Lipids at membrane contact sites: cell signaling and ion transport

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Abstract

Communication between organelles is essential to coordinate cellular functions and the cell's response to physiological and pathological stimuli. Organellar communication occurs at membrane contact sites (MCSs), where the endoplasmic reticulum (ER) membrane is tethered to cellular organelle membranes by specific tether proteins and where lipid transfer proteins and cell signaling proteins are located. MCSs have many cellular functions and are the sites of lipid and ion transfer between organelles and generation of second messengers. This review discusses several aspects of MCSs in the context of lipid transfer, formation of lipid domains, generation of Ca^{2+} and cAMP second messengers, and regulation of ion transporters by lipids.

Keywords contact sites; ion transport; lipid transfer; signaling

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See the Glossary for abbreviations used in this article.

Introduction

The numerous and diverse cellular functions are regulated by a limited set of second messengers. Often a single second messenger regulates opposite or several cell functions of different spatial and temporal characteristics [1–3]. These obviate the need for extreme specificity in second messengers' generation and action. Segregation of key initiators and modulators of cell functions and generation of second messengers to MCSs is a major mean cells use to achieve this. Cell functions are accomplished by highly coordinated activity of several membrane-enclosed organelles that exchange chemicals and information. To achieve fidelity and specificity in signaling and to coordinate their specific functions in the cell, cell membranes communicate through the formation of membrane contact sites (MCSs). The membrane that is present in all parts of the cell and makes contacts with all other organellar membranes is the endoplasmic reticulum (ER). Indeed, most if not all MCSs are formed by the ER membrane, which forms MCSs with the plasma membrane (PM), mitochondria, endosomes, lysosomes, peroxisomes, and the

vacuole of yeast [4–8]. MCSs are tethered by specialized tether proteins that have several key features: (i) being anchored in the ER either by a transmembrane domain, by a lipid anchor or by interaction with the ER protein VAMP-associated protein-B (VAP-B) through FFAT (two phenylalanine in acidic tract) motifs, (ii) having a large cytoplasmic domain that spans the 15–20 nm distance between the two membranes, and (iii) having an interacting site that associates in trans with the opposing membrane, which is often a positively charged stretch of amino acids that bind to negatively charged phospholipids. Many tethering proteins have additional functions, such as lipid transfer or regulation of ion channels.

MCSs are few tens to hundreds nm in size and typically use a small fraction of the membrane of each organelle, namely 2–3% in the resting state that may increase to 5–10% after cell stimulation, except in yeast in which the ER-PM contacts are formed with as much as 40% of the PM [9]. Until recently, almost all information on the formation and properties of MCSs was obtained in yeasts, where they were mainly studied in the context of lipid synthesis and transfer, and yeasts remain a major model to study MCSs. In recent years, the development of new tools, in particular lipid sensors for PI(4)P, PI(4,5)P₂, phosphatidic acid (PA), and phosphatidylserine (PS) [10], and genetically encoded cell signaling sensors [11,12] have led to rapid and major progress in understanding MCSs in mammalian cells (for recent reviews, see [5,7,13]).

This review focuses on cell signaling and regulation of ion channels and transporters by lipids at MCSs. We briefly discuss lipid transfer at MCSs by lipid transport proteins. These determine the lipid composition of cellular membranes, but likely also generate a specific lipid environment at MCSs [8,13]. We discuss several aspects of cell signaling and regulation of ion channels/transporters by the specialized lipid environment that may exist at MCSs [5,7,13]. These include Ca^{2+} [14] and adenylyl cyclases/cAMP pathways [15], channels and exchangers [16,17], and the Orai1 and STIM1 systems [18–21].

The ER/mitochondrial and ER/PM MCSs—composition and lipid transfer

Cellular synthesis of phospholipids is compartmentalized and thus requires transfer of the different lipid species between organelle compartments (reviewed in [4,6,8,13,22]). Figure 1 highlights several

Glossary

AA	arachidonic acid
AC	adenylyl cyclases
AKAP	A-kinase anchoring proteins
ANOs	anoctamins
ARC channels	AA-regulated Ca^{2+} current
DAG	diacylglycerol
DGK	diacylglycerol kinase
EMC	ER membrane protein complex
ER	endoplasmic reticulum
ERMES	ER-mitochondria encounter structure
E-Syt	extended synaptotagmin
FFAT	two phenylalanine in acidic tract
FHIT	fragile histidine triad
GPI	glycosylphosphatidyl inositol
IRBIT	IP_3 receptor binding protein released with IP_3
Ist2	increased sodium tolerance protein 2
LTPs	lipid transfer proteins
MAMs	mitochondrial-associated membranes
MCSs	membrane contact sites
MCU	mitochondrial Ca^{2+} uniporter
MFN	mitofusin
NAADP	nicotinic acid adenine dinucleotide phosphate
OMM	outer mitochondrial membrane
ORD	OSBP-related ligand-binding domain
ORP	OSBP-related protein
OSBP	oxysterol-binding protein
PA	phosphatidic acid
PC	phosphatidylcholine
PDE	phosphodiesterase
PH	Pleckstrin homology
PI	phosphatidylinositol
PIPs	phosphatidylinositol phosphates
PITD	PI transfer domain
PM	plasma membrane
PS	phosphatidylserine
PTPIP51	protein tyrosine phosphatase-interacting protein 51
SARAF	SOCE-associated regulatory factor
SMP	synaptotagmin-like mitochondrial and lipid binding protein
STARD3	StAR-related lipid transfer domain protein 3
STIM1	stromal interaction molecule 1
TPC	two-pore channel
VAP	vesicle-associated membrane protein (VAMP)-associated protein
VDAC1	voltage-dependent anion-selective channel 1

lipid synthesis pathways in the PM, ER, and in mitochondria and the proteins involved in their transport. $\text{PI}(4,5)\text{P}_2$ is mainly synthesized at the PM by phosphorylation of phosphatidylinositol (PI) to $\text{PI}(4)\text{P}$ and $\text{PI}(4)\text{P}$ to $\text{PI}(4,5)\text{P}_2$. Hydrolysis of $\text{PI}(4,5)\text{P}_2$ at the PM generates IP_3 and diacylglycerol (DAG). DAG is converted to PA by DAG kinase, which is a precursor for the synthesis of many phospholipids in the ER and mitochondria [13,22]. PA is transferred from the PM to the ER in exchange for PI by Nir2 to supply the PM with PI [23], and it is transferred from the ER to the mitochondria by as yet unknown lipid transfer protein(s) (Fig 1). At the ER, PA is used to synthesize PI and PS. The ER supplies PI to the mitochondria and PS to the PM [24] and mitochondria [25]. The latter are transferred by the lipid transfer proteins ORP5 and ORP8, which mediate PS/ $\text{PI}(4)\text{P}$ exchange to supply the PM with PS and the ER with $\text{PI}(4)\text{P}$. The PS/ $\text{PI}(4)\text{P}$ exchange is facilitated by a Sac1-type $\text{PI}(4)\text{P}$ phosphatase [24]. In the

mitochondria, PS is used to synthesize phosphatidylethanolamine (PE); part of each is then transferred to the ER for synthesis of phosphatidylcholine (PC). These lipid transfers take place at ER/PM and ER/mitochondria MCSs (Fig 1).

Knowledge of the ER/mitochondrial MCSs components and their functions is more advanced than other MCSs. The most detailed information comes from studies in yeast in which two ER/mitochondrial MCSs complexes are known: the ER-mitochondria encounter structure (ERMES) and the ER membrane protein complex (EMC) [13] (Fig 1). The ERMES complex consists of four components, with two residing in the mitochondrial outer membrane, a soluble protein subunit and a glycosylated ER-resident membrane protein [13,22]. The three transmembrane ERMES subunits that reside in the mitochondria and the ER have a synaptotagmin-like mitochondrial and lipid binding protein (SMP) domain [26,27]. The SMP domain is found in many tethering proteins in various cellular compartments and was shown to mediate lipid transfer between bilayers [28,29]. Apart from lipid transfer, the ERMES complex appears to be involved in many cellular functions, as suggested by the numerous phenotypes associated with ERMES mutants [30]. The highly conserved EMC is composed of multiple ER subunits that interact with a subunit of the mitochondrial translocase of the outer membrane complex [31] (Fig 1). The EMC was shown to participate in lipid metabolism or trafficking between the ER and mitochondria [31].

The tethers of mammalian ER/mitochondria MCSs are not defined as well as in yeast (Fig 2). Mitofusin (MFN) 1 and mitofusin 2 were considered well-established tethers [5]. MFN2 is a dynamin-related protein that binds to the ER and mitochondria at Ca^{2+} transfer sites between the organelles and determines their distance [32]. However, recently the exact role of MFN2 became somewhat uncertain [33], specifically whether MFN2 keeps the ER away from [34] or closer to the mitochondria [35] remains controversial. Other proteins found at the ER/mitochondria MCSs are voltage-dependent anion-selective channel 1 (VDAC1) and the IP_3 receptors [36] that are functionally coupled by the chaperone GRP75 [37] and the mitochondrial Ca^{2+} uniporter (MCU) complex [38]. Several proteins regulate the formation, distance, stability, or number of ER/mitochondria MCSs (see Fig 2). The interaction of the ER VAP-B with the mitochondrial protein tyrosine phosphatase-interacting protein 51 (PTPIP51) is required for the formation of MCSs [39]. The outer mitochondrial membrane protein FIS1 interacts with the ER protein BAP31 to allow transmission of apoptotic signals from the mitochondria to the ER [40]. The regulator of ER tubular structure NOGO-B modulates the distance between the ER and mitochondria at the MCSs [41]. The IP_3 receptor binding protein released with IP_3 , IRBIT, which regulates many cellular functions [42] and expands and stabilizes the ER/mitochondria MCSs [43]. The mitochondrial membrane fragile histidine triad (FHIT) protein enhances the number of ER/mitochondria MCSs [44]. All these modulators of ER/mitochondria MCSs affect Ca^{2+} transfer between the ER and mitochondria and thereby influence cell metabolism and function.

