

# Geometry and cellular function of organelle membrane interfaces

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## Abstract

A vast majority of cellular processes take root at the surface of biological membranes. By providing a two-dimensional platform with limited diffusion, membranes are, by nature, perfect devices to concentrate signaling and metabolic components. As such, membranes often act as “key processors” of cellular information. Biological membranes are highly dynamic and deformable and can be shaped into curved, tubular, or flat conformations, resulting in differentiated biophysical properties. At membrane contact sites, membranes from adjacent organelles come together into a unique 3D configuration, forming functionally distinct microdomains, which facilitate spatially regulated functions, such as organelle communication. Here, we describe the diversity of geometries of contact site-forming membranes in different eukaryotic organisms and explore the emerging notion that their shape, 3D architecture, and remodeling jointly define their cellular activity. The review also provides selected examples highlighting changes in membrane contact site architecture acting as rapid and local responses to cellular perturbations, and summarizes our current understanding of how those structural changes confer functional specificity to those cellular territories.

## Introduction

Cellular membranes occupy a large fraction of the eukaryotic cellular space and enclose between 30 and 90% of the cell volume in separate intracellular compartments (Diekmann and Pereira-Leal, 2013). These membranes consist of lipids and proteins of different shapes, sizes, forms, and functions, assembled together in a meticulous arrangement leading to the formation of semi-permeable bilayers (Sonnino and Prinetti, 2010; Cheng and Smith, 2019). The molecular organization of membranes and their physical properties confer biological membranes the ability to set apart different subcellular compartments, and as such to

### ADVANCES

- MCS molecular and membrane architecture is diverse.
- Membrane geometry is emerging as a critical parameter for MCS function and specificity.
- Changes in MCS architecture, and underlying modification in molecular and cellular activities, are essential for cellular adaptation including sensing and responding to environmental stresses.

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define functional territories within the cell (Bigay and Antonny, 2012; Ernst et al., 2018). Because of the space they occupy, as well as their intrinsic physical and chemical properties, membranes tend to congregate a large repertoire of activities, ranging from signal perception, integration and transduction to lipid metabolism, cytoskeleton remodeling, and the formation of transport vesicles (Schmick and Bastiaens, 2014; Bozelli and Eband, 2020). Membranes operate by concentrating functional elements at strategic points within the cell, acting as information centralizers and multimodal processing cores. The activities at those cores often require bidirectional information processing across lipid bilayers (Hurtley, 2005), but also between juxtaposed organelle membranes at specialized subdomains known as membrane contact sites (MCSs; Scorrano et al., 2019).

The functional diversity of cellular membranes comes along with the broad multiscale structuring and forms they take, which range from local nanometer-scale lipid–protein domain organizations to broad micrometer-scale organelles with configurations stretching from flat-, curved-, and tubular-shape structures (Jarsch et al., 2016). Membranes also present differences in terms of charge densities, lipid packing, and membrane thickness (Bigay and Antonny, 2012), and these simple physical and geometrical parameters are used by the cell to generate sharp spatial–temporal responses (Bozelli and Eband, 2020; Noack and Jaillais, 2020; For a detailed description of anionic lipid gradient, subcellular activities, and plant development, see Dubois and Jaillais in this focus issue). In this review, we bring into focus how membrane shape and dynamics facilitate specialized responses to cellular perturbations within the structural framework of the endoplasmic reticulum (ER)-MCSs. These are ubiquitous membranous structures in eukaryotic cells that represent seemingly examples of how membrane geometrical attributes are intimately intertwined with specialized cellular functions.

Before leaping further into this review, we direct the reader to the unifying features and essential functions assigned to MCSs. MCSs can be defined as sites of close membrane apposition between different organelles (although examples of homotypic MCSs between membranes within the same organelle also exist; Scorrano et al., 2019). The term “contact” conveys the notion of physical interaction between membrane-bound organelles, whether bi- or monolayered [as for lipid droplets (LDs)], which is usually achieved by supramolecular protein assemblies that populate the intermembrane gap and generate tethering forces through protein–protein or protein–lipid interactions (For a detailed description of protein–lipid interactions in plants, see de Jong and Munnik in this focus issue). These protein assemblies confer MCSs with functional specificity and prevent membrane fusion by maintaining an intermembrane distance in the 10–80 nm range (although larger or even smaller MCS intermembrane distances have also been described; Ping et al., 2016; Nicolas et al., 2017). The vast majority of MCSs that were initially described characterized

interactions between the ER and other membrane compartments [ER–Plasma membrane (PM); ER–Golgi; ER–LDs; ER–mitochondria, etc.], but over the years, it has become clear that most organelles establish MCSs, and these structures are dynamic in time and space. The dominant view in the field has long been that MCSs were solely dedicated to direct nonvesicular exchange of molecules, such as lipids or  $\text{Ca}^{2+}$  (Phillips and Voeltz, 2016; Wu et al., 2018). The latest research in the field, however, has proven this concept too restrictive, and MCS functions expand much wider than originally thought. These functions now include, in addition to the bidirectional transport of molecules, membrane remodeling activities, such as organelle biogenesis and dynamics, autophagy, organelle fission, and regulation of enzymatic activities (Scorrano et al., 2019). This multiplicity of functions comes along with a diversity in MCS membrane geometry, shape, and dynamics, which is the focus of this review.

## Cellular function depends on membrane geometrical parameters

As stated earlier, membrane geometry and cellular processes are intimately linked. The former helps to define local membrane environments holding unique properties through curved configurations (whether positive or negative, sharp or blunt), overall shape, and 3D architecture (McMahon and Boucrot, 2015). By building up microenvironments, which concentrate macromolecular assemblies dedicated to specific functions and assist protein activity, membrane geometry directly impacts cellular activities (Aimon et al., 2014; Schmick and Bastiaens, 2014; Iversen et al., 2015; Bozelli et al., 2018; Haucke and Kozlov, 2018; Lou et al., 2019; Bozelli and Eband, 2020; Larsen et al., 2020). MCSs are excellent examples of how membrane geometry influences cellular function. Their 3D organization enables the juxtaposition of two membranes over a short distance, and this tethered apposition is central to their function, for instance (but not only) for lipid transfer (Lahiri et al., 2015). Local membrane shaping and curvature also modifies MCS interfacial properties (Vanni et al., 2014), which in turn strongly impact protein recruitment, membrane deformation and budding, and lipid distribution within the same membrane and even across MCSs (as illustrated below). In other words, MCS membrane geometry and 3D organization are fundamental factors clenching on both specificity and versatility of function. Below are some examples, mainly extracted from yeast and mammals, which illustrate our point.

