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ER–PM connections: sites of information transfer and inter-organelle communication

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Abstract

Eukaryotic cells are divided into distinct membrane-bound organelles with unique identities and specialized metabolic functions. Communication between organelles must take place to regulate the size, shape, and composition of individual organelles, as well as to coordinate transport between organelles. The endoplasmic reticulum (ER) forms an expansive membrane network that contacts and participates in crosstalk with several other organelles in the cell, most notably the plasma membrane (PM). ER–PM junctions have well-established functions in the movement of small molecules, such as lipids and ions, between the ER and PM. Recent discoveries have revealed additional exciting roles for ER–PM junctions in the regulation of cell signaling, ER shape and architecture, and PM domain organization.

Introduction

The transmission of signals between cells and tissues is fundamental for normal cell growth and development. Only recently have we begun to appreciate the significance of information transfer between intracellular organelles at membrane contact sites. The endoplasmic reticulum (ER) is the origin of the secretory pathway and it has essential roles in protein modification and quality control, lipid biosynthesis, and calcium signaling. Numerous proteins and lipids synthesized in the ER are ultimately destined for transport to the plasma membrane (PM). The PM undergoes remodeling, in response to both intrinsic and extrinsic cues, by the delivery of new material via the secretory pathway and removal of proteins and lipids by endocytosis. Consequently, biosynthesis in the ER must be modulated, corresponding to changes in PM composition. However, secretory vesicles derived from the ER do not directly fuse with the PM, and endocytic vesicles derived from the PM do not fuse with the ER, implying that rapid transfer of information between the ER and PM occurs independent of vesicle trafficking (Figure 1a). To balance ER metabolism with changes in PM composition, the ER and PM engage in crosstalk at membrane junctions — where these two organelles become closely apposed without undergoing membrane fusion (Figure 1a). The ER consists of a continuous system of membrane sheets and tubules that form contacts with several organelles in the cell (e.g. the PM, Golgi compartments, endosomes, lysosomes, and mitochondria) [1-3]. In this way, the ER coordