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Recent Insights into Mammalian ER-PM Junctions

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Abstract

ER-PM junctions are subcellular sites where the endoplasmic reticulum (ER) and the plasma membrane (PM) are kept in close appositions, providing a platform for inter-organelle contact. These membrane contact sites are important for many physiological functions in mammalian cells, including excitation-contraction coupling, store-operated Ca^{2+} entry, and non-vesicular transfer of lipids between the ER and the PM. Here we review recent insights into the 3D structure and spatial organization of ER-PM junctions in mammalian cells as well as molecular mechanisms underlying the formation and functions of mammalian ER-PM junctions.

Keywords

ER-PM junctions; membrane contact sites; Ca^{2+} signaling; non-vesicular lipid transfer; cholesterol transport

Introduction

The endoplasmic reticulum (ER) is an extensive membrane organelle important for Ca^{2+} storage, biosynthesis of proteins and lipids, and protein folding. Communication between the ER and the plasma membrane (PM) is important for cells to properly respond to extracellular stimulation and maintain cellular homeostasis. Close appositions between the ER and the PM at ER-PM junctions, also referred to as ER-PM contacts or ER-PM contact sites, enable direct contacts between these two membrane compartments for signaling and molecular trafficking [1].

ER-PM junctions were first observed using electron microscopy (EM) in muscle cells and referred to as dyads and triads [2]. Dyads and triads are found to be essential for excitation-contraction (E-C) coupling underlying muscle contraction [3]. At dyads and triads, depolarization of voltage-gated Ca^{2+} channels at the PM triggers the juxtaposed ryanodine receptors at the ER to release stored Ca^{2+} stimulating contraction of muscle cells. Junctophilins (JPHs) enriched at dyads and triads are important for the formation of these ER-PM junctions and E-C coupling [4]. Deficiency in JPHs leads to cardiac arrests and lethality in mice.

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Nearly five decades after, ER-PM junctions were identified as the spatial platform of the ubiquitous store-operated Ca^{2+} entry (SOCE) pathway for Ca^{2+} signaling and homeostasis [5, 6]. Following a decrease in ER Ca^{2+} , SOCE is activated by translocation of the ER membrane protein STIM1 to ER-PM junctions, enabling its interaction with the PM Ca^{2+} channel Orai1 [7]. SOCE is essential for numerous physiological processes such as the immune response, secretion, cell migration, and tissue development. Humans deficient in SOCE suffer severe combined immunodeficiency, skeletal myopathy, and ectodermal dysplasia [8].

More recently, it was found that lipid exchange at ER-PM junctions is pivotal to the phosphatidylinositol (PI) cycle, a fundamental process first observed in 1953 in receptor-stimulated pancreas slices [9]. The PI cycle involves transfer of phosphatidic acid (PA), which is converted from diacylglycerol (DAG) generated by stimulation-induced breakdown of PI 4,5-bisphosphate ($\text{PI}(4,5)\text{P}_2$), from the PM to the ER where it can be converted to PI and transferred back to the PM for $\text{PI}(4,5)\text{P}_2$ re-synthesis to sustain cell signaling [10, 11]. The production of PA following receptor stimulation induces translocation of the cytosolic PI/PA transfer protein Nir2 to ER-PM junctions to mediate the PI cycle [12-14]. The PI cycle is not only important for sustaining receptor-induced signaling, but also required to maintain numerous $\text{PI}(4,5)\text{P}_2$ -dependent cellular functions such as ion transport, membrane trafficking, and cytoskeleton dynamics.

The known functions of mammalian ER-PM junctions continue to expand. Additional proteins that constitutively or dynamically enriched at ER-PM junctions have been identified, including extended synaptotagmins (E-Syts), TMEM24, Kv2 voltage-gated K^+ channels, oxysterol-binding protein (OSBP)-related proteins (ORP) 5 and ORP8, and GRAM domain-containing proteins (GRAMDs). Moreover, recent developments in imaging technologies and tools enable the visualization of the 3D structure of ER-PM junctions and their spatial organization in mammalian cells, advancing our understanding of these membrane junctions. The aim of this review is to discuss recent insights into this fast growing field of research on the structure, functions, and regulation of mammalian ER-PM junctions, complementing several comprehensive reviews on this topic [1, 10, 15, 16].

3D structure and spatial organization of mammalian ER-PM junctions

Early EM studies on the structure of mammalian ER-PM junctions were mostly performed using muscle and neuronal cells. ER-PM junctions in muscle cells were observed in specific regions of sarcomere, the contraction unit of the muscle [3]. The gap distance between the ER and the PM at these junctions is kept at 9 to 12 nm. Correlations between the localization, number, and gap distance of ER-PM junctions with the contraction speed of different types of muscles were observed, consistent with an important role of ER-PM junctions in E-C coupling and muscle contraction. In neurons, ER-PM junctions were observed and described as close appositions between the PM and the subsurface cisterns (SSC) with a narrow ER lumen [17]. These SSC-PM junctions can be several microns long with a 5-8 nm gap between the SSC and the PM.

Compared with their counterparts in muscles and neurons, ER-PM junctions in non-excitable cells are of low abundance and more difficult to detect using thin-section EM. The average size of ER-PM junctions in Jurkat T cells is 150 nm with a gap of 10-25 nm [6]. In adherent HeLa cells, ER-PM junctions are 100-200 nm in size and preferentially localize at the basal/adhesion surface of the cell [18]. Interestingly, the number, but not the size, of ER-PM junctions increases during Ca^{2+} signaling [6].

Recently, a genetically-encoded ER-PM junctional marker named MAPPER was developed based on STIM1 [1, 12]. While thin-section transmission EM (TEM) reveals the gap distance of ER-PM junctions, MAPPER enables visualization of the whole contacting area of ER-PM junctions in living cells using light microscopy techniques such as confocal and total internal reflection fluorescence (TIRF) imaging (Figure 1). In addition, MAPPER greatly increases the throughput in analyzing ER-PM junctions by simultaneously visualizing hundreds of ER-PM junctions in a typical adherent mammalian cell. Analysis of ER-PM junctions using MAPPER in HeLa cells suggest that in addition to an increase in the number, a decrease in the gap distance also occurs during Ca^{2+} signaling [12]. This decrease is regulated by translocation of E-Syt1 to ER-PM junctions following receptor-induced increase in cytosolic Ca^{2+} , and facilitates Nir2 targeting to ER-PM junctions to mediate the PI cycle. Consistently, a decrease in gap distance from 21.8 nm to 14.8 nm was detected using cryo-electron tomography in E-Syt1-overexpressing COS-7 cells during Ca^{2+} signaling [19].

The size and shape of the contact area as well as the spatial organization of mammalian ER-PM junctions were examined using MAPPER and three super-resolution imaging techniques. Stimulated emission depletion (STED) microscopy was used to track the same ER-PM junctions in live HeLa cells, revealing that the size of individual ER-PM junctions remains the same during Ca^{2+} signaling [12]. Photoactivated localization microscopy (PALM), which provides superior lateral resolution, determined ER-PM junctions in HeLa cells to be oblong-shaped with the dimensions of $\sim 120\text{nm} \times \sim 80\text{nm}$ [20]. Based on these measurements, a single ER-PM junction is predicted to accommodate up to several hundred proteins. Lastly, TIRF-structured illumination microscopy (TIRF-SIM) revealed that cortical actin contributes to the spatial distribution of ER-PM junctions, with most ER-PM junctions in close proximity to F-actin [20]. Disruption of cortical actin results in decreased stability of ER-PM junctions, manifested as increased lateral motility and reduced number of junctions, suggesting intimate interactions between ER-PM junctions and actin cytoskeleton proximal to the PM.

A recent study using focused ion beam scanning electron microscopy (FIB-SEM) revealed the 3D architecture of ER-PM junctions in neuronal cells [21]. The size of neuronal ER-PM junctions was found to be generally larger than those observed in other cells. Notably, ER-PM junctions are larger in cell bodies than those in dendrites and axons. In addition, ER-PM junctions were shown to be highly abundant, representing over 10% of the PM in neurons, compared with 2~4% in non-excitable cells [1].