Autophagy is an evolutionarily conserved catabolic process characterized by the formation of a double-membrane vesicle, the autophagosome, which engulfs cytoplasmic components and delivers them to the lysosome or vacuole for recycling (Enrique Gomez et al., 2018; Nakatogawa, 2020). This essential cellular process requires the establishment of MCSs and heavily relies on membrane geometrical parameters such as curvature induction. For instance in mammals,

and within the frame of selective ER-phagy (also called reticulophagy), several receptors acting on selective ER-phagy display membrane-shaping functions (Grumati et al., 2017; Bhaskara et al., 2019; Chen et al., 2019). Among the best studied examples, the mammalian receptor for ER turnover, Family with sequence similarity 134 member B (FAM134B), oligomerizes at autophagy puncta and induces membrane curvature in the ER while physically bridging the ER and the phagophore membranes through its interaction with MAP1LC3B (Bhaskara et al., 2019). By combining membrane tethering and curvature, FAM134B oligomerization promotes the recruitment of the autophagy machinery at these transitory MCSs and helps pinching-off targeted ER membranes for subsequent degradation (Jiang et al., 2020). In other words, through their membrane remodeling function, membrane-shaping proteins set the stage for creating localized and temporally regulated organelle responses.

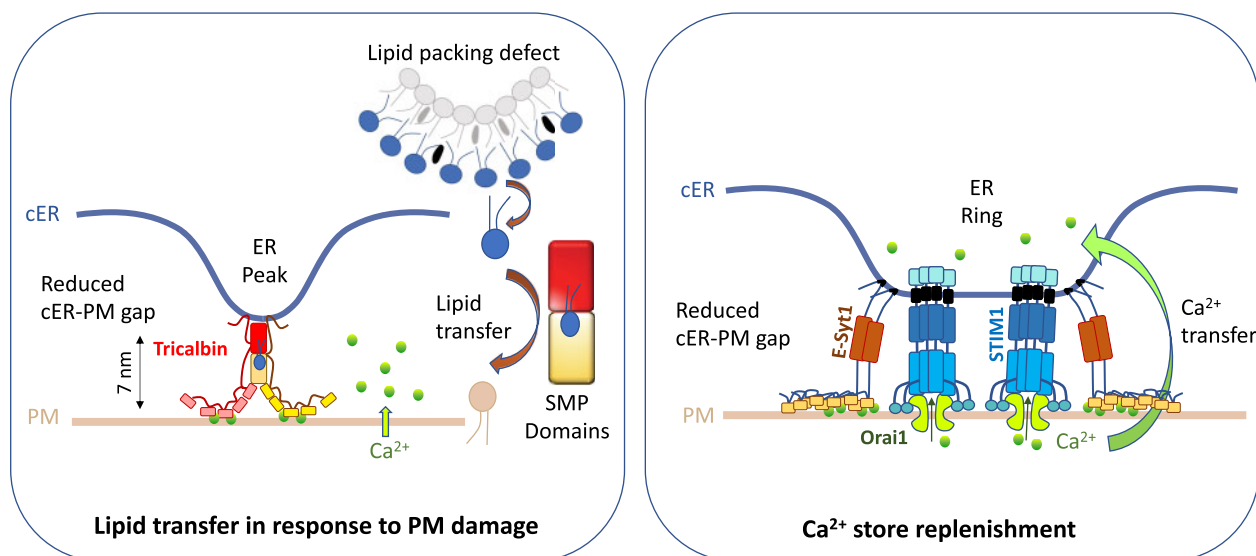
LD biogenesis provides another example of how membrane shaping and MCS activity are intrinsically linked. In mammals, the LD biogenesis process initiates at localized ER subdomains where neutral lipids accumulate between the ER leaflets and coalesce to form lens-like structures (nascent LDs; Walther et al., 2017). These first steps of LD initiation have been imputed to the high membrane curvature of tubular ER, which favors the condensation of triglycerides by lowering the energetic barrier for lipid de-mixing, thereby leading to neutral lipid accumulation in nascent LDs (Roberts et al., 2020; Santinho et al., 2020). Shortly after initiation, LDs grow and extend toward the cytosol through a continuous neutral lipid flux from the ER to the LD, while remaining connected to the ER. This process involves the oligomeric seipin complex, which accumulates at LD lens initiation sites, possibly owing to its ability to recognize phospholipid packing defects (i.e. when lipids are loosely packed) generated by local membrane deformation (Sui et al., 2018). Seipin acts in anchoring and stabilizing nascent LDs to the ER and generates a neck region of high membrane curvature between the two structures (Salo et al., 2016, 2019; Greer et al., 2020). This process might be linked to seipin's propensity to bind and locally stabilize the cone-shaped lipid phosphatidic acid (Yan et al., 2018). Although this remains to be experimentally confirmed, these ER tubes have been proposed to facilitate flow of neutral lipids from the ER to the LD (Salo et al., 2019; Santinho et al., 2020). Despite being structurally unrelated, the biogenesis of peroxisomes from the ER in mammals similarly requires local ER remodeling by membrane-shaping proteins (Joshi et al., 2018; Wang et al., 2018), suggesting that organelle buddings from the ER share common structural and molecular bases. Remarkably, the spatial organization of mammalian LD tethers goes beyond the formation of bi-organellar contacts during LD biogenesis. Indeed, mature LDs display the structural capacity to form tri-organellar MCSs that facilitate bulk lipid flux by positioning lipid metabolism enzymes next to LD contacts (Bohnert, 2020). This is exemplified by the mitoguardin 2 tether in mammalian adipocytes that interacts with ER-localized

vesicle-associated membrane protein-associated proteins (VAPs) and coordinates the lipid transfer activities of mitochondria, LDs, and the ER within a single MCS (Freyre et al., 2019).