As outlined above, lipids are exchanged between ER and mitochondria at the MCSs. Oxysterol-binding protein-related protein (ORP) 5 and ORP8 mediate PS/ $\text{PI}(4)\text{P}$ exchange between the two membranes (Fig 1) [25]. It is unknown at present if these proteins are also involved in the formation and/or tethering of the ER/mitochondrial MCSs. PTPIP51 and VAP-B may mediate the transfer of phosphatidylinositol phosphates in addition to their structural role [45] (Fig 1).

Figure 1. Organellar lipid synthesis and transport.

Biosynthetic lipid pathways are localized in specific compartments, the plasma membrane (PM), the endoplasmic reticulum (ER), or the mitochondria. At the PM, phosphatidylinositol phosphate (PI) is phosphorylated to PI(4)P and then to PI(4,5)P₂ by PI4 kinase (PI4K) and PI5K, respectively, and PI(4,5)P₂ is hydrolyzed to DAG and IP₃ by phospholipase C (PLC). DAG is converted to PA by diacylglycerol kinase (DGK), the precursor for PI and phosphatidylserine (PS) in the ER, and prostaglandins (PG) in the mitochondria. Lipids are transferred between compartments at membrane contact sites (MCSs). In mammals, ER/PM MCSs are tethered by the extended synaptotagmins (E-Syts) and likely by the lipid transfer proteins ORP5/8 and Nir2/3. The yeast ER/mitochondria are tethered by the ER–mitochondria encounter structure (ERMES) and ER membrane protein complex (EMC) complexes. In mammalian cells, the ER/mitochondria are tethered by interaction of VAMP-associated protein-B (VAP-B) with protein tyrosine phosphatase-interacting protein 51 (PTPIP51) and perhaps by ORP5/8. Extensive phospholipid transfer takes place between the PM and ER and between the ER and mitochondria, likely mediated by specific lipid transfer proteins, the identity of most of which is not known at present. However, ORP5/8 mediate PI(4)P/PS exchange between the ER/PM and ER/mitochondria and Nir2 mediates PA/PI exchange between the ER/PM.

The ER and PM contact sites [the ER/PM junctions (Fig 1)] were identified as the site of lipid synthesis and transfer between the ER and PM. In yeast, the ER/PM junctions encompass nearly 40% of the PM and are tethered by multiple proteins: the three Tricalbins, the increased sodium tolerance protein 2 (Ist2), and the ER-resident Scs2/22 [4,9]. All six proteins appear to be required to maintain intact ER/PM junctions [9], although other tethers may exist at the junctions. For example, the lipid transfer proteins Lam1/3 and Lam2/4 localize to the ER/PM junctions in yeast [6], are anchored at the ER, and have a PI(4,5)P₂ binding ligand, and thus, they are likely to participate in tethering the ER/PM junctions. Recent studies identified several mammalian homologues of the yeast tether proteins. The

Tricalbins homologues are the three extended synaptotagmins (E-Syt1, E-Syt2, and E-Syt3) [45,46], and the Scs2/22 homologues are VAP-A and VAP-B [7] (Fig 1). The Ist2 homologue is not known at present. However, the Ist2 domain structures are shared with those of the Anoctamins, a family of 10 members with diverse functions. ANO1 and ANO2 function as Ca²⁺-activated Cl[−] channels [47], while ANO6 functions as lipid scramblase, although other ANOs show some scramblase activity as well [48–50]. Whether an ANO member has a function similar to Ist2 and whether Ist2 has lipid transfer, exchange, or scramblase activities remains to be determined.

Several lipid transfer and exchange proteins are found at the ER/PM junctions that may reside in the same or adjacent to ER/PM

Figure 2. Ca^{2+} transfer at the ER/mitochondria MCSs.

The ER and outer mitochondrial membrane (OMM) are tethered by interaction of the ER VAP-B and Fis1 with the OMM PTPIP51 and BAP31, respectively, while mitofusin 2 (MTF2) determines the distance between the ER and OMM to form the ER/mitochondria MCSs. The IP_3Rs -VDAC1-MCU complex at the ER/mitochondria MCSs mediates Ca^{2+} transfer from the ER to the mitochondria, while mitochondrial specific AKAP/PKA at the ER/mitochondria MCSs controls PKA activity at the OMM.

junctions associated with Ca^{2+} signaling (Fig 1). All E-Syts have an SMP domain [51] that transfers glycerolipids between bilayers [28,29]. Other lipid transfer proteins at the ER/PM junctions are ORP5 and ORP8 [24,52,53] and Nir2 and Nir3 [54,55]. ORP5 and ORP8 have the domain structure of a tether: a C-terminal transmembrane domain that spans the ER, a long cytoplasmic domain with a lipid binding OSBP-related ligand-binding domain (ORD) that spans the space between the ER and PM, and a Pleckstrin homology (PH) domain that binds to $\text{PI}(4)\text{P}$ and $\text{PI}(4,5)\text{P}_2$ at the PM. ORP5 and ORP8 exchange ER PS with PM $\text{PI}(4)\text{P}$ [24,52,53]. Continued synthesis of $\text{PI}(4)\text{P}$ at the PM and its hydrolysis at the ER is required for the exchange to proceed. Nir2 and Nir3 bind to the ER-resident VAP-B through their FFAT motifs and interact with the PM by binding of their C-terminal Lipin/Ned1/Smp2 (LNS2) domains to PA [7]. After $\text{PI}(4,5)\text{P}_2$ depletion by cell stimulation, Nir2 and Nir3 replenish the PM $\text{PI}(4,5)\text{P}_2$ and $\text{PI}(3,4,5)\text{P}_3$ in a process that requires their PI transfer domain (PITD) [7,54,55]. An important function of Nir2 is the exchange of ER PI with PM PA, which is required for replenishing PM $\text{PI}(4,5)\text{P}_2$ [23]. It is likely that other tether and lipid transfer proteins that are present in the ER/PM junctions will be identified in the near future.