Molecular mechanisms underlying formation and functions of mammalian ER-PM junctions

The ER and the PM are kept in close apposition at ER-PM junctions by protein-protein and protein-lipid interactions between the two membrane compartments. Many proteins have been reported to support the formation and functions of mammalian ER-PM junctions. Here we discuss seven groups of proteins that harbor well-characterized dual targeting mechanisms for the ER and the PM, enabling their constitutive or dynamic enrichment at ER-PM junctions (Figure 2).

JPHs:

JPHs are four ER membrane proteins highly expressed in excitable mammalian cells [4]. JPH1 and JPH2 are selectively expressed in muscles, whereas JPH3 and JPH4 are highly expressed in neural tissues. JPH contains multiple cytosolic membrane occupation and recognition nexus (MORN) motifs that mediate their localization to ER-PM junctions by binding to phospholipids at the PM. JPH1 knockout mice died shortly after birth with reduced number of triad junctions in skeletal muscles [22], demonstrating the essential role of ER-PM junctions in E-C coupling as described in the introduction. JPH2 knockout mice exhibited embryonic lethality with cardiac arrest and reduced number of ER-PM junctions in cardiac myocytes [23]. JPH3 and JPH4 double knockout mice exhibited impaired motor coordination and learning [24]. These findings demonstrate the important role of JPHs in the formation of ER-PM junctions and functions of muscle and neuronal cells.

Kv2 channels:

Kv2.1 and Kv2.2, also known as KCNB1 and KCNB2, are PM voltage-gated K⁺ channels abundantly expressed in mammalian brain and also found in other excitable tissues including muscles and pancreatic islets. Unique among the 7 groups of ER-PM junctional proteins discussed here, Kv2 channels are currently the only known PM proteins mediating ER-PM tethering. Kv2.1 and Kv2.2 were initially observed to localize in large clusters in the PM at proximal dendrites, axon initial segments, and with particular abundance, in the soma of neurons [25]. These clusters were later shown to localize at ER-PM junctions via the binding of the proximity restriction and clustering (PRC) domain in the cytosolic region to the ER [26, 27]. Notably, Kv2.1 and Kv2.2 enrichment is independent of ion conductance [28], and clustered Kv2.1 channels do not efficiently conduct K⁺ [29].

Recently, two independent groups employed proteomic analyses and identified the ER membrane VAMP-associated proteins (VAPs), VAPA and VAPB, as interactors of Kv2.1 [30, 31]. VAPs bind double phenylalanine in an acid tract (FFAT) motif-containing proteins, and localize them to junctions between the ER and other membrane compartments such as the PM, Golgi, and endosomes [32]. Interestingly, VAP-Kv2 interaction is mediated by a noncanonical phosphorylation-dependent FFAT motif in the PRC domain of Kv2.1 [30, 31]. Conversely, Ca²⁺-dependent dephosphorylation induced by excessive excitatory stimulation by exposure to noxious conditions such as hypoxia result in Kv2 cluster dispersal [33]. While the ER-PM targeting mechanism has been identified, the biological function of Kv2 channels localizing at ER-PM junctions remains to be addressed.

STIMs:

STIM1 and its family member STIM2, are ubiquitously expressed singlepass transmembrane ER proteins important for SOCE as described in the introduction. Following ER Ca^{2+} depletion triggered by receptor stimulation, dissociation of Ca^{2+} from the EF-SAM domain in the luminal region induces STIM1 oligomerization and conformational change [5, 34]. An exposed C-terminal polybasic (PB) motif in activated STIM1 binds to $\text{PI}(4,5)\text{P}_2$ at the PM, resulting in STIM1 enrichment at ER-PM junctions [35]. Consistently, significant reduction of PM $\text{PI}(4,5)\text{P}_2$ level abrogated STIM1 localization to ER-PM junctions, limiting SOCE in Ras-association domain family 4 (RASSF4) knockdown cells [36]. Activated STIM1 at ER-PM junctions interacts with the PM Ca^{2+} channel Orai1, leading to Orai1 enrichment at ER-PM junctions and SOCE for propagation of Ca^{2+} signaling and refill of ER Ca^{2+} store [6]. Interestingly, STIM1 dynamically tracks microtubule plus ends by binding to the EB1 protein via its TRIP motif near the C-terminus [37]. EB1 binding limits STIM1 targeting to ER-PM junctions, providing a mechanism that shapes local Ca^{2+} signaling in cellular regions with growing microtubules [38].