The structural mechanism by which membrane curvature could facilitate lipid exchanges *in trans* between two organelles at MCSs is illustrated by recent analyses of ER shaping functions mediated by the yeast tricalbins, which are homologous to the animal extended synaptotagmins (E-Syts) and plant synaptotagmin ER-PM MCS tethers. These studies showed that, in addition to tethering the cortical ER (cER) to the PM, tricalbins create peaks of high membrane curvature which extrude from the cER facing the PM, with the consequence of reducing the cER-PM gap from 22 to 7 nm (Collado et al., 2019; Hoffmann et al., 2019). These peaks would promote lipid export between membranes by first facilitating lipid extraction from the positively curved cER, and second, reducing the intermembrane gap, hence the transport distance (Figure 1). The importance of membrane curvature in nonvesicular lipid transfer was previously discussed and experimentally tested within the frame of the sterol/PI4P lipid exchange at mammalian ER-trans-Golgi MCSs (Von Filseck et al., 2015). In this example, the mammalian oxysterol-binding homology protein Osh4p, which mediates sterol/PI4P exchange between the ER and trans-Golgi network, shows faster transport efficiency *in vitro* when lipid-packing defects (which depend on membrane curvature and lipid composition) increase. The proposed mechanism underlying this observation is that sterol desorption from the ER is facilitated by lipid-packing defects that make the membrane more permissive for lipid extraction. By generating an asymmetrical geometry of the membranes positioned face to face at MCSs (for instance, curved membrane at the ER and flat at the PM or Golgi), membrane shape could theoretically regulate directional lipid transfer (For details of how this process is powered by lipid gradients between the closely apposed membranes, see Dubois and Jaillais in this focus issue). It is currently unknown whether ER shaping is a conserved function of plant tethers, but the evolutionary conservation of the proteins involved in the process, and the multiple phenotypes and activities associated with their loss of function strongly suggest that the actions of MCS tethers in plants go beyond mechanical anchoring.

### Highly specialized protein arrangements underlie ER-MCS architecture and function

The wide variety of cellular and physiological processes that take place at ER-MCSs demonstrates their importance in the normal development and physiology of eukaryotic organisms. The emerging picture in the field, whether in yeast, mammal, or plant, is that MCS functions are essentially determined by their molecular composition (Perez-Sancho et al., 2016; Scorrano et al., 2019). Hence, the importance to characterize the unique protein (and lipid) make up that shapes those cellular microdomains and



**Figure 1** ER shaping accompanies lipid and Ca<sup>2+</sup> transfer at ER–PM MCSs. Left: The yeast tethers tricalbins generate high curvature at the cER (cER peaks) through their transmembrane domains and reduce inter-organelle distance possibly by binding to the PM in a Ca<sup>2+</sup>-dependent manner (most likely not all C2s interact with the PM). High membrane curvature at cER peaks induces lipid packing defects and may facilitate lipid extraction by the tricalbin SMP lipid transfer domains, which would then transfer phospholipids to the PM from the ER. This response may occur during heat stress, when PM integrity has lost generating Ca<sup>2+</sup> entry in the cell and subsequent tricalbin-mediated cER peak at ER–PM MCS to facilitate lipid transport and PM repair. Adapted from Hoffmann et al. (2019) and Collado et al. (2019). Right: In nonexcitable animal cells, depletion of the ER Ca<sup>2+</sup> stores induces the formation of cER–PM MCS to activate SOCE and refill the ER. The drop in luminal ER Ca<sup>2+</sup> causes STIM1 to oligomerize and to transfer to ER–PM MCS where it interacts with and traps the Ca<sup>2+</sup> channel Orai1. Subsequently, Ca<sup>2+</sup> influx via SOCE induces E-Syt1 to reshape the originally formed MCSs into ring structures. These rings have been proposed to facilitate Ca<sup>2+</sup> store replenishment by reducing the ER–PM gap and stabilizing the structure. Adapted from Kang et al. (2019).

confers them with functional specificity. In an attempt to develop a unified terminology for the MCS field, Scorrano et al. (2019) classified the putative MCS protein residents into four possible categories based on molecular functions either assigned or predicted for those MCS components. In this classification, MCS proteins could have: (1) structural roles carried out by tethers that hold the two organelles together or spacers that keep the two membranes at a defined distance; (2) sorting roles carried out by proteins that define the contact site proteome and lipidome through active recruitment or exclusion of proteins into the contact site microdomain; (3) roles in the bidirectional transfer of materials mediated by ion channels, lipid transfer proteins (LTPs), or metabolite transporters; and (4) signaling roles mediated by sensors that integrate environmental and developmental cues and modulate the extent of the MCS itself, as well as the relative positioning and activity of the proteins within (Scorrano et al., 2019).

Remarkably, the repertoire of structural functions and biochemical activities assigned to MCS components keeps growing in the literature, making their classification into a single category increasingly difficult. This difficulty is clearly exemplified by the multiplicity of functions associated with the activity of the E-Syts at mammalian ER–PM contact sites. Early genetic and cell biology analyses showed that E-Syts form heteromeric complexes that act as tethers regulating the establishment of ER–PM contacts, a process which depends on both cytosolic Ca<sup>2+</sup> and the anionic lipid

