. The field has grown so much in the last decade that an encyclopedic review would be a book length. Instead, we focus here on the major conceptual advances in cell biology that have occurred as we have come to appreciate the various roles of MCSs in cellular processes. The first part of the review discusses how the study of MCSs has changed our view of intracellular lipid trafficking, lipid metabolism, and phosphatidylinositol (PI) phosphate (PIP) signaling. Next, we describe our emerging understanding of the roles of MCSs in calcium signaling. Finally, we discuss how MCSs have been found to facilitate the regulation of cellular processes: the formation of lipid droplets (LDs), motor-protein-mediated membrane dynamics, and organelle division. We focus on MCSs formed by the cell-wide continuous ER with other organelles that commonly consist of many discrete structures. There are contacts between pairs of organelles that we will highlight that do not include the ER, but these contacts are conceptually similar to those formed by the ER.

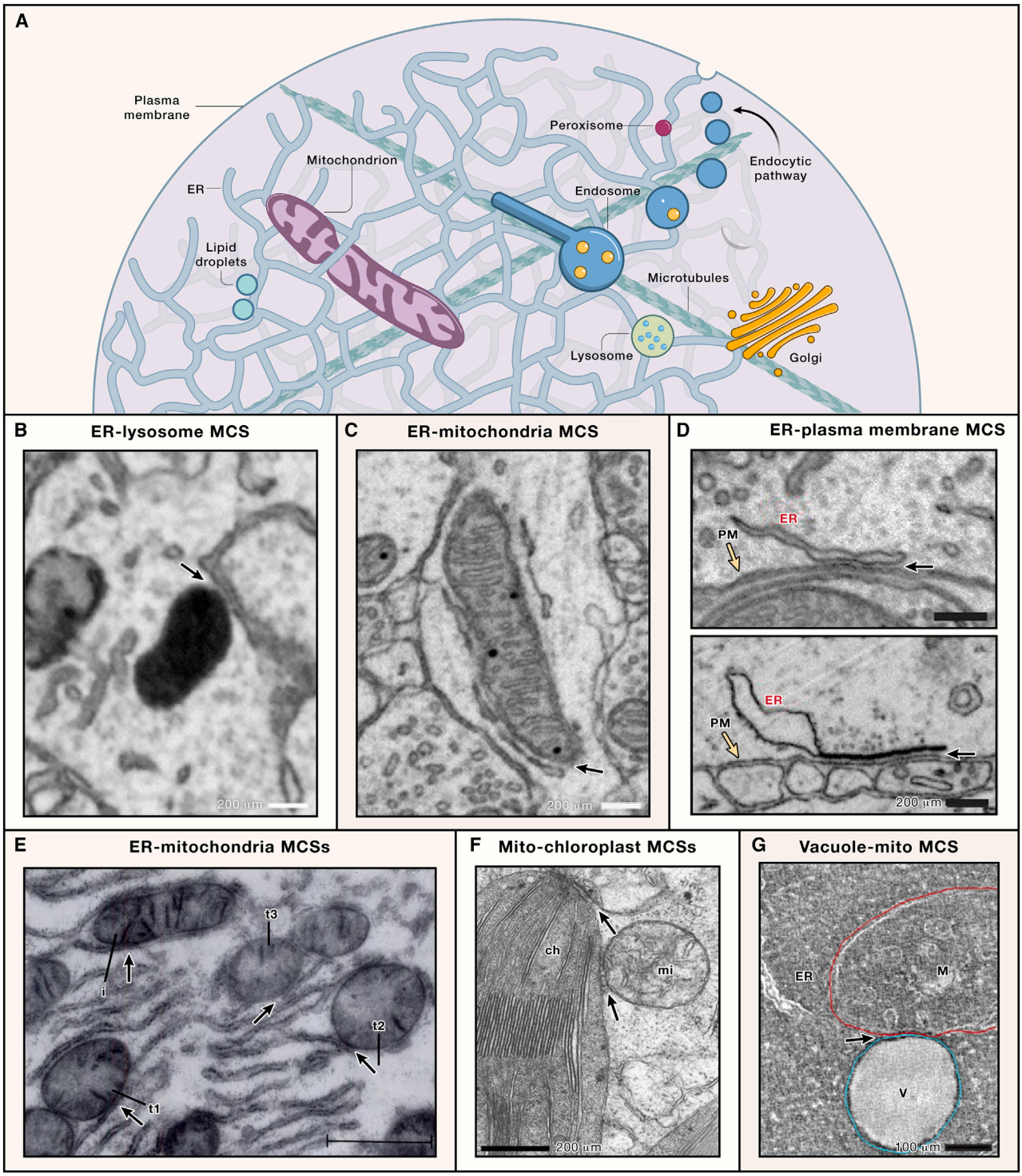

Figure 1. Organelles are highly organized and interconnected

(A) Organelles do not exist in isolation. Their locations are organized by the cytoskeleton (microtubules) and contacts formed between the membranes of different organelles. The endoplasmic reticulum (ER), in particular, forms extensive contacts with other organelles. These contacts are major regulatory hubs in the cytoplasm.

(B–F) Electron micrographs reveal that organelle membranes contact each other within molecular distances (arrows).

(B) ER-lysosome MCS in the soma of a neuron viewed by focused ion beam milling coupled to scanning electron microscopy. Reproduced with permission.[11]

Scale bars, 200 nm.

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NON-VESICULAR TRAFFICKING OF LIPIDS AT MCSs

Cells obtain lipids from two sources: they produce them, primarily in the ER, or take them up from outside. In animals, most external lipids are delivered by endocytosis of lipoproteins, where lipoproteins are degraded in lysosomes and the released lipids are trafficked to the rest of the cell. How lipids are moved between organelles and what determines the lipid compositions of cellular compartments is incompletely understood.

It has long been known that lipids are moved between cellular compartments by a combination of vesicular and non-vesicular mechanisms. Classic work from the Simoni group, for example, showed that blocking vesicular trafficking did not stop the transport of newly synthesized cholesterol from the ER, where it is made, to the PM.[15] This is true of some other classes of lipids as well. The mechanism and function of this transport were unknown and are only now becoming clear. We now understand that MCSs play a central role in nonvesicular transport of cholesterol and other lipids between cellular compartments. The transport requires proteins called lipid transport proteins (LTPs), which are defined by their ability to facilitate lipid exchange between membranes *in vitro*. There are many families of LTPs, and some have been studied for decades.[16,17] A critical advance in our understanding of intracellular lipid trafficking is the discovery that many LTPs operate at contact sites.

LTP families are structurally diverse but mechanistically fall into two broad categories: lipid-shuttling transporters and proteins that form lipid conduits. Most shuttling transporters bind a single lipid monomer in a hydrophobic cleft or groove. In some cases, the bound lipid is also shielded from the aqueous phase by a lid-domain. These LTPs can extract a lipid from a membrane, transfer it through the aqueous phase, and deliver it to a second membrane (Figure 2A). Many LTPs also have accessory domains that localize them to contact sites. For example, LTPs enriched at ER-PM contact sites often have a domain that binds ER-resident proteins called vesicle-associated membrane-protein-associated proteins (VAPs) and a domain that binds PI 4,5-bisphosphate, a lipid highly enriched in the PM.