Lipid domains at MCSs and their recognition—evidence for lipid codes

The potential existence and importance of lipid microdomains has been appreciated for quite some time as a major element of membrane rafts and caveolae [56]. Membrane rafts and caveolae are smaller than 100-nm domains enriched in cholesterol, ceramide,

and glycosphingolipids [57]. Vesicular transport is a major pathway for cholesterol transport to the PM. However, cholesterol is also transported by lipid transfer proteins (LTPs) at MCSs mediated by members of the OSBPs and ORP proteins that transfer cholesterol and cholesterol derivatives in exchange with $\text{PI}(4)\text{P}$ [58]. The presence and role of LTPs in cholesterol enrichment at caveolae and PM lipid rafts need to be further established. Yet, such transport is suitable for generation of cholesterol and other sterol microdomains. Although caveolae and lipid rafts differ from MCSs by not forming clear contact with the ER and in lipid composition, they do form defined lipid domains and are thus part of the lipid code.

The clearest evidence for lipid domains at MCSs exists for PIs, in particular $\text{PI}(4)\text{P}$, $\text{PI}(4,5)\text{P}_2$, $\text{PI}(3)\text{P}$, and $\text{PI}(3,5)\text{P}_2$ [16,59]. $\text{PI}(4)\text{P}$ is synthesized at the Golgi and PM by Type III PI4 Kinases α and β [60]. $\text{PI}(4)\text{P}$ is also the precursor for $\text{PI}(4,5)\text{P}_2$ at the PM, where $\text{PI}(4)\text{P}$ is phosphorylated by PI5 -kinases [61]. As indicated, during cell stimulation the level of $\text{PI}(4)\text{P}$ and $\text{PI}(4,5)\text{P}_2$ is maintained by Nir2 and Nir3 [23,54,55]. $\text{PI}(3)\text{P}$ is found in early endosomes [62], and $\text{PI}(3,5)\text{P}_2$ in late endosomes and lysosomes [59]. Distribution of the PIs is not uniform. The existence of PI microdomains is strongly suggested by localization of $\text{PI}(4)\text{P}$ and $\text{PI}(4,5)\text{P}_2$ binding proteins at the PM MCSs, as evident from the punctate localization of $\text{PI}(4)\text{P}$ and $\text{PI}(4,5)\text{P}_2$ binding proteins at the PM. One classical example is the localization of K-Ras to distinct regions of the PM that is dependent on the interaction of its polybasic domain with $\text{PI}(4,5)\text{P}_2$ and PS [63]. Another example is localization of STIM1, which is discussed in details below. More direct evidence for $\text{PI}(4)\text{P}$ and $\text{PI}(4,5)\text{P}_2$ microdomains is becoming available with development of adequate tools and the use of super resolution microscopy. Such an approach revealed punctate localization of $\text{PI}(4,5)\text{P}_2$ at the PM

[64,65]. PIs levels at MCSs are not static and dynamically change during cell stimulation. For example, the level of PI(4,5)P₂ oscillates during cell stimulation to induce Ca²⁺ oscillations [66,67]. Endosomal PI(3)P is hydrolyzed, and PI(4)P is synthesized and required for delivery of endosomes to and their fusion with the PM [68].

Phosphatidylserine (PS) is a major phospholipid in the inner leaflet of the PM. As outlined earlier, PS is synthesized in the ER and is transferred to the PM in exchange for PI(4)P by ORP5 and ORP8 at the ER/PM junctions ([24,52,53] and Fig 1). There is no clear evidence for PS microdomains. However, domains of ordered lipid microdomains with higher PS content function as signaling sites. For example, PS-rich domains cluster non-palmitoylated Gα1 subunits [69] and Ebola virus VP40 [70], and they are required to maintain clustering of Ras proteins [71]. Moreover, polarized distribution of PS was shown to be required for Cdc42 localization and for development of cell polarity [72]. PS transport at MCSs likely generates the PS-rich domains that can persist at least while ORP5 and ORP8 are actively mediating the PI(4)P/PS exchange.

The generation of lipid microdomains appears to set up a lipid code that is “read” by proteins and that functions to cluster ion channels and transporters and thus the exchange of materials between membranes and organelles [4,5,13,59]. Proteins can be targeted to specific lipids domains by lipidation, that is, by palmitoylation, myristoylation, prenylation, cholesterylation, or the addition of a Glycosylphosphatidyl inositol (GPI) anchor [73]. Each of these modifications targets the proteins to distinct lipid domains, affecting their function and many signaling pathways [74].