E-Syts and TMEM24:

E-Syt1, E-Syt2, and E-Syt3 are ER membrane proteins with a cytosolic region containing a synaptotagmin-like mitochondrial-lipid binding protein (SMP) domain followed by multiple C2 domains [39]. E-Syt2 and E-Syt3 constitutively enrich at ER-PM junctions with their C2 domains interacting with PM $\text{PI}(4,5)\text{P}_2$, whereas E-Syt1 dynamically translocates to ER-PM junctions following a significant increase of cytosolic Ca^{2+} that releases its auto-inhibition [12, 39, 40]. Knockdown of all three E-Syts or a decrease in PM $\text{PI}(4,5)\text{P}_2$, as in RASSF4-knockdown cells, resulted in a significant reduction of ER-PM junctions [36, 39]. Structural and biochemical studies indicated that the SMP domain of E-Syts can transfer glycerolipids and DAG [41, 42]. Nevertheless, mice deficient in all E-Syts exhibited no obvious defects, suggesting redundancy in the functions of these ER-PM tethering lipid transfer proteins [43, 44]. A recent study identified TMEM24, another SMP domain-containing ER membrane protein with a preference to transfer PI [45]. TMEM24 localizes at ER-PM junctions by binding to the PM via its positively-charged C-terminal region. It was proposed that Ca^{2+} -dependent phosphorylation and dephosphorylation of TMEM24 result in oscillatory localization of TMEM24 at ER-PM junctions, regulating pulsatile insulin secretion in insulinoma cells.

Nir2 and Nir3:

Nir2 and Nir3, also known as PITPNM1 and PITPNM2, are widely expressed cytosolic lipid transfer proteins that mediate the PI cycle as described in the introduction. Nir2 and Nir3 contain an N-terminal PI transfer protein (PITP) domain, an FFAT motif that can bind VAPs in the ER, a DDHD domain with unknown functions, and a C-terminal LNS2 domain that can bind PA. The production of PA at the PM following receptor-induced $\text{PI}(4,5)\text{P}_2$ hydrolysis leads to recruitment of Nir2 and Nir3 to ER-PM junctions within a minute [12-14]. Interestingly, the Nir2 mutant with mutations in the FFAT and LNS2 domains failed to mediate $\text{PI}(4,5)\text{P}_2$ replenishment following the hydrolysis [13], indicating that enrichment at ER-PM junctions is important for Nir2 to mediate the PI cycle. Nir2 knockout mice were

embryonic lethal [46], consistent with its role in maintaining the level of PM PI(4,5)P₂ important for numerous cellular processes.

ORP5 and ORP8:

ORP5 and ORP8 are ER integral membrane proteins containing an N-terminal pleckstrin homology (PH) domain and an OBSP-related domain (ORD). It was proposed that ORP5 and ORP8 enrich at ER-PM junctions by binding to PI 4-phosphate (PI4P) at the PM via the PH domain, and mediate transport of PM PI4P to the ER and phosphatidylserine (PS) from the ER to the PM via the ORD [47]. Recent studies revealed that PM binding of ORP5 and ORP8 involves both the N-terminal PB region and the PH domain [48-50]. In addition, structural studies uncovered that the PH domain of ORP5 and ORP8 binds PI(4,5)P₂, and enrichment of ORP5 and ORP8 at ER-PM junctions depends on the level of PM PI(4,5)P₂ [48, 50]. Notably, ORP5 constitutively localizes at ER-PM junctions, whereas ORP8 enriches at ER-PM junctions when PM PI(4,5)P₂ level is elevated. Knocking down both ORP5 and ORP8 resulted in elevated PM PI(4,5)P₂. Based on these findings, a rheostat model was proposed in which ORP5 and ORP8 countertransport PS and PI4P between the ER and the PM at ER-PM junctions to maintain the homeostasis of PM PI(4,5)P₂ by limiting its precursor, PI4P [50].