In the last few years, a new family of LTPs has been characterized that seems to work by a different mechanism.[18] Proteins in the family, which have been called Vps13-like or repeating β-groove (RBG) motif bridge-like proteins, are thought to form large (10–25 nm) rigid structures that have a hydrophobic groove that runs the length of the protein (Figure 2B). The groove has been proposed to allow lipids to flow between membranes. This would require the LTPs to bind two membranes simultaneously, which has been observed in cells.[19] Whether lipids flow through the proteins in this way has not been definitively shown, but some studies have found that introducing charged residues into the groove prevents the proteins from functioning in cells, consistent with the idea that the LTPs are conduits.[20,21] LTPs in this family have been proposed to facilitate high-volume lipid transport. Although shuttling LTPs transport lipids at rates of up to about 1 lipid per LTP per second,[22] at least *in vitro*, one recent estimate of transport by the Vps13-like LTP Atg2 indicates that the protein moves about 200 lipids per second.[23] High volume transport may be necessary for large-scale changes in membrane lipid composition; examples include autophagosome formation,[24] changes in intracellular lipid distribution necessary to main membrane fluidity in low temperature,[25,26] and membrane repair.[21]

Some LTPs outside the Vps13 family probably also function as bridge-like transporters that allow lipids to flow between membranes at contact sites. The ER-mitochondria encounter structure (ERMES) tethers the ER and mitochondria in *S. cerevisiae* and facilitates lipid movement between these organelles.[27,28] The complex contains four proteins. A recent remarkable *in situ* structural analysis of ERMES using cryoelectron microscopy (cryo-EM) revealed that three synaptotagmin-like mitochondrial lipid-binding domains in ERMES components assemble to form a conduit between the ER and mitochondria that could allow lipids to move between them.[29] Whether other LTPs complexes can from similar conduits at contact sites is an important question.

Lipid transport between organelles at contact sites by shuttling LTPs has several functions. One is that it may increase the speed of transport, at least in cases where the rate of transport is diffusion limited; LTPs have only a short distance to diffuse between membranes at MCSs. This could be important to allow cells to rapidly move lipids in response to stress or when vesicular transport is not functioning. It has even been proposed that lipid exchange at contact sites preceded vesicular transport in the evolution of cells with multiple internal organelles.[30]

Another role for lipid exchange at MCSs is to channel lipids to a particular organelle or enzyme. This can be driven by four mechanisms. One is the consumption of a lipid by enzymes in one of the membranes at MCSs. An example is the production of sphingomyelin from ceramide. Ceramide is synthesized in the ER and transported to the Golgi complex at the ER-Golgi MCS by the LTP ceramide transfer protein (CERT).[31] Once ceramide reaches the Golgi, it can be converted to sphingomyelin by enzymes there. Because sphingomyelin is not transported by CERT, the net movement of ceramide from the ER to the Golgi complex is driven by the conversion of ceramide to sphingomyelin. A second mechanism that can promote lipid exchange at the MCS is the production of lipids at a contact site. For example, the

(C) ER-mitochondria MCS in a dendrite viewed by focused ion beam milling coupled to scanning electron microscopy. Reproduced with permission.[11] Scale bars, 200 nm.
(D) ER-plasma membrane (PM) MCSs in the soma of neurons viewed by focused ion beam milling coupled to scanning electron microscopy. Reproduced with permission.[11] The ER at PM contacts sometimes contains lumenal content (top) and other times has tightly constricted membranes without lumen (bottom). Scale bars, 200 nm.
(E) In this classic image (reproduced with permission[12]), abundant ER-mitochondria MCSs are visible in a rat liver cell. I: mitochondrion shown in longitudinal section. t_1, t_2, t_3, mitochondria shown in transverse section.
(F) In plants, chloroplasts (ch) and mitochondria (mi) form MCSs marked by electron-dense bridges. Reproduced with permission.[13] Scale bars, 200 nm.
(G) Mitochondria (M) form MCSs with the lysosome-equivalent vacuole (v) in budding yeast. Reproduced with permission.[14] Scale bars, 100 nm.

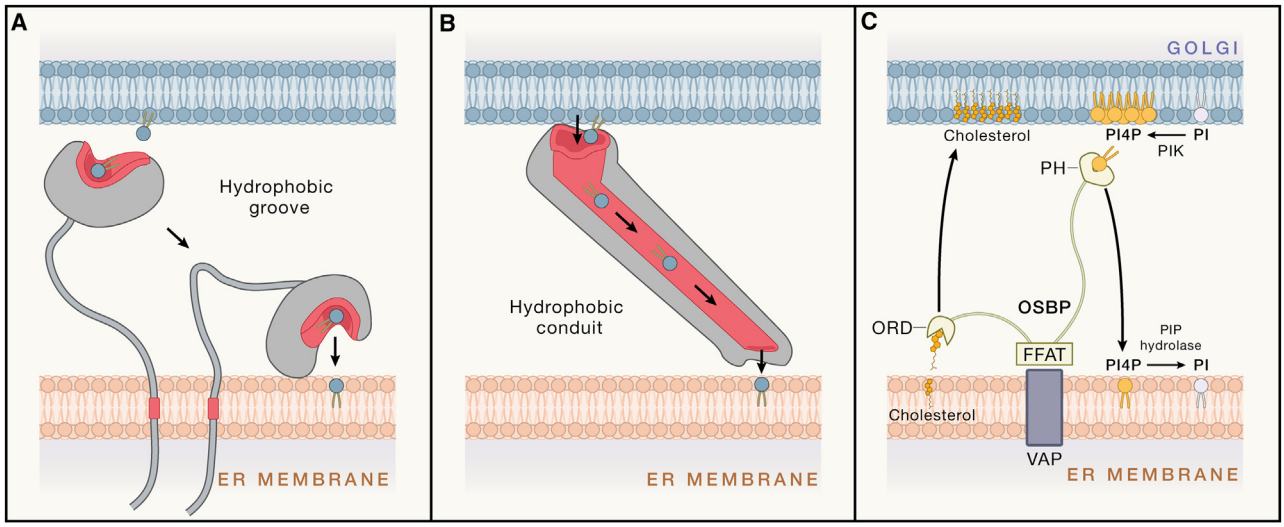

Figure 2. Membrane contact sites facilitate lipid transfer between organelles

(A) Lipid transporters with hydrophobic grooves or pockets extract phospholipids from a donor organelle membrane (blue) and catalyze their transfer to a recipient organelle membrane (gray). The hydrophobic groove shields the lipid from the cytosol during transport.

(B) Lipid transporters with tubular, hydrophobic conduits form stable routes for lipid transfer between tethered organelle membranes.