Additionally, the lipid code can be read by lipid recognition motifs and domains. Cholesterol is recognized by the CRAC motif (L/V-(X)(1–5)-Y-(X)(1–5)-R/K, where (X)(1–5) represents between 1 and 5 residues of any amino acid) and the CARC (inverted CRAC) motif [75]. The CRAC motif tends to bind cholesterol in the inner leaflet and the CARC motif in the outer leaflet and the presence of both motifs in a protein enhances interaction with cholesterol [76]. However, the presence of CRAC and/or CARC motifs is not sufficient to infer that the protein indeed binds cholesterol and any regulatory interaction needs to be verified experimentally [77]. As indicated above, polybasic motifs recognize PIs, in particular PI(4)P and PI(4,5)P₂, which is thought to involve electrostatic interactions [59,78]. A recent study provided evidence that the polybasic domain of K-Ras acts together with S-farnesyl cysteine carboxymethyl ester to determine lipid binding specificity [79]. The C-terminus of K-Ras4b (referred to as K-Ras) interacts with the PM through the polybasic motif and farnesyl-cysteine-methyl-ester¹⁷⁵KKKKKK SKTKCVIM¹⁸⁸ domain. Activated K-Ras shows similar affinities for PS, PI(4,5)P₂, and PI(3,4,5)P₃. Neutralizing the charges of K175 or K179 resulted in domains that preferred PI(3,4,5)P₃, neutralizing the charge of K178 resulted in a domain that preferred PI(4,5)P₂, while mutating the CVIM motif to CCIL that cause geranylgeranylation rather than farnesylation of K-Ras resulted in a domain that preferentially bound PS. Hence, the code specifies preference for binding PI(4,5)P₂ or PS, which then determines the signaling output [79]. These important findings indicate the existence of a lipid domain deciphering code that is more complex than electrostatic interactions of proteins with charged phospholipids. Similarly, the lipid composition at MCSs can be read by specific lipid binding modules within proteins. For example, various PH domains recognize specific phosphoinositides [80] and PI(4,5)P₂ is also recognized by C2

[81] and ENTH/ANTH domains [82]. In addition, PI(4,5)P₂ is recognized by the FERM domain [83], and PI(3) is recognized by the FYVE [62] and PX domains [84], while PI(3,5)P₂ is recognized by the PROPPIN domain [85]. Several PDZ domains bind phospholipids, including PI(4,5)P₂, with various affinities [86] (for further details on modules binding lipids see [78,80]). In summary, the establishment of lipid microdomains represents a lipid code that, in combination with incredibly diverse modes of deciphering this code, allows for the intricate regulation of cellular activity by lipids and lipid microdomains at MCSs.

Second messengers at MCSs: Ca²⁺ and cAMP

Although the exact mechanism is not understood well, many Ca²⁺ transport proteins and Ca²⁺ signaling are affected by lipids and lipid mediators beyond generation of IP₃ from PI(4,5)P₂. Below, we discuss Ca²⁺ signaling at MCSs and the potential role of lipids in regulating the signaling. The best understood signaling pathway at MCSs is Ca²⁺ signaling where all critical steps take place at MCSs. Most studies of Ca²⁺ signaling at MCSs were done in model systems, although localization of Ca²⁺ signaling proteins at MCSs in native cells has been demonstrated. For example, in muscle and neurons the voltage-activated Ca²⁺ channels communicate with the ryanodine receptors at MCSs [87]. In polarized epithelial cells, all Ca²⁺ signaling proteins are clustered at the apical pole MCSs where they form complexes [88,89]. Thus, physiological cell stimulation evokes Ca²⁺ signals confined to the apical pole [90]. The Ca²⁺ transporters generating the receptor-evoked Ca²⁺ signal interact with various lipids at MCSs. The PM Ca²⁺ ATPase pump (PMCA) is prominently regulated by PI(4,5)P₂ that interacts with the autoinhibitory domain to stimulate the pump [91]. The interaction of STIM1 with PI(4,5)P₂ is critical for its function [21,92] and STIM1 and Orai1 interact with and are regulated by cholesterol [93,94]. These forms of regulation are discussed in more detail below.

Ca²⁺ signaling at the ER/mitochondria takes place at the mitochondrial-associated membranes (MAMs) and involves the ER IP₃ receptors Ca²⁺ release channels; the mitochondrial outer membrane VDAC1 channel that mediates passage of Ca²⁺ released by the IP₃ receptors across the outer mitochondrial membrane; and the inner mitochondrial membrane Ca²⁺ influx MCU channel complex that mediates mitochondrial Ca²⁺ influx [1] (Fig 2). Ca²⁺ signaling is sensitive to the integrity of the ER–mitochondrial contact site and to its lipid composition. Manipulation of ER/mitochondrial MCSs by changing expression of the interacting ER VAP-B and mitochondrial PTPIP51 modulates mitochondrial Ca²⁺ signaling and autophagy [39]. Presenilin-2 and Parkin-2 also affect the ER/mitochondria MAM integrity and distance and the activity of IP₃ receptors and therefore ER to mitochondria Ca²⁺ transfer (reviewed in [33]). The ER/mitochondrial MCSs appear to have a specific lipid environment [95] that interacts with VDAC1 and perhaps other Ca²⁺ signaling proteins to affect their function. VDAC1 is the major cholesterol binding protein at the MAMs, and VDAC1 channel function is regulated by cholesterol [96]. Cholesterol, phosphatidylglycerol, and cardiolipin control VDAC oligomerization [97], thereby affecting its selectivity [98] and voltage-dependent gating [99].