PI(4,5)P<sub>2</sub> (Giordano et al., 2013; Idevall-Hagren et al., 2015). Subsequent cryoelectron tomography studies outlined E-Syts as spacers capable of maintaining the intermembrane cER–PM distance of about 20 nm (Fernández-Busnadiego et al., 2015; Collado et al., 2019). Besides their function as tethers/spacers, E-Syt1 and E-Syt2 also facilitate the nonvesicular transfer of phospholipids and diacylglycerol between the cER and the PM through their synaptotagmin-like mitochondrial lipid-binding protein (SMP) domains (Schauder et al., 2014; Saheki et al., 2016; Yu et al., 2016; Jeyasimman and Saheki, 2020). During this process, E-Syt1 complexes release the auto inhibitory conformation of their SMP domains, reducing the cER–PM intermembrane gap (Saheki et al., 2016; Bian et al., 2018), and the E-Syt2 SMP domain dimerizes to form a 9-nm-long hydrophobic cylinder that enables the nonvesicular transfer of glycerophospholipids (Schauder et al., 2014). These findings illustrate how the E-Syt activity in mammals results from a combination of molecular actions that secure lipid transfers at cER–PM MCSs, presumably as a specific inter-organelle response to elevated Ca<sup>2+</sup> (Chang et al., 2013; Idevall-Hagren et al., 2015; Bian et al., 2018; Kang et al., 2019). Adding to this complexity, the internal MCS architecture often creates asymmetric distribution of proteins along the membrane interface that generates territories with varied composition and specific function (Pérez-Sancho et al., 2016; Perico and Sparkes, 2018; Baillie et al., 2020; Prinz et al., 2020; Zaman et al., 2020). This is exemplified by the yeast ER–PM contact sites

where membrane curvature restricts the localization of VAP tethers to relatively flat cER sheets and the localization of tricalbin tethers to the tubular cER and curved edges of the cER sheets (Collado et al., 2019; Hoffmann et al., 2019). The information gathered from yeast and mammals suggests that to fully understand how MCS components trigger specific and conditionally regulated ER activities in plants, we will need to first identify their repertoire of activities, but also define the structural parameters conferring functional specificity within the plant MCS territories.

### Architectural changes of cellular membranes act as a dynamic response to the environment

Cellular membranes are deformable material that can be quickly and easily remodeled. This property represents a significant advantage when it comes to physiological adaptations at the cellular level. In fact, cells commonly modify the geometry of their membranes in response to physiological perturbations with the consequence of directly affecting membrane-based signaling and metabolic reactions. A prime example of membrane re-shaping is the profound remodeling of the cER polygonal network in response to the developmental cues or external perturbations. In plant cells, the remodeling of cER membranes is largely driven by cER–cytoskeleton interactions (Wang et al., 2014); ER-shaping and curvature stabilizing proteins, such as reticulons (Sparkes et al., 2010; Breeze et al., 2016; Brooks and Dixon, 2020) and lunaparks (Kriechbaumer et al., 2018; Sun et al., 2020); ER fusogens, such as the membrane-anchored root hair defective 3 family of GTPases (Chen et al., 2011; Zhang et al., 2013; Ueda et al., 2016); and molecular anchors, such as Synaptotagmin1 (SYT1) and Vesicle-Associated membrane Protein-associated 27 (VAP27) tethering complexes. The coordinated action of some of these components has been deemed essential to maintain the structural stability of the polygonal ER network (Siao et al., 2016), and to create remodeling nodes (ER–PM MCSs) where ER tubules connect and “wrap” around (Bayer et al., 2017; Lin et al., 2017).

Like any cellular membranes, the ER–PM MCS diversity in shapes, hence functions, directly relates to their plastic nature. As previously described, the emerging consensus is that the specialized functions assigned to a particular MCS are determined by a cooperative phenomenon involving changes in its 3D membrane architecture and molecular organization (Fernández-Busnadiego, 2016; Petit et al., 2019). This dynamic interplay involves, among others, cytoskeletal components, specific membrane lipids, and MCS protein residents, which altogether modulate the MCS biochemical activities at the membrane interface and ultimately determine its functional specificity. For instance, multiple studies in eukaryotes, including yeast, mammals and plants, have shown that ER–PM MCSs have the ability to regulate the MCS intermembrane gap and to change their overall architecture in response to cellular perturbations. Some of these studies relied on super-resolution microscopy and single-

particle tracking techniques to analyze the nanoscale dynamics of MCS-resident proteins, as well as the subsequent changes in MCS membrane architecture driven by their activity. For example, in mammalian cells, single particle tracking was used to study changes in the patterns of diffusion and trapping of stromal interaction molecule 1 (STIM1) and Orai1  $\text{Ca}^{2+}$  channels (named “Orai” after the Greek keepers of the gates of heaven) during stress-induced store-operated calcium entry (SOCE) at ER–PM MCSs (Wu et al., 2014). In yeast, cryoelectron tomography coupled with correlative light and electron microscopy was used to show that tricalbins generate peaks of extreme curvature on the cortical ER, presumably to sustain lipid transfer and maintain PM integrity during heat stress (Collado et al., 2019). Finally, in plants, time lapse imaging techniques and persistence mapping were used to analyze the changes in architecture and dynamics of ER–PM MCSs in response to ionic stress (Lee et al., 2019; For detailed information on membrane imaging techniques in plant systems see Liu et al., in this focus issue). The plethora of structural data obtained in these studies, combined with genetic and functional analyses of MCS components, clearly establish that MCSs serve as dynamic platforms hosting a variety of regulatory activities (Lewis and Lazarowitz, 2010; Mehrshahi et al., 2013; Levy et al., 2015; Pérez-Sancho et al., 2015; Ishikawa et al., 2018, 2020; Lee et al., 2019; Wang et al., 2019). In the next section, we will provide mechanistic information of the multiple factors that contribute to the establishment, stabilization, tightening, and remodeling of different ER–MCS structures in response to cellular and environmental perturbations.

### Changes in MCS membrane shape and intermembrane distance allow rapid and local responses to cellular perturbations

By using examples from selected eukaryotic organisms, we now illustrate our current understanding of how the cortical cytoskeleton, lipids, and cellular and environmental signals influence MCS architecture and re-shaping activity and how this relates to MCS-specialized functions.