(C) Lipid transporters regulate organelle membrane lipid compositions at MCSs. OSBP transports cholesterol from the ER to the Golgi, despite cholesterol levels in the ER being much lower than in the Golgi. To accomplish this, OSBP exchanges the Golgi lipid PI4P for ER cholesterol. In the ER, PI4P is hydrolyzed into PI to maintain the sharp PI4P gradient. OSBP has an ORD domain to bind cholesterol, a PH domain to bind PI4P, and an FFAT domain to bind to VAP.

production of phosphatidylserine at ER-mitochondria contacts promotes the transfer of this lipid to mitochondria.[32] LTP enrichment at contact sites is a third mechanism of fostering lipid exchange at contacts and channeling lipids to a cellular compartment. This has been suggested by several studies in which LTPs are artificially enriched at contact sites. The enrichment of CERT, for example, at ER-mitochondria contacts promotes ceramide transfer to mitochondria, leading to apoptosis.[30]

The fourth mechanism cells use to drive lipid transport at MCSs makes use of gradients in PIP levels between the ER, where PIP levels are low, and organelles, where PIP levels are significantly higher. PIPs play critical roles in many cellular processes, and specific PIP species are enriched in various cellular membranes; for example, the Golgi complex has high levels of PI 4-phosphate (PI4P), and some endocytic membranes are enriched in PI 3-phosphate.[33] Differences in PIP levels in the ER and other cellular compartments are the result of the localization of enzymes that produce and degrade PIPs. The precursor of PIPs is PI, which is produced in the ER, whereas the kinases that generate PIPs are located outside the ER in the PM, Golgi complex, and endosomal membranes. In addition, when PIPs reach the ER, they are degraded by lipases. Sustaining PIP levels in various cellular compartments was long known to require interorganelle lipid exchange, both to produce PIPs and for PIP degradation and recycling precursors to the ER.[34] We now know that the transport of PIPs and PIP precursors at MCSs requires LTPs operating at these sites (Figure 2C). In addition, PIPs in one of the two membranes at an MCS can be used to drive the transport of a second lipid against a concentration gradient. This mode of transport, often called "counter transport," was first shown for the LTP oxysterol-binding protein (OSBP), which can exchange cholesterol and PI4P at MCSs between the ER and the *trans*-Golgi network (TGN).[35] Because PI4P is produced in the TGN and degraded in the ER, the difference in PI4P concentration in the ER and TGN is used by the OSBP to drive cholesterol from the ER, which has low levels of cholesterol, into the TGN, where cholesterol levels are high (Figure 2C). Thus, PIP metabolism at contact sites is used to enrich cholesterol in the TGN. A similar mechanism was later shown for other LTPs in the same family as OSBP; they use the difference in PIP levels in the ER and PM to drive the transport of phosphatidylserine into the PM.[36,37]

As we study lipid transport at contact sites, one notable emerging finding is that intracellular lipid transport pathways seem to be highly redundant and adaptable. This is best illustrated by studies on the ER-mitochondria lipid trafficking ERMES complex. Many were perplexed when it was determined that the ERMES complex is not essential—this was unexpected because *S. cerevisiae* cannot survive without mitochondria, and mitochondria require membrane phospholipids for biogenesis. Later, this led to an interesting surprise: when ERMES is not present, phospholipids probably move from the ER to mitochondria by a circuitous MCS-dependent route. Phospholipids synthesized in the ER move to the vacuole via MCSs and then to the mitochondria at a vacuole-mitochondria contact termed the vacuole-mitochondria patch or vCLAMP.[14,38]

vCLAMP was identified in parallel by two groups using two different strategies. One strategy sought alternative pathways for transferring essential lipids to the mitochondria that would explain why the ERMES complex is not essential. Screening for mutations that would lead to a pronounced increase in contact between the ER and mitochondria (more ERMES foci)[38] revealed that mutations in *VPS39* increased ERMES contacts and showed that cells lacking *VPS39* and ERMES were not viable.[38]

Independently, fluorescent microscopy (FM) revealed sites of colocalization between the vacuole and mitochondria, and cryo-EM confirmed that these contacts were within tethering distance (~10 nm, Figure 1G).[14] A candidate screen of vacuolar components required to maintain vacuole-mitochondria contacts identified Vps39 as well as a vacuole-mitochondrial contact site protein that can promote vCLAMP expansion and compensate for ERMES function.[14] To make matters more complex, a second vacuole-mitochondria MCS that probably also facilitates lipid exchange between these organelles via the LTP Vps13 was later discovered.[39] Together, these complementary studies suggest an important principle: nonvesicular trafficking of lipids through contact sites is complex because cells apparently have multiple redundant routes available to move lipids between organelles. Indeed, visual studies reveal that contact sites can be observed between almost every pair of organelles, at least in some growth or stress conditions,[40–42] suggesting that there may be many possible routes for nonvesicular lipid exchange among cellular compartments.

CALCIUM SIGNALING AT MCSs

ER-PM contact sites

Movement of Ca^{2+} ions across membranes is a common feature in cell signaling. How can a Ca^{2+} signaling event that occurs in one compartment evoke a response at another? MCSs provided the simple and logical answer to the mysterious mechanism behind store-operated Ca^{2+} entry (SOCE), a process whereby depletion of lumenal Ca^{2+} stores within the ER opens PM channels to promote Ca^{2+} flow back into the ER. The first challenge was to understand how PM Ca^{2+} channels sense the concentration of Ca^{2+} in the ER lumen. The second mystery was how SOCE can replenish Ca^{2+} into the ER lumen without causing cytoplasmic Ca^{2+} levels ($[Ca^{2+}]c$) to rise.[43] A big clue came in 2005 when two independent RNAi screens determined that the ER membrane protein Stim was required for SOCE.[44,45] Stim is a single-pass ER membrane protein with a Ca^{2+}-binding EF hand domain in the ER lumen, suggesting it is a lumenal Ca^{2+} sensor.[44,45] Shortly thereafter, its functional partner Orai was identified as the channel subunit of the PM CRAC complex.[46–49] How could a domain of Stim located in the ER lumen activate Orai channel opening on the PM? The solution was simple, but not obvious back then without further experimental data: these machineries directly interact at MCSs in their Ca^{2+}-depleted conformation.

Stim localization provided a great deal of insight because it was altered upon manipulation of lumenal Ca^{2+} concentration in an informative way. Under resting conditions, Stim was found throughout the ER, but when ER Ca^{2+} was depleted, Stim translocated to puncta that appeared by total internal reflection fluorescence (TIRF) microscopy and immuno-electron microscopy to be located near the PM.[44,45] Mutation of a key residue in the Ca^{2+} binding domain, which was predicted to prevent it from binding Ca^{2+}, strikingly caused Stim to locate constitutively to puncta.[44,45] Together, these results indicated that Stim localization is sensitive to ER lumenal Ca^{2+} levels. TIRF and electron microscopy revealed that Stim puncta are at regions where the ER and PM are closely apposed (within 10–25 nm).[43] Furthermore, by combining TIRF imaging with fluorescent Ca^{2+} sensors it was possible to show that Stim puncta overlap with sites of Ca^{2+} influx through open CRAC/Orai channels.[50]

The mechanism of action for the Stim Ca^{2+} sensor has been further unraveled. When lumenal ER Ca^{2+} is reduced, the Stim lumenal domain is not bound to Ca^{2+} and this conformation oligomerizes.[51] This clustering causes the Stim cytoplasmic domain to directly hetero-oligomerize with Orai channels at sites of ER-PM juxtaposition (Figure 3A).[51,52] Thereby, Stim oligomerization is sufficient to cause it to translocate to ER-PM junctions. Patch clamp experiments have demonstrated that this translocation triggers Orai channel opening and Ca^{2+} influx.[51,52] Ca^{2+} influx then can locally feed Ca^{2+} into the ER lumen without causing global $[Ca^{2+}]c$ to rise. This mechanism presented an elegant MCS solution to the SOCE mystery.