Lysosomal Ca²⁺ release at ER/endolysosome MCSs affects ER Ca²⁺ release, and the endolysosomal two-pore channel (TPC) is

localized at and regulates ER/endolysosome MCSs [100]. Lipid transfer between ER and lysosomes is mediated by ORP5 interacting with the NPC1/2 system that transports cholesterol and possibly by the lipid transfer proteins StAR-related lipid transfer domain protein 3 (STARD3) and ORP1L, which are targeted to these MCSs [5]. The TPCs that control formation of ER/endolysosomal contact sites are activated by nicotinic acid adenine dinucleotide phosphate (NAADP) and the lipid PI(3,5)P₂ [101,102]. Although it is not known how PI(3,5)P₂ activates the TPCs, the dynamic regulation of PI(3,5)P₂ levels at the ER/endolysosomal MCSs [10,59] will likely affect TPCs activity. Whether the lipid transfer proteins also function as tethers and the role of lipids transported by these proteins in lysosomal signaling remains to be determined.

Several observations based on targeted cAMP probes suggest that also cAMP/PKA signaling occurs specifically at MCSs [15]. Components of the cAMP signaling pathway adenylyl cyclases (ACs), PKA and A-kinase anchoring proteins (AKAPs), and phosphodiesterase (PDE) isoforms are found in membrane rafts [103] and caveolae [104]. This confined localization of cAMP signaling components generates spatially restricted and segregated cAMP signals. Interestingly, the cAMP generated in the raft resides in a region that is depleted for PI(4,5)P₂ [105]. In addition, depletion of ER Ca²⁺ that clustered STIM1 at the ER/PM junctions recruited AC3 to the puncta and increased cAMP [106] and STIM1 was shown to activate AC5 [107]. Notably, the Ca²⁺-activated AC8 is specifically activated by Ca²⁺ entering the cells through the store-operated Ca²⁺ influx channel Orai1, and cell stimulation results in recruitment of AC8 to the ER/PM junctions where it directly interacts with Orai1 [108]. The Ca²⁺-activated AC1 is likely regulated by a similar mechanism [109]. The physiological significance of such regulation of ACs at the MCSs was shown recently by the finding that Orai1-mediated Ca²⁺ influx increases cellular cAMP, which activates the cAMP/PKA responsive transcription factor CREB to increase expression of the neutral lipases that control lipolysis [110]. The ER Ca²⁺ sensor STIM2 that clusters at the ER/PM junctions in response to Ca²⁺ release from the ER regulates cAMP/PKA-dependent phosphorylation of AMPA receptor GluA1 subunit by coupling PKA to AMPA receptors at the ER/PM junctions [111]. In summary, these limited observations suggest that the cAMP/PKA signaling pathway does take place at MCSs and should provide the impetus to examine the localization and function of the many AKAPs, PKA, ACs, and PEs at MCSs.

Ion transporters and channels at MCSs

Localization of ion channels and transporters at MCSs has not been examined extensively, although regulation by lipids points to such localization. Direct evidence for localization and role of ion channels as tethers at MCSs are sparse. In mitochondria IP₃ receptors, VDAC1 and the MCU complex are organized at MCSs [1]. In muscles, excitation–contraction coupling is mediated by close apposition of L-type voltage-activated Ca²⁺ channels and the ryanodine receptors at the triad, the muscle ER/PM junctions [112]. The triad junctions are tethered by Juncophilins 1 and 2, while other Juncophilin isoforms function in other cell types, mainly neurons, by coupling other L-type and IP₃ or ryanodine isoforms at MCSs [113]. In neurons and non-excitable cells, the K⁺ channel Kv2.1 [114] and Orai1 when recruited by STIM1 (discussed below) localize at

ER/PM MCSs. Here, Kv2.1 functions as dynamic tether independent of its channel function and recruits the L-type Ca²⁺ channel Cav1.2 to the ER/PM junctions [114]. Many channels and transporters have been localized to caveolae and plasma membrane raft domains [115,116]. The Ca²⁺-activated Cl[−] channel ANO1 is activated by localized Ca²⁺ signals generated by IP₃-mediated Ca²⁺ release [117] and Ca²⁺ influx through Orai1 [118]. Unlike Kv2.1, most channels and transporters do not function as tethers. However, localization at MCSs allows interaction with and regulation by lipids enriched at MCSs. As discussed below, a good example is regulation by PI(4,5)P₂ [16,17] that is also indicative of stable or transient localization at MCSs. In a feedback loop, ion transporters and channels can affect their lipid environment, and thus their regulation by lipids, by modulating the membrane potential to affect PM PI(4,5)P₂ and PS reorganization. PM depolarization specifically clusters PI(4,5)P₂ and PS that is reversed by depolarization to affect cell signaling [119].