#### Regulation of MCS architecture by the cytoskeleton

The narrow width of the cytosolic sleeve, combined with the molecular crowding at ER–PM MCSs, can impose steric restrictions on the cortical cytoskeleton network. This phenomenon has been described structurally for the contractile ring assembly in the fission yeast *Schizosaccharomyces pombe*, where prominent ER–PM contacts restrict actomyosin kinetics and limit the PM accessibility for the initial allocation of ring precursors along the cell cortex (Zhang et al., 2016; Zhang 2020). Conversely, cortical cytoskeleton components can create trapping mechanisms regulating MCS architecture and expansion. For example, in HeLa cells, the microtubule (MT) plus-end binding protein EB1 traps and delays the translocation of the ER-localized  $\text{Ca}^{2+}$  sensor STIM1 to ER–PM MCSs, effectively preventing  $\text{Ca}^{2+}$  overaccumulation at the ER lumen (Chang et al., 2018). The

extension of ER–PM membrane contacts in mammals also depends on F-actin dynamics. Thus, following  $\text{Ca}^{2+}$  depletion in ER and subsequent cytosolic  $\text{Ca}^{2+}$  elevation, the ER stress sensor PERK-related endoplasmic reticulum kinase (PERK) dimerizes and forms a complex with the actin-binding protein Filamin A. This complex modifies F-actin remodeling and polymerization dynamics with the consequence of favoring the expansion of ER–PM MCSs. Cells that do not express PERK accumulate F-actin at the cell edges, which obstructs ER–PM contact expansion (Van Vliet and Agostinis, 2016).

In plants, the ER–PM MCSs and the cortical MT network create mutual exclusion zones (Pérez-Sancho et al., 2015; Wang et al., 2016; McFarlane et al., 2017). This exclusion is likely determined by the narrow ER–PM MCS intermembrane gap, as well as by the molecular crowding at the ER–PM MCS interface. Remarkably, MT depolymerization (stress or pharmacologically induced) influences the lateral diffusion of SYT1 tethers within the ER–PM MCS but does not affect the morphology and/or expansion of pre-existing ER–PM MCSs (Pérez-Sancho et al., 2016; Lee et al., 2019). Furthermore, ER–PM MCSs expand in response to the PM lipid composition in a process that does not disrupt the cortical MTs, suggesting that the cortical MT network does not create physical constraints to ER–PM MCS expansion (Lee et al., 2019). Although these studies propose additional processes (e.g. local  $\text{Ca}^{2+}$  or lipid signaling) as the drivers of ER–PM MCS remodeling in response to stress, they cannot rule out that a functional MT network could still be required for the establishment and/or formation of de novo ER–PM MCSs. For example, these studies did not address whether MT disruption during cell division, a process that heavily relies on membrane trafficking and remodeling through MT-associated proteins, influences the generation of ER–PM MCS at the nascent cell plate.

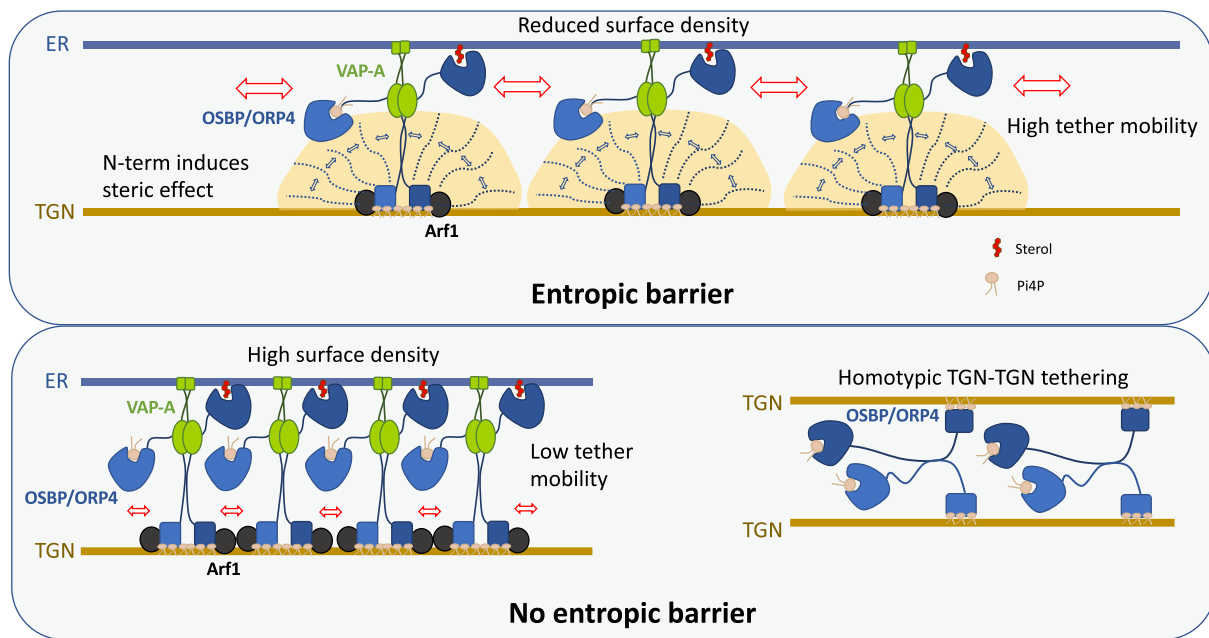
In contrast, the spatial proximity and physical associations between ER–PM MCS components and the actin cytoskeleton in plants are essential for the establishment and function of ER–PM MCSs (Lewis and Lazarowitz, 2010; Stefano et al., 2018; Wang et al., 2019). These interactions are mediated by members of the plant-specific NETWORKED (NET) family that act as membrane cytoskeleton adaptors (Deeks et al., 2012) and localize actin filaments in close proximity to ER–PM MCSs (Wang et al., 2014). For instance, the Arabidopsis (*Arabidopsis thaliana*) actin-binding protein NET3C interacts with the lipid-binding protein VAP27-1 at punctate ER–PM junctions creating actin patch assemblies where autophagy intersects with endocytic processes (Stefano et al., 2018; Wang et al., 2019). In the next section, we describe how these and additional factors, such as local lipid composition and the activity of LTPs, establish the MCS architecture that facilitates the assembly of autophagy and endocytosis machineries in plants.