ER-mitochondria contact sites

It has been long known that several ATP-producing enzymes and cell survival regulators respond to Ca^{2+} concentration ($[Ca^{2+}]$) increases in the mitochondrial matrix. Additionally, mitochondria have been shown to contain Ca^{2+} conducting channels in both their outer and inner membranes: voltage-dependent anion channels (VDACs) in the outer membrane and the mitochondrial calcium uniporter (MCU) in the inner membranes.[53] However, until the discovery of the importance of ER-mitochondria contact sites for calcium signaling within mitochondria, it was puzzling that mitochondrial Ca^{2+} uptake seemed to be activated only above the physiological range of $[Ca^{2+}]c$, raising skepticism about the relevance of calcium signaling within mitochondria. By using genetically targeted Ca^{2+} reporters in intact cells, physiological $[Ca^{2+}]c$ signals were shown to enter the mitochondria[5] and, in turn, to tune metabolism.[54] This was soon attributed to the proximity of mitochondria to the ER Ca^{2+} release channels (inositol 1,4,5-trisphosphate receptors [IP3Rs]), which sense a higher local $[Ca^{2+}]$ at contact sites, rather than the global $[Ca^{2+}]c$ levels of the cytoplasm (Figure 3B).[6]

Evidence for the functional relevance of ER-mitochondrial contact sites in Ca^{2+} signaling came with the discovery of tethers between these organelles and the finding that disrupting these tethers interfered with Ca^{2+} signal propagation to the mitochondria. Conversely, synthetic ER-mitochondrial linkers were found to enhance the local Ca^{2+} transfer.[55] High Ca^{2+} nanodomains were detected both at the contacts and on the mitochondrial surface.[56,57] Since then, the identification of the ER-mitochondrial tethering molecules and the relevance of contact sites beyond local Ca^{2+} transfer has become a major area in cell biology.[58] The IP3Rs of the ER were first reported to interact with VDAC, forming a potential Ca^{2+} tunnel into the mitochondrion.[59] Genetic data reinforce the role of IP3Rs as physical support to ER-mitochondrial contacts.[60]

Many IP3R-independent ER-mitochondrial tethers have also been reported.[58] These tethers might be primarily involved in other functions at ER-mitochondrial contacts, such as lipid transfer and organelle dynamics, at least some of which might also affect local ER-mitochondrial Ca^{2+} transfer. For example, tethers that are <10 nm long might support lipid transfer but create ER-mitochondrial gaps that exclude the bulky IP3Rs.[56] In summary, tethering of IP3Rs to the mitochondrial surface

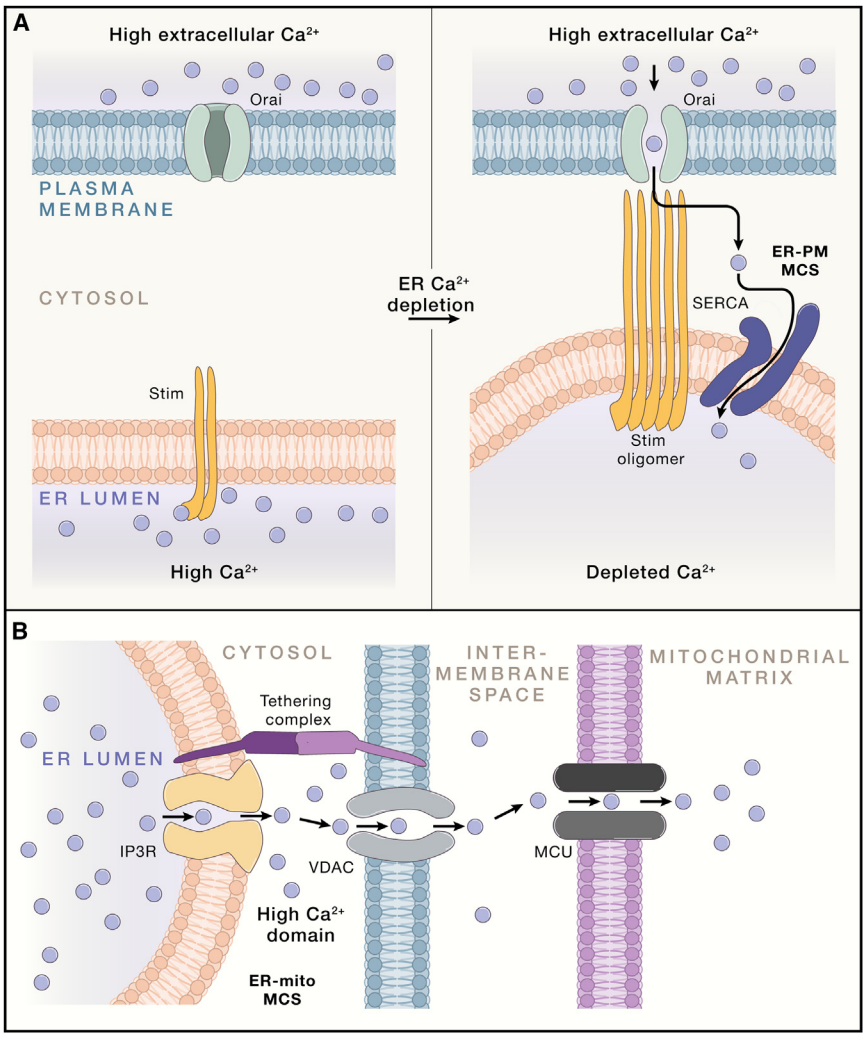

Figure 3. Calcium ion transport is a critical function of membrane contact sites

(A) Cells normally contain high concentrations of Ca^{2+} within the ER lumen and in the extracellular space, with very low levels in the cytosol. Under these conditions, the Ca^{2+} channel Orai in the plasma membrane is closed, and the ER Stim1 protein is bound to Ca^{2+} and distributed throughout the ER. When ER Ca^{2+} stores are depleted, Stim1 loses its bound Ca^{2+}, which stimulates oligomerization. In its oligomeric state, Stim1 binds the plasma membrane Orai channel and stimulates Ca^{2+} influx from the extracellular space at ER-plasma membrane contact sites. Ca^{2+} is transported back into the ER through the SERCA pump at the contact site.

(B) IP3 receptors (IP3Rs) in the ER membrane release local accumulations of cytosolic Ca^{2+}, which are taken up by mitochondria through the voltage-dependent anion channel (VDAC) in the outer mitochondrial membrane. Then, Ca^{2+} ions are transported into the mitochondrial matrix by the mitochondrial calcium uniporter (MCU). This arrangement allows Ca^{2+} signaling between the ER and mitochondria at membrane contact sites without globally increasing cytosolic Ca^{2+} levels.

provides a "highway" for Ca^{2+} transfer into the mitochondria (Figure 3B). The relevance of other contact site proteins as restrictors or facilitators of the ER-mitochondrial Ca^{2+} traffic needs further investigation.