GRAMDs:

GRAMD1a-c and GRAMD2a and b comprise a family of mammalian proteins with structural similarity to the yeast Ltc/Lam ER membrane proteins [51-53]. GRAMDs contain an N-terminal GRAM domain—found in glucosyltransferases, Rab-like GTPase activators, and myotubularins—that resembles a PH domain, and a single C-terminal ER-anchoring transmembrane domain. Both GRAMD1a and GRAMD2a require the GRAM domain for ER-PM junction localization [54]. Additionally, GRAMD2a localizes at ER-PM junctions in a PI(4,5)P₂-dependent manner. In a recent study, Sandhu et al. redefined the exon-intron topology of *Gramd1a-c*, characterized a central domain resembling steroidogenic acute regulatory protein (STAR)-related lipid transfer (START) domain, and renamed the protein products Aster-A, -B, and -C [55]. Asters bind cholesterol in vitro, and the crystal structure of the central domain in Aster-A reveals a 7-stranded beta-sheet pocket for cholesterol occupancy. Aster-B is recruited to ER-PM junctions in response to cholesterol and the GRAM domain of Aster-B is sufficient for cholesterol induced recruitment to the PM. Interestingly, the Aster-B GRAM domain pelleted with liposomes containing PS independent of cholesterol content. Lastly, mice lacking Aster-B exhibited altered adrenal sterol homeostasis, consistent with a role of Asters in facilitating cholesterol transport from the PM to the ER downstream of the high-density lipoprotein receptor. These findings implicate GRAMD/Aster proteins in sterol transport via ER-PM junctions, yet the relationship linking PS and sterol levels at the PM is currently unknown.

Perspectives

Recent findings indicate that mammalian ER-PM junctions are membrane contact sites supported by cortical actin and multiple families of proteins at a given time. Since the enrichment of these proteins at ER-PM junctions can be regulated by various factors such as

ER/cytosolic Ca^{2+} levels, PM PA/PS/PI4P/PI(4,5) P_2 /cholesterol levels, or phosphorylation, it is likely that the composition of single ER-PM junctions is heterogeneous and controlled by the local environment and stimulated state of a cell. Also, it is difficult to completely disrupt mammalian ER-PM junctions, given many different proteins contribute to the formation and stability of these structures. It is of interest to investigate the crosstalk among these proteins at ER-PM junctions, determine the factors that control the size and shape of ER-PM junctions, characterize the molecules that mediate the close association between cortical actin and ER-PM junctions, examine the distribution of ER-PM junctions during actin reorganization and cell migration, and continue to identify new tethers and functions of ER-PM junctions.

Arguably, mammalian ER-PM junctions are the best characterized membrane contact sites with many insights into their structure, regulation, and physiological functions. The knowledge derived from studying mammalian ER-PM junctions may be applied to understand inter-organelle signaling at other membrane contact sites.

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Figure 1. Structure and spatial organization of ER-PM junctions.

(A) A TEM image of an ER-PM junction (red arrow head) in a MAPPER-expressing HeLa cell. (B-C) Images of the adherent surface of a MAPPER-expressing HeLa cell acquired using TIRF microscopy (TIRFM) and PALM. (D) A close-up from (C). (E) TIRF-SIM image of a HeLa cell with ER-PM junctions labeled by MAPPER (green) and stained with AF568-phalloidin for F-actin (orange). (F) A close-up from (E). (G) Diagram depicting spatial distribution of single ER-PM junctions and cortical actin. Scale bars: 1 (B), 0.2 (D), 10 (E) and 2 (F) μm . Figure 1A and 1B-F were originally published in [10] and [20].

Figure 2. Proteins enriched at mammalian ER-PM junctions.

JPH, Junctophilin; MORN, membrane occupation and recognition nexus motif; PRC, proximity restriction and clustering domain; EF-SAM, EF hand and sterile alpha motif; CC, coiled-coil domain; PB, polybasic domain; SMP, synaptotagmin-like mitochondrial-lipid binding protein domain; PITP, phosphatidylinositol transfer protein domain; FFAT, two phenylalanines (FF) in an acidic tract motif; DDHD, domain characterized by these conserved residues; LNS2, Lipin/Ned1/Smp2 domain; ORD, OSBP-related domain; PH, pleckstrin homology domain; GRAM, glucosyltransferases, Rab-like GTPase activators and myotubularins domain; ASTER, START (StAR-related lipid-transfer)-like domain.