The function of ion transporters and channels depends on lipids. Structural evidence from channels, pumps, and exchangers suggests that the direct interaction with lipids can affect their structure and function [17,120,121]. However, specific lipids can regulate transporter function also in a more dynamic process in response to certain stimuli. Such regulation was reported for DAG [122], arachidonic acid (AA) and other AA and fatty acid metabolites [123–125], PI(3,5)P₂ [126], and PI(4,5)P₂ [16]. DAG regulates most TRPC channels [122], although their regulation at MCSs is only suggestive at present. DAG is generated by PLC-mediated hydrolysis of PI(4,5)P₂, which is found at MCSs. TRPC1 and TRPC3 are present at or are recruited to MCSs in response to cell stimulation [88,127] and are activated by STIM1 [128]. TRPC4 and TRPC5 have a PDZ ligand that interacts with PDZ scaffolds like NHERF1 to confer regulation of TRPC4/TRPC5 by DAG [129]. PDZ scaffolding proteins localize in cell junctions like tight junctions, a form of MCSs. The activation of the ARC channel, which is composed of a STIM1-assembled Orai1/Orai3 heteromer, was reported to occur in response to AA [123]. Subsequent studies indicated that the physiological activation of the ARC channels is mediated by LCT4 rather than AA (discussed in [130]). Since STIM1 activates the Orai channels at the ER/PM junctions, also ARC channel activation might occur at the ER/PM junction [131]. PI(3,5)P₂ activates the endolysosomal Ca²⁺-permeable TPC and TRPML channels [126] and TPCs in turn localize to and stabilize the ER/endolysosomal MCSs [100].

Regulation of ion transporters by PI(4,5)P₂ is the best understood form of regulation by any lipid. It was discovered by Hilgemann *et al* [132], who showed that PI(4,5)P₂ activates the Na⁺/Ca²⁺ exchanger NCX1 and several inward rectifying K⁺ channels [133]. Since then, regulation by PI(4,5)P₂ has been demonstrated for many channels, including K⁺, Ca²⁺, TRP, ANO1 and ENaC Na⁺ channels, P2X receptors (see [16] for a comprehensive list), Na⁺/H⁺ exchangers [134], Na⁺-HCO₃[−] cotransporters [135,136], and the PMCA pumps [91]. Several modes of regulation by PI(4,5)P₂ have been reported. Interaction with PI(4,5)P₂ can be obligatory for the activity of a transporter. This mode is observed with the Kir2.2 and Kir3.2 channels, in which PI(4,5)P₂ is within the channel structure and the channels are not functional without PI(4,5)P₂ [137,138]. PI(4,5)P₂ is obligatory for activation of Kir3.2 even when it is bound to its activator Gβγ [139]. The second mode of regulation by PI(4,5)P₂ involves receptor-stimulated reduction in PM PI(4,5)P₂ levels. The activity of almost all Kv7 channels follows PM PI(4,5)P₂ levels with

reduction of PI(4,5)P₂ in response to PLC stimulation reducing channel activity that is restored only after restoration of PI(4,5)P₂ levels [140]. PI(4,5)P₂ can also function to inhibit channel activity. Although most TRP channels are activated by PI(4,5)P₂ [141], including TRPC4β [142], TRPC4α is inhibited by PI(4,5)P₂ [143]. TRPC5 is both activated and inhibited by PI(4,5)P₂ depending on the experimental setting, suggesting a more complex form regulation by PI(4,5)P₂ [144]. Regulation by PI(4,5)P₂ synthesis and breakdown likely occurs at MCSs, suggesting that the regulation of TRPC channels also occurs at MCSs.

A novel form of regulation by PI(4,5)P₂ is by translocation into and out of high PI(4,5)P₂ domains at MCSs, rather than by synthesis and breakdown. This form of regulation has been described recently for regulation of the STIM1-Orai1 complex by PI(4,5)P₂ [21] (Fig 3). The ER Ca²⁺ sensor STIM1 activates the Orai and TRPC channels and acts as a *bona fide* tether [128]. STIM1 functions as a dynamic tether that clusters at the ER/PM junctions in response to ER Ca²⁺ release and binds to PI(4,5)P₂ through its C-terminal polybasic domain to expand and stabilize the junctions [18,19]. However, STIM1 binding to PI(4,5)P₂ is not essential for activation of the Orai1 channels, which are fully activated by the short STIM1 SOAR domain that is unable to bind PI(4,5)P₂ [145]. Rather, binding of STIM1 to PI(4,5)P₂ is required for the interaction of STIM1 with the ER-resident SARAF protein [21,146]. SARAF interacts with the SOAR domain of STIM1 and facilitates the Ca²⁺-dependent