Other MCSs

The bulk of intracellular Ca^{2+} transport is likely mediated across the PM and ER membranes. However, like mitochondria, other organelles, including lysosomes and endosomes, also possess Ca^{2+} transport mechanisms that might support local calcium signaling at their MCSs with other organelles. For example, the lysosome-mitochondria and endosome-ER MCSs Ca^{2+} coupling are subjects of growing interests in terms of both the underlying mechanisms and functional relevance.[61,62]

THE ROLES OF BRIDGE-LIKE CONTACTS IN ER-LD BIOGENESIS AND FUNCTION

LDs are organelles found in all eukaryotic cells. They store neutral lipids, i.e., uncharged lipids, and play central roles in lipid metabolism, serving as a lipid reserve and location to store excess lipids. LDs differ structurally from other membrane-bound organelles; they have a core of neutral lipids surrounded by a phospholipid monolayer. This structure allows them to form contact sites with the ER that are significantly different from other known contact sites. At standard contact sites, the two organelles come in close contact but do not fuse. By contrast, at some ER-LD contacts, the lipid monolayer surrounding an LD is continuous with one of the leaflets of the ER membrane (Figure 4A). These bridging contacts allow lipids and proteins to flow between the ER and LDs.[63–65] Indeed, to date, these bridging ER-LD contacts are the only MCSs where proteins have been shown to flow between membranes at contact sites.

Bridging ER-LD contacts form by at least two mechanisms. One is that they form during LD biogenesis. New LDs are thought to form when neutral lipids in the ER, particularly triacylglycerides, phase separate and form lenses in the ER membrane (Figure 4A).[67] When the lens grows larger, it emerges into the cytoplasm but remains attached to the ER, forming a contact site. The protein seipin is thought to play a central role in the process. It forms an oligomer that is believed to be at the neck of the ER-LD contacts and may regulate the flow of lipids and proteins between the ER and LD (Figure 4B).[68] Structurally similar ER-LD bridging contacts may also form with mature LDs by processes that do not seem to require seipin. One way in which they form may be dependent on the Arf1/COPI machinery.[69] These proteins play a well-known role in vesicle formation, but how they facilitate contacts between the ER and LDs remains to be determined. More recent work has shown that other protein

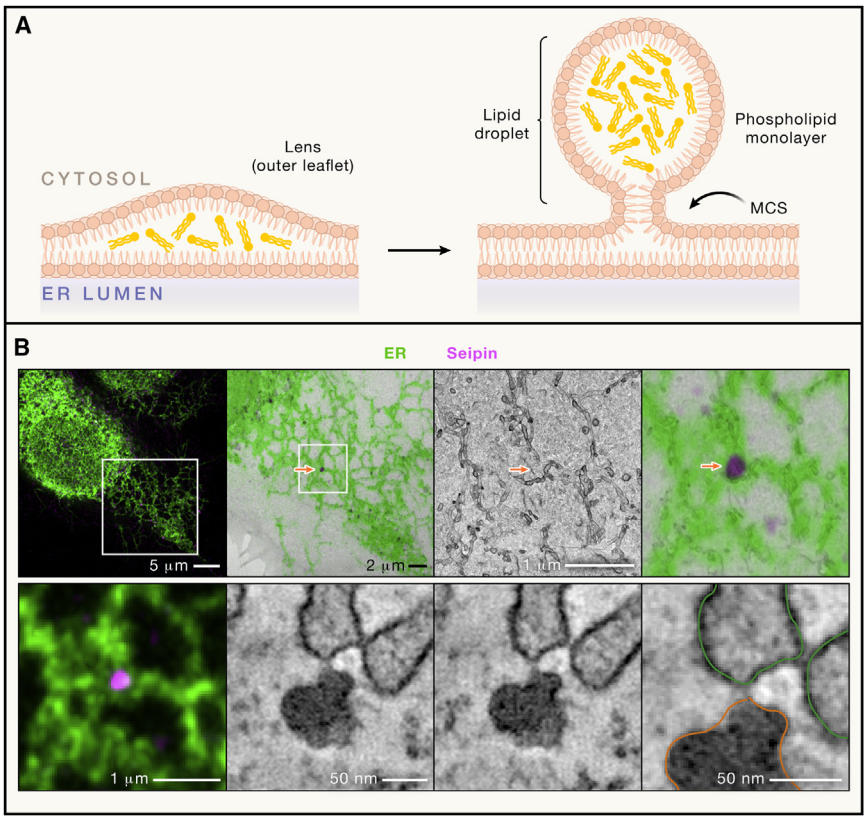

Figure 4. Lipid droplets grow from and are continuous with the ER

(A) Model of lipid droplet (LD) biogenesis. Neutral lipids such as triglycerides accumulate within ER membranes, leading to leaflet displacement (lens). The LD grows outward from the cytosolic face of the ER, eventually forming a distinct droplet encapsulated by a phospholipid monolayer. LDs can form special contacts sites (indicated with an arrow) that allow them to remain connected to the ER. They also form regular contact sites with other organelles.

(B) Seipin localizes to sites of nascent LD biogenesis in contact with the cytosolic surface of ER membranes. Reproduced with permission.[66] Top row: correlated light and electron microscopy. Bottom row: correlated light and electron tomography.

complexes known to participate in vesicular trafficking, namely components of the COPII machinery, SNARES, and Rab-based tethers, facilitate the formation of contact sites with mature LDs.[70]

Seipin is thought to play a critical role in regulating the flow of lipids and proteins between the ER and LDs, particularly membrane proteins and triglycerides, one of the primary neutral lipids stored in LDs.[65,66,70,71] Membrane proteins that flow between the ER and LD via bridging contacts are initially inserted into the ER and then move between the ER and LDs. These proteins have unusual membrane-imbedded segments that do not completely traverse the ER bilayer, which allow them to localize to the surface of LDs. The mechanistic details behind how seipin regulates the movement of proteins between the ER and LDs remain to be determined. Whether the flow of proteins between the ER and LD is regulated at contact sites not containing seipin is another important question.

ORGANELLE MOTILITY DYNAMICS ARE DIRECTLY REGULATED BY MCSs

All the major cytoplasmic organelles traffic on the cytoskeleton at the speed of molecular motors.[72–77] But, it was generally assumed that organelles trafficked independently of one another. With the advent of live high-resolution microscopy and the multitude of spectrally diversified fluorescent protein fusion possibilities, this view changed as it became feasible to simultaneously image the coupled dynamics of multiple organelles on microtubules (MTs; Figures 5A and 5B).[40,78–83] The result never fails to amaze: a movie of an endosome trafficking along an MT pulling ER tubules around with it (Figure 5B).[84] Indeed, as you watch organelles like the ER, mitochondria, and endosomes trafficking together, it can become quite difficult to determine which organelle is the driver and which is the passenger. Some smaller organelles, like peroxisomes and RNA granules, also use larger organelles like endosomes and lysosomes to "hitchhike" around the cytoplasm (Figure 5A).[85,86] Thus, to understand how each organelle traffics, it is increasingly important to understand how, why, and when they are coupled to each other.