### Regulation of MCS architecture by lipids and LTPs

In mammals, lipid transfer activities are required at ER–autophagosome MCSs to sustain the omegasome growth

into its typical highly curved structure (Axe et al., 2008; Biazik et al., 2015). This process necessitates the regulated partition of incoming lipids in time and space (Von Filseck et al., 2015; Nascimbeni et al., 2017; Hsieh and Yang, 2019). The regulatory role of MCSs in the process has been inferred by the suppression of autophagosome formation upon knockdown of tethering factors at ER–mitochondria MCSs (e.g. mitofusin 2; Hamasaki et al., 2013) or ER–PM MCSs (e.g. E-Syts1/2/3; Nascimbeni et al., 2017) which presumably prevents, among other activities, lipid intake (Schütter et al., 2020). Multiple studies in mammals also point toward MCS-localized LTPs, which contain two phenylalanines in an acidic tract motif and oxysterol-binding protein (OSBP)-related proteins (ORPs), as essential contributors for the correct positioning and transfer of different lipid species onto the autophagosome membrane (Hammond and Pacheco, 2019; Ye et al., 2020). In this context, the activity of intrinsically disordered regions within the LTPs' 3D structure and their interactions with specific phosphoinositides underlie the MCS' ability to change its geometry and regulate lipid transfer. For example, in HeLa cells, the unfolded N-terminus of the two related oxysterol-binding proteins, OSBP and ORP4, acts as entropic barrier whose thermal motion limits OSBP surface density at ER–trans-Golgi network MCSs, preventing their accumulation and immobilization by protein crowding. A second effect of the N-terminus is to prevent the two PH domains from simultaneously bridging over the two membranes through PI4P interaction and to promote heterotypic membrane tethering between VAP-containing and PI4P-containing membranes at the ER–trans-Golgi network interface (Jamecna et al., 2019; Figure 2).

In yeast and mammals, MCS-localized ORPs and additional components, such as the StArkin family of sterol-binding proteins, regulate the local accumulation of phospholipids and sterols in lipid membranes (Schulz et al., 2009; Gatta et al., 2015; Quon et al., 2018; Tong et al., 2018). These accumulations generate liquid-ordered membrane micro-domains exhibiting positive and negative membrane curvature (Bacia et al., 2005; Bigay and Antonny, 2012) that could serve as positional cues for the assembly of autophagy and/or endocytosis machineries (Hirama et al., 2017; Nguyen et al., 2017). Studies in yeast have shown that the interaction between ORPs and VAP27 homologs is a requirement for the generation of ER-derived autophagic or endocytic structures (Encinar del Dedo et al., 2017). Through these interactions, ORPs could provide the lipid environment that generates the shape and curvature of the nascent autophagosome/endocytic membrane, and VAPs could act as membrane anchors that attach those nascent structures to the contact site (Murphy and Levine, 2016). This model has a mechanistic precedent in plants where tomboviruses co-opt ORP and VAP proteins from ER–PM MCSs to generate sterol-rich membrane surfaces with high curvature, presumably to facilitate virus replication through local lipid channeling (Barajas et al., 2014). The ER–PM MCSs in plants also host an alternative autophagy-dependent pathway that is



**Figure 2** An entropic barrier controls membrane tethering geometry. Top: OSBP/ORP4 (blue dimers) transports cholesterol from the ER to the *trans*-Golgi network (TGN) and back transfer PI4P in exchange. They interact via their PH domain, with PI4P and the small G protein Arf1 (black) on the TGN membrane and with the ER-resident protein VAP-A (Green) via their FFAT motif. Both OSBP and ORP4 present a long N-terminal unstructured region (N-term), which through steric effect controls OSBP/ORP4 density at MCS by limiting the surface density of the downstream PH domain. This in turn facilitates protein diffusion within MCS and protein recycling between MCS. The N-term region also controls the geometry of ER–TGN MCSs and protein orientation by favoring heterotypic membrane tethering (TGN–ER) between VAP-containing and PI4P-containing membranes. Bottom: In the absence of the N-term region, protein density is higher and mobility reduced (left). Homotypic configuration (TGN–TGN), where two PI4P-rich TGN membranes are tethered by the two PH domains of OSBP/ORP4, is now favored. Adapted from [Jamecna et al. \(2019\)](#). Arf1: adenosine diphosphate-ribosylation factor; FFAT: two phenylalanines in an acidic tract; PH: pleckstrin homology; TGN: *trans*-Golgi network; VAP-A: vesicle-associated membrane protein-associated protein A.

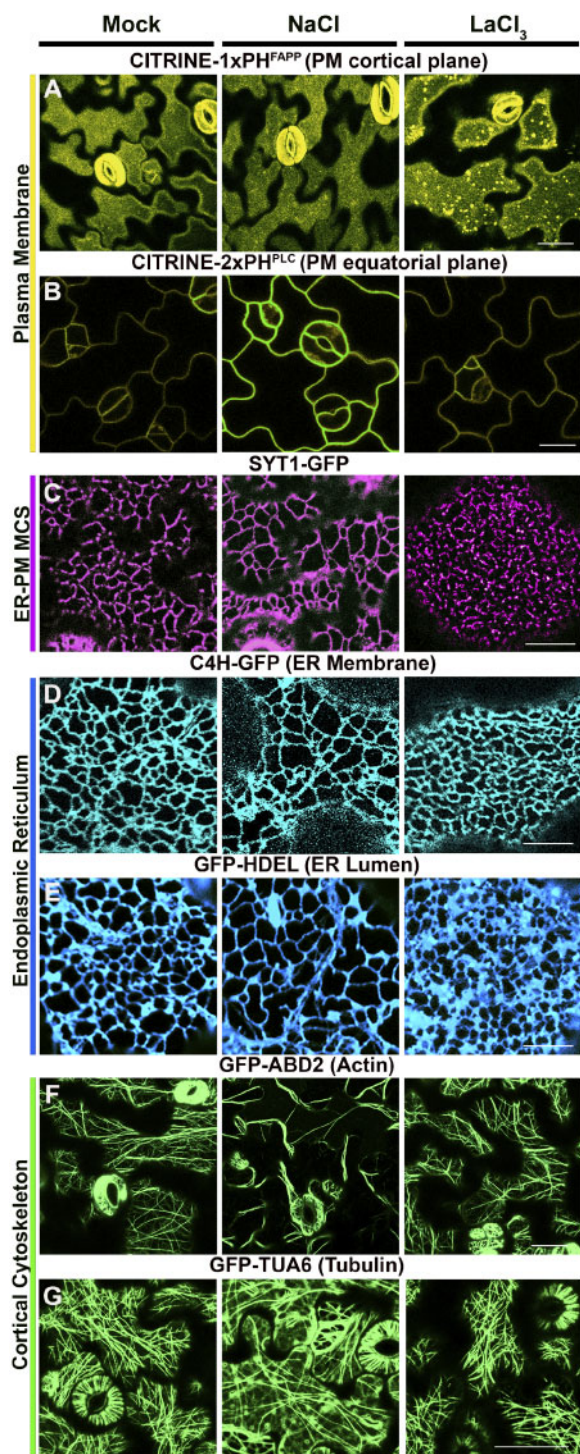
activated during nutrient starvation ([Stefano et al., 2018](#); [Wang et al., 2019](#)) and salt stress ([Fox et al., 2020](#)). In these pathways, VAP27s associate with essential endocytic machineries, such as the AtEH1/Pan1 and AtEH2/Pan1 components of the TPLATE complex ([Stefano et al., 2018](#); [Wang et al., 2019](#)), as well as water transporters, such as aquaporins ([Fox et al., 2020](#)), to target stress-induced autophagy and endocytosis to ER–PM MCSs ([Stefano et al., 2018](#); [Wang et al., 2019](#)). The examples above clearly illustrate how MCS machineries act as multi-functional scaffolds generating compatible membrane geometries that can be used by conventional endocytic trafficking pathways ([Stanhope and Derré, 2018](#); [Wang and Hussey, 2019](#)) and enable localized water transport in response to stress ([Fox et al., 2020](#)).