inhibition of Orai1, which limits Ca²⁺ influx and cell toxicity. Interaction of SARAF with SOAR is dependent on (i) formation of ER/PM junctions by E-Syt1, (ii) stabilization of STIM1 clusters by interaction of STIM1 with Orai1, (iii) binding of the STIM1 polybasic domain to PI(4,5)P₂, and (iv) presence of the STIM1-Orai1 complex in a PI(4,5)P₂-rich PM domain [21]. Most notably, upon initial Ca²⁺ release from the ER, the STIM1-Orai1 complex is formed at a PI(4,5)P₂-poor domain where SARAF does not bind to STIM1, allowing for maximal Ca²⁺ influx. Only then the STIM1-Orai1 complex translocates to a PI(4,5)P₂-rich domain (or PI(4,5)P₂ translocates to the STIM1-Orai1 complex) where SARAF interacts with STIM1 to limit Ca²⁺ influx by Orai1 [21]. Hence, regulation of Ca²⁺ influx by the Orai1 channel through interaction of SARAF with STIM1 is by translocation between PI(4,5)P₂ domains, rather than by PI(4,5)P₂ hydrolysis and re-synthesis.

Whether other lipids are also enriched at the ER/PM junctions and are required for optimal functioning of the STIM1-Orai1 complex remains to be determined. However, the fact that PS synthesis takes place at ER MCSs [147], ORP5 and ORP8-mediated PI(4)P/PS exchange delivers PS to the PM at ER/PM junctions [52], and the recent discovery of a lipid code that distinguishes between PI(4,5)P₂ and PS [79] raise the possibility that PS might regulate channel function at MCSs. As discussed above, polybasic sequences together with a prenyl group comprise a combinational code with the specificity determined by the precise polybasic sequence [79].

Figure 3. Regulation by translocation between PI(4,5)P₂ domains.

The MCSs at ER/PM junctions are tethered by E-Syt1 and E-Syt2 and Ist2/ANO, with E-Syt1 affecting STIM1-Orai1 complex localization and E-Syt2 may control plasma membrane PI(4)P levels through the PI(4) phosphatase Sac-1 to determine PI(4,5)P₂ levels of the PI(4,5)P₂-rich domain at the ER/PM junctions. The AC/PKA/cAMP signaling pathway is also present at the ER/PM junctions. The ER/PM junctions are dynamic and are likely formed or at least stabilized in response to cell stimulation, and the signaling proteins are recruited to the ER/PM junctions after their formation/stabilization. Prior to cell stimulation, STIM1 is in an inactive conformation and is not clustered at the ER/PM junctions. The STIM1 inhibitor SARAF is also at the ER and does not interact with STIM1. AC1, AC3, and AC8 may not be localized at the ER/PM junctions, but at a nearby domain and AC8 is not associated with Orai1. All proteins are at a PM PI(4,5)P₂-poor domain. In response to depletion of ER Ca²⁺, STIM1 clusters at the ER/PM junctions to expand and stabilize the junctions. STIM1 clusters first at a PI(4,5)P₂-poor domain and recruits Orai1 to this domain to activate Ca²⁺ influx. Subsequently, the STIM1-Orai1 complexes translocate to the PI(4,5)P₂-rich domain where SARAF interacts with STIM1 to initiate the Ca²⁺-dependent inhibition of Ca²⁺ influx and prevent Ca²⁺ toxicity. Once at the PI(4,5)P₂-rich domain, STIM1 and Orai1 interact with the Ca²⁺-activated ACs to trigger generation of cAMP and activation of PKA. The ACs may also be recruited to the PI(4,5)P₂-rich domain after its stabilization by STIM1, and thus, translocation between PI(4,5)P₂-poor and PI(4,5)P₂-rich domains regulates both Ca²⁺ and cAMP signaling.

Box 1: In need of answers

- (i) Determining the full complement of tether proteins in MCSs.
- (ii) Defining the specificity of MCSs formed between the same membranes but different tethers.
- (iii) Identifying the lipid transfer proteins for the various phospholipids at all MCSs.
- (iv) What are the lipid recognition codes used by lipid-binding and lipid-regulated proteins?
- (v) How are signaling proteins and ion transporters recruited to MCSs?
- (vi) How is protein function affected by selective movement in and out of MCSs?

In this respect, the polybasic and prenyl code of K-Ras PM targeting domain prefers PM domains with high levels of PS and PA [148]. When this code is transferred to STIM1, it clusters STIM1 at the ER/PM junctions that recruit Orai1 to the clusters to activate Ca^{2+} influx in the absence of ER Ca^{2+} store depletion [21]. Thus, a better understanding of lipid composition at the ER/PM junctions and other MCSs is needed to clarify the regulation of ion transporters, signaling and cell function by lipids. Moreover, it is likely that multiple types of MCSs with specific functions are formed between the same membranes. A clue may be provided by the differential localization of the three E-Syts [46] and their specific functions. E-Syt1, but not E-Syt2 and E-Syt3, are required for the interaction of SARAF with STIM1 [21], while E-Syt2, but not E-Syt1 and E-Syt3 are required for synthesis of PM PI(4)P, which replenishes PI(4,5)P₂ [149]. The diversity, specificity, and relationship between the various MCSs formed by the same membranes need to be explored.

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Conflict of interest

The authors declare that they have no conflict of interest.

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