Elegant studies have unraveled that ER MCSs can directly influence the trafficking of other organelles (for review see Bonifacino and Neefjes[87] and Striepen and Voeltz[88]). One of the most compelling examples is the ability of ER MCSs to determine which molecular motor(s) are recruited to endosomes.[89,90] Following internalization, early endosomes traffic toward the minus end of MTs and mature into late endosomes (LEs). Coincident with maturation, they acquire larger, more acidic, lose lumenal Ca^{2+}, and become tightly tethered to the ER through MCSs.[81,82,91–93] LEs are actively transported on MTs in both directions by molecular motors. The association of LEs with opposing molecular motors has been shown to be regulated by at least two different ER contact site proteins that function by slightly different mechanisms to promote plus-end-directed trafficking (Figures 5C and 5D).

One function for these ER MCSs is to disrupt minus-end-directed trafficking and redirect LEs toward the PM. Minus-end-directed transport of endosomes is regulated by cholesterol through an endosomal sterol sensor—OSBP-like 1 (ORP1L). When endosomal cholesterol levels are high, ORP1L is more likely to be bound to cholesterol via its C-terminal OSBP-related ligand-binding domain (ORD).[89] This is referred to as the "closed" conformation of ORP1L, and in this conformation it interacts with a Rab7/RILP/p150 dynactin complex to recruit dynein motors to the surface of LEs (Figure 5C). Dynein-loaded

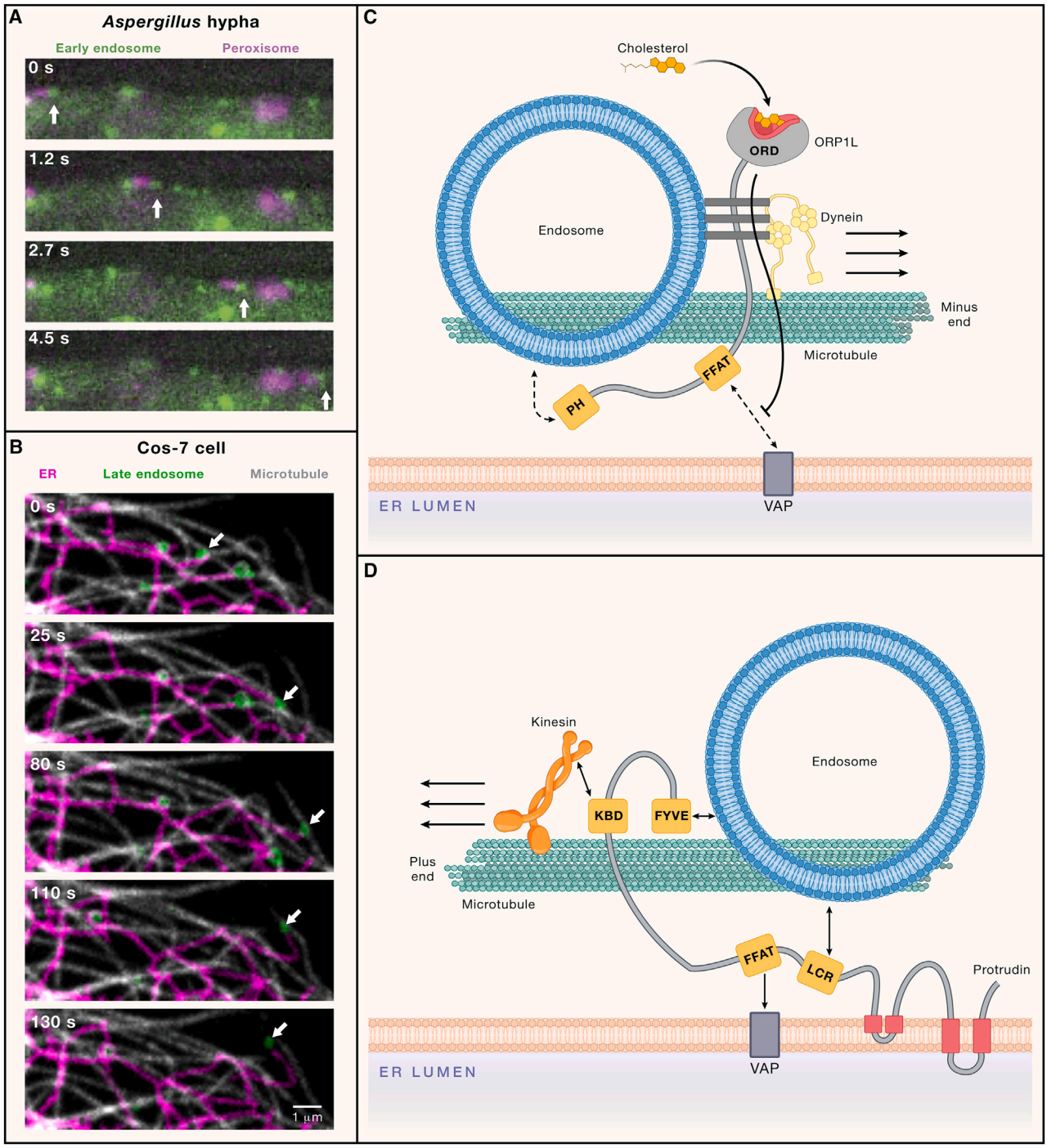

Figure 5. Membrane contact sites regulate organelle trafficking

(A) In the extended hyphae of filamentous fungi, peroxisomes, hitchhike on early endosomes moving along polarized microtubules. The endosomal protein PxdA is required for perixosome, but not endosome, transport, acting as a molecular link between the two organelles. Reproduced with permission.[85] Scale bars, 5 μm.

(B) In mammalian cells, trafficking late endosomes move along microtubules while maintaining contact with ER tubules (see arrowhead). Images courtesy of Haoxi Wu. Scale bars, 1 μm.

(C) ORP1L regulates endosome transport and contacts. Cholesterol is a ligand for ORP1L. ORP1L contains a PH domain to bind endosomal lipids, an FFAT domain to bind the ER protein VAP, and an ORD domain to bind cholesterol. Under high cholesterol concentrations, ORP1L adopts a conformation that favors dynein recruitment to endosomes, leading to their transport to microtubule minus ends. This conformation also disfavors ER contact. By contrast, low cholesterol levels inhibit dynein recruitment and facilitate endosome-ER membrane contact site formation.

(D) The integral ER membrane protein protrudin assembles at a tripartite ER-endosome-microtubule interface through its multiple interaction surfaces. Protrudin contains FYVE and LCR domains to bind to the endosome, a kinesin binding domain (KBD), and an FFAT domain that could allow it to bind VAP. In doing so, protrudin integrates the trafficking of endosomes and the ER together along microtubules.