### Regulation of MCS architecture by calcium and mechanical signals

At the core of many stress-induced changes in ER–MCS architecture, dynamics, and function is the sensing and transduction of stress-specific  $\text{Ca}^{2+}$  signatures achieved by an extensive array of MCS-localized proteins. In mammalian cells, the  $\text{Ca}^{2+}$ -dependent changes in ER–PM MCS architecture are mediated by families of  $\text{Ca}^{2+}$  sensing proteins that are localized either in the ER lumen (e.g. STIMs, ([Liou](#)

et al., 2005) or the cytosol (e.g. E-Syts, ([Giordano et al., 2013](#)) and TMEM24 ([Lees et al., 2017](#))). A classical example of  $\text{Ca}^{2+}$ /MCS interplay is the previously mentioned SOCE in mammalian cells. During stress episodes,  $\text{Ca}^{2+}$  depletion in the ER lumen induces changes in the ER–PM MCS architecture, and these changes subsequently modulate the activity of ER–PM MCS-localized  $\text{Ca}^{2+}$  transporters. At the molecular level,  $\text{Ca}^{2+}$  depletion induces a conformational change in STIM1 that exposes its polybasic C-terminus. This change in architecture promotes its oligomerization and translocation to ER–PM MCSs ([Liou et al., 2007](#); [Gudlur et al., 2019](#)) where it recruits the PM  $\text{Ca}^{2+}$  channel Orai1 ([Park et al., 2009](#); [Gudlur et al., 2019](#)). In parallel, the E-Syt1 tethers reshape the originally formed ER–PM MCSs into ring-shaped structures. This ring-shaped configuration is thought to facilitate STIM1/Orai1  $\text{Ca}^{2+}$  gating and  $\text{Ca}^{2+}$  store replenishment through the stabilization of the ER–PM MCS structure and the reduction of the cER–PM intermembrane distance ([Kang et al., 2019](#); [Figure 1](#)).

The mammalian E-Syts and the plant orthologs SYTs share a common structural organization and regulation. Both use homotypic and heterotypic tethering complexes with their N-terminal domains anchoring the ER and their C-terminal C2 domains establishing  $\text{Ca}^{2+}$ -dependent interactions with PM phospholipids ([Schapire et al., 2008](#); [Yamazaki](#)



**Figure 3** Architectural and compositional changes at the ER-PM MCS interface. Confocal microscopy images of *Arabidopsis* cotyledons expressing the indicated fluorescent markers in Mock (water or 5mM EGTA) treatments (left), 100 mM NaCl (center) and 500  $\mu$ M  $\text{LaCl}_3$  (right). For all treatments, the CITRINE-1xPH<sup>FAPP</sup> marker indicates the accumulation of PI4P at the PM (Simon et al., 2016) (A). The CITRINE-2xPH<sup>PLC</sup> marker indicates the accumulation of PI(4,5)P<sub>2</sub> at the PM (Simon et al., 2016) (B). The SYT1-GFP marker indicates the distribution of SYT1 tethers at ER-PM MCS (Pérez-Sancho et al., 2015) (C). The GFP-HDEL marker indicates the morphology of the ER lumen (Batoko et al., 2000) (D). The GFP-ABD2 marker labels actin filaments (Sheahan et al., 2004) (E). The TUA6-GFP marker labels the microtubules network (Ueda

et al., 2010; Giordano et al., 2013; Xu et al., 2014; Pérez-Sancho et al., 2015). Despite the mechanistic similarities, clear differences in the temporal regulation of ER-PM MCS remodeling between these organisms exist. For example, following an stress-induced increase in  $[\text{Ca}^{2+}]_{\text{cyt}}$ , the mammalian E-Syts aggregate and concentrate at membrane junctions within minutes (Giordano et al., 2013; Idevall-Hagren et al., 2015; Saheki et al., 2016), but similar changes in MCS architecture require hours in plants (Ishikawa et al., 2018; Lee et al., 2019; Lee et al., 2020). This difference suggests that additional processes, such as stress-induced changes in the lipid composition in the inner leaflet of the PM (which control E-Syts and SYTs localization), could be a limiting factor controlling the dynamics of the ER-PM remodeling in plants. In agreement with this model, recent studies in *Arabidopsis* have shown that the slow changes in ER-PM connectivity in response to different stresses are reversible and correspond with the kinetics of accumulation of specific PM phosphoinositides, such as PI(4,5)P<sub>2</sub> in the cytoskeleton-independent responses to NaCl stress (Lee et al., 2019) or PI4P in the cytoskeleton-dependent responses to  $\text{Ca}^{2+}$  signaling surrogates in the cytosol (Lee et al., 2020; Figure 3). In addition, biochemical studies and structural analyses in vitro suggest that this process is mediated by  $\text{Ca}^{2+}$ -dependent electrostatic interactions between negatively charged phosphoinositides at the PM and Lysine/Arginine-rich polybasic clusters conferring electropositive potential to C2-containing SYT tethers (Schapire et al., 2008; Pérez-Sancho et al., 2015; Lee et al., 2019, 2020). Last, genetic and physiological studies using *syt* loss-of-function mutants and SYT fluorescent markers suggest that these connectivity changes could function as an adaptive response providing mechanical stability to the PM during sustained periods of stress (Pérez-Sancho et al., 2015; Lee et al., 2019). Changes in connectivity could also create a molecular platform for unconventional secretion or targeted exocytosis (Harrison and Ivanov, 2017; Bellucci et al., 2018) or regulate cell-to-cell communication in plants through plasmodesmata (Uchiyama et al., 2014; Levy et al.,