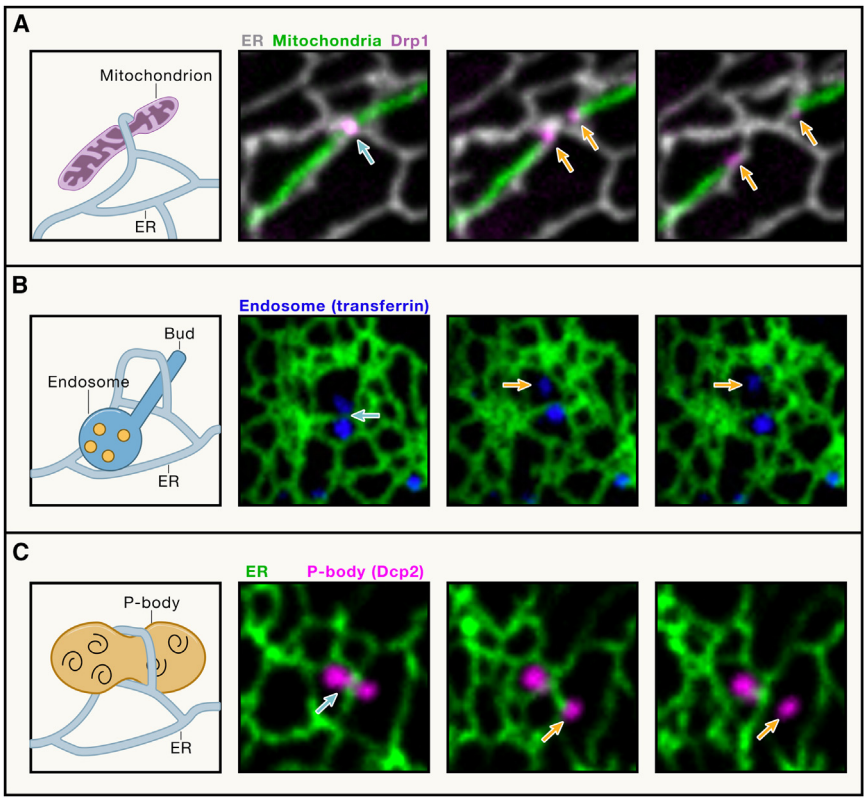

Figure 6. ER tubules induce organelle division at membrane contact sites

(A) ER tubules wrap around and constrict mitochondria at sites of mitochondrial fission. Drp1 accumulates at the ER-mitochondrial MCS and further constricts the mitochondrion to promote fission at the MCS. Images courtesy of Jason Lee.

(B) ER tubules mark sites where recycled cargos accumulate in endosome buds as well as the sites of endosome fission, where buds scission from the endosome vacuole. Here, an early endosome marked by its cargo, transferrin, forms an MCS with the ER (cyan arrow) and then undergoes ER-associated fission (yellow arrowhead). Reproduced with permission.[98]

(C) ER tubules contact P-bodies prior to their division. Unlike mitochondria and endosomes, P-bodies are membraneless organelles. The biological significance and molecular mechanism of P-body division are not known. Images courtesy of Jason Lee.

LEs then traffic toward the minus end of MTs, where the endosomes can fuse with lysosomes and be degraded. This allows cholesterol in the endosomes to traffic to other cellular compartments or to be stored in LDs. However, when the ORD of ORP1L is "open" and not bound to cholesterol (when cellular cholesterol levels are low), the ORP1L open conformation exposes an FFAT domain that can bind to the ER-resident protein VAP, bridging the ER-LE MCS (Figure 5C).[89,94,95] VAP binding disrupts the RAB7/RILP/p150/dynein complex. Multiple lines of evidence support this hypothesis: minus-end-directed trafficking of endosomes is disrupted when ORP1L lacks its ORD (and cannot bind cholesterol) or when drugs are used to deplete cellular cholesterol; this effect can be rescued by VAP depletion. Together, the data suggest that when endosomal cholesterol concentrations are low, ORP1L has an open conformation that can bridge through its FFAT domain to form an MCS with the ER protein VAP instead of cholesterol, and this promotes dynactin complex disassembly to disrupt dynein trafficking to the MT-organizing center (MTOC).[89]

One function of plus-end-directed trafficking of LEs is to promote their fusion with the PM and to supply new membranes to growing protrusions formed at the leading edge of migrating cells. Another ER MCS complex that promotes plus-end trafficking of LEs on MTs is mediated by the ER protein protrudin. Protrudin (which gets its name because it is enriched in protrusions of LEs) is an ER-LE contact site protein that loads kinesin onto LEs to stimulate plus-end-directed trafficking (Figure 5D). Structure function studies reveal that protrudin contains multiple domains that each interact with organelles or motors. Their arrangement within a single protein allows protrudin to integrate organelle motility into the context of an MCS. Protrudin contains a protein-binding low complexity region (LCR) and a PIP-binding FYVE zinc-finger domain to simultaneously interact with two components found on LEs: Rab7 and PI3P, respectively. Protrudin also contains a Kif5b (kinesin heavy chain)-binding domain to help recruit Kif5b/kinesin to ER-LE MCSs. Protrudin cooperates with a kinesin adaptor protein FYCO1 to load LEs on kinesin.[90] Together, protrudin, FYCO1, and Rab7 are all required to recruit Kif5b to LEs for trafficking to the PM and to promote Syt7-dependent fusion with the PM.[90] Interestingly, protrudin also contains an FFAT domain, which might be important for it to interact with VAP; this is worth noting because it could link protrudin to ORP1L sites to couple kinesin recruitment with dynein displacement, perhaps making both complexes sensitive to cholesterol levels.

THE ER REGULATES THE DIVISION OF OTHER ORGANELLES VIA MCSs

The biogenesis of many organelles depends on their ability to constantly undergo fission (and fusion) reactions in interphase cells. Until a little over a decade ago, our mechanistic understanding of organelle fission and fusion was mostly focused on understanding how cytoskeletal elements, lipid composition, tethers, and curvature-generating proteins drive the constriction, fission, and/or fusion of the target membranes. Every organelle was considered in isolation. Serendipity led us to consider how an additional variable could be involved: ER contact sites. While using EM tomography to visualize the three-dimensional (3D) architecture of the elaborate interconnected ER network in yeast, we noticed something unexpected: tubular ER clamps wrapped in a C shape around constrictions in mitochondria (Figure 6A).[96,97] These structures suggested an exciting new function for smooth (i.e., ribosome free) ER tubules. Subsequent

descriptive and paradigm shifting experiments using confocal fluorescence microscopy of live cells revealed that mitochondrial constriction and division were almost always where ER tubules wrapped about mitochondria.[96] This correlation was found in organisms from yeast to mammals.

Accumulating evidence suggests that mitochondria undergo fission (and fusion) to ensure mitochondrial metabolic and genomic health. Mitochondria and their genomes can be damaged by oxidation, and cells have evolved mechanism to ensure that daughter cells receive functional mitochondria, keeping damaged mitochondria in the mother. Visualizing the order of events that lead to ER-associated mitochondrial constriction and division revealed details of how this process is regulated but also unearthed many surprises. The first was that the inner mitochondrial membrane (IMM) is constricted at ER contact sites, even in the absence of the mitochondrial Drp1/Dnm1 constriction/division machinery,[96] which had been thought to initiate mitochondrial membrane deformation during division. Second, IMM constriction at ER contact sites precedes outer mitochondrial membrane (OMM) constriction.[99] And third, mitochondrial DNA nucleoids accumulate at the position of ER-associated division.[100] These data suggest either a model whereby replicating nucleoids in the matrix directly signal ER recruitment to the OMM or, alternatively, that ER contact with the OMM somehow remodels the IMM and recruits nucleoids from the matrix to the position of contact. Because the nucleoids are in the matrix and the ER is wrapped around the outside of the OMM, there must be several multi-membrane-spanning complexes that link the ER, OMM, IMM, and nucleoids in the matrix, allowing constriction to begin with the IMM and then occur in the OMM in a coordinated fashion. The composition of the IMM and matrix-associated layers of these proposed multi-membrane-spanning tethering complexes remains unknown. However, several studies have probed possible ways in which ER contact sites could stimulate OMM constriction.

The first mechanism proposed was that an ER-localized inverted formin (INF2) localizes to ER-mitochondrial contact sites and recruits an actin-myosin ring to the interface to push against the OMM and initiate its constriction.[101,102] We know this must be one of the later steps in mitochondrial division because OMM constriction follows IMM constriction. It also remains an appealing possibility that ER-mitochondrial contact sites alter the phospholipid composition of regions of the mitochondrial membranes in a way that promotes membrane deformation and constriction. An ER acyl hydrolase, ABHD16A, was recently identified through proximity biotinylation of ER-mitochondria contact site proteins.[103] ABHD16A was proposed to generate cone-shaped lipids (lysophospholipids) on the ER that are transported to the mitochondria at contact sites to drive membrane curvature. This model is supported by the findings that depletion of ABHD16A blocks IMM/OMM constriction, prevents recruitment of both mitochondrial fission and fusion machineries to ER contact sites, and that rescue requires the acyl hydrolase domain of ABHD16A.[103] An important and still only partially answered question is what cells gain by linking mitochondrial division with ER-mitochondria contact sites? A common theme is that the ER-mitochondrial contact site establishes a local ''hybrid'' lipid/protein environment that is pre-constricted and primed to recruit the fission machineries.

Other organelles have also been localized to sites of ER-associated mitochondrial division, including lysosomes and Golgi-derived vesicles.[104,105] According to recent data, mitochondria that undergo ER- versus lysosome-associated fission events have different fates: biogenesis versus mitophagy, respectively.[106] Specifically, lysosome-marked mitochondrial division sites tend to occur near the periphery of a mitochondrial tubule and result in a mitochondrial compartment that has a reduced membrane potential, higher Ca^{2+}, lacks nucleoids, and contains autophagic markers like Parkin.[106] By contrast, midzone mitochondrial fission events occur during healthy mitochondrial proliferation and are consistently marked by the ER.[96,106] How these two pathways are coordinated remains to be determined.

ER MCSs also define the position and timing of endosome fission, which functions to sort cargoes within the endocytic pathway (Figure 6B).[98] Endosomes undergo fission and fusion events as they traffic from their inception at the PM toward their perinuclear destination, where they fuse with lysosomes. The purpose of these endocytic fission and fusion events is to sort cargo to be recycled—back to the PM or Golgi and away from cargo that will be degraded in lysosomes. Cargo destined for degradation is internalized into intra-lumenal vesicles (ILVs) within the vacuolar compartment, whereas cargo that will be recycled is pulled into budding domains of the lysosome membrane by the budding machinery. The divide between these two compartments during fission is defined by contact sites with ER tubules.[98]

How ER-endosome contacts contribute to or respond to cargo sorting in endosomes remains a fascinating question. The answer has begun to emerge for retromer-dependent cargo sorting from LEs to the Golgi complex. Retromer-dependent bud formation and fission of LEs can be visualized quite vividly by live-cell imaging as a sequence of productive events: first, dynein helps pull a tubule out of the endosome and these tubules are stabilized by a sorting nexin. Sorting nexins are cytoplasmic proteins that can be recruited to membranes where they have domains that stabilize membrane tubules/buds and bind cargo receptors; for review, see Daly and Cullen.[107] The sorting nexins (Snx2) then recruit the retromer complex, so called because it is a receptor that facilitates retrograde traffic of proteins back to the Golgi complex.[108–110] The retromer then recruits the Wiscott-Aldrich syndrome protein and scar homolog (WASH) complex onto the bud, which binds to the branched actin nucleator Arp2/3[109,111–114]; Arp2/3 nucleates branched actin assembly, which is thought to stabilize the bud to allow more time for cargo sorting[115]; and finally, Arp2/3 also recruits a negative regulator of branched actin assembly, coronin,[115,116] which functions to clear an actin exclusion zone at the base of the bud and recruit ER tubules to the bud.[115,116] The beauty of this system is that coronin localizes to the bud after cargo sorting and is required to recruit dynamic ER tubules to MCSs (through the TMCC1 protein tether) to trigger ER-associated fission.[116,117] This multi-step process is an example of how events are ordered to ensure that ER MCSs are recruited to promote bud fission only after cargo has been sorted. However, what is still missing in this

pathway is the final murky step: how do the ER contact sites drive constriction and division, and what is the division machinery?

The trend is that many different types of cytoplasmic organelles divide at contact sites. However, there are no shared machineries between ER-associated mitochondrial division and ER-associated endosome division. Even membraneless organelles like P-bodies and stress granules have been shown to divide at contact sites with ER tubules (Figure 6C).[118] The machinery necessary for this process has not yet been discovered but could be quite different from that for membrane-bound organelles.[118] Possibly, there are multiple ways to divide an organelle, and it simply requires some way of providing mechanical force; indeed, an interesting study by the Kornmann lab has shown this to be true for mitochondria.[119]

THE FUTURE OF ORGANELLE BIOLOGY

The discovery of ubiquitous contact sites between all organelles has changed our view of how organelles function and integrate their operations. Many fundamental questions remain. One is how contacts are formed and broken. Some contacts last the life a cell, while others persist for only a few seconds. Many tethers have been identified, but there are surely more to find. Whether there are general rules that govern contact site tethering is not yet clear, though it does seem that there are almost no proteins whose sole function is to tether; most tethers have functions beyond tethering. New methods to identify proteins at contact sites and identify tethers are necessary.

Another major question is how protein machineries that operate at contact sites between the same pair of organelles coordinate their functions. For example, there are ER-mitochondrial contact sites that facilitate the transfer of Ca^{2+}, mitochondrial fission, intracellular signaling, and lipid metabolism. Do all of these activities happen at the same ER-mitochondria contacts, and how are these different activities coordinated?

The bigger conceptual question about MCSs is whether they benefit cells beyond facilitating intracellular communication. Cells contain only one large ER, made of a single continuous membrane enclosing the lumen. If the ER is tethered to all organelles in at least one or multiple locations, and if the organelles themselves are often tethered to one another, then cytoplasmic organelles could be considered to form an "electrical" network. Does this allow multiple organelles to respond in unison to signaling, stimuli, or stresses? How is this network coordinated? What is the functional advantage of cell-wide communication?

In summary, the study of MCSs has fundamentally changed our view of how organelles integrate their operation, even though this is not yet reflected on the covers of cell biology textbooks. As we learn more about how these contacts form, are regulated, and their roles in helping cells respond to challenges, there are sure to be many more exciting discoveries about how organelles work together.

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G.K.V. is an editorial board member for *Cell* journal.

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