et al., 1999) (F). Compared to Mock (left), the NaCl treatment (center) induces the homogeneous accumulation of PI(4,5)P<sub>2</sub> at the PM, promotes the formation of ER-PM MCS along cortical ER tubules, increases the average area of the cortical ER polygons, and induces actin filament bundling and cortical microtubules depolymerization. Compared to Mock (left), the  $\text{LaCl}_3$  treatment (right) induces the heterogeneous accumulation of PI4P labeled vesicles at the PM, increases the number of ER-PM MCS puncta, reduces the number of tubular-shape ER-PM MCSs, reduces the average area of the cortical ER polygons, and does not cause visible morphological defects in the actin filaments or microtubule networks. Scale bars = 50  $\mu$ m. Adapted from Lee et al., (2019, 2020). ABD2: actin binding domain 2; FAPP: four-phosphate-adaptor protein; GFP: green fluorescent protein; HDEL: ER retention motif;  $\text{LaCl}_3$ : lanthanum chloride; NaCl: sodium chloride; PH: pleckstrin homology; PM: plasma membrane; PLC: phospholipase C; TUA6: tubulin alpha-6 chain.



2015; Ishikawa et al., 2020; Brault et al., 2019). Although the common outcome of different plant stress responses is clear (changes in ER–PM connectivity), the specific sequence of events underlying this remodeling process (changes in PM phospholipid composition, ER–PM MCS tether re-localization, changes in ER morphology, and cytoskeleton re-organization) has not been established and remains an open question in the plant MCS field. In structural terms, NaCl stress expands the cER localization of SYT1 tethering complexes, which are normally restricted to immobile ER tubules (Ishikawa et al., 2018), to the whole cER tubular network (Lee et al., 2019). A consequence of this process is the overall reduction of the cER–PM intermembrane distance along the cortical ER tubules (Lee et al., 2019). This process potentially facilitates the nonvesicular transfer of lipids via their SMP domains as described in mammalian E-Syts (Schauder et al., 2014; Reinisch and De Camilli, 2016; Saheki et al., 2016), the *cis/trans* activity of MCS-localized enzymes as discussed for the PI4P phosphatase Sac1p in yeast (Manford et al., 2010; Stefan et al., 2011; Zewe et al., 2018; Venditti et al., 2019), or the activation receptor-mediated signaling pathways through physical interactions with receptor-like kinases as described in the plant stomatal lineage (Ho et al., 2016). It is still unknown whether NaCl stress also creates physical forces (e.g. tensile stress) that could contribute to the observed changes in MCS architecture. Indeed, the influence of physical forces in MCS remodeling has been illustrated by changes in ER–PM MCS architecture in response to external pressure (Pérez-Sancho et al., 2015). Another example is the accumulation of ER–PM MCS components around rapidly expanding symbiosomes where membrane tension directs targeted lipid transfer and exocytosis toward the symbiotic interface (Gavrin et al., 2017; Harrison and Ivanov, 2017; Bellucci et al., 2018). Those studies clearly convey the importance of creating mechanically sensitive MCS assemblies to properly sense and integrate a wide variety of physical/mechanical cues; however, the specific architectural changes induced by physical forces and the putative role of MCS-localized and/or MCS-associated  $\text{Ca}^{2+}$  mechanosensors in the process are currently unknown and represent an exciting field of study in plant MCS research.

## Concluding remarks

Despite the evolutionary advantages of subcellular compartmentation, the successful metabolic activity of eukaryotic cells requires spatially controlled and integrated interactions between organelles in response to environmental and developmental cues. The eukaryotic ER–MCSs provide such highly specialized microenvironments for  $\text{Ca}^{2+}$  homeostasis regulation (ER–PM MCSs), inter-organelle lipid transfer (ER–PM, ER–mitochondria, ER–Golgi, ER–LDs), organelle biogenesis (ER–peroxisome, ER–LD MCSs), and endocytic and autophagy processes

## OUTSTANDING QUESTIONS

- What are the molecular mechanisms controlling MCS shape, intermembrane distance, and dynamics in plants?
- Is lipid transfer activity at MCS directly related to membrane curvature?
- What is the relationship between MCS dynamics and physical forces or membrane tension?
- Do changes in MCS geometry and dynamics regulate biochemical activities in *cis/trans* between apposed organelles?
- Can geometrical parameters and functions derived from yeast/mammalian MCS be extrapolated to characterize plant MCS?

(ER–PM, ER–mitochondria MCSs). Discoveries spanning several decades have identified an assortment of factors ranging from cytoskeletal components to  $\text{Ca}^{2+}$  sensors, LTPs, and tethering factors as modulators that enable the fast and dynamic rearrangement of the organelle membrane interface in response to cellular perturbations. What transpires from these studies is that MCS structures are highly sophisticated, dynamic, and responsive to environmental and developmental cues, and that the MCS individual molecular and 3D architecture precludes functional specialization. We envision that a deeper understanding of the regulatory and functional activities coordinating the remodeling and 3D architecture of membrane interfaces at MCSs (See outstanding questions box) will create new paradigms about fundamental communication mechanisms that enabled organelle specialization and functional diversification in eukaryotic lineages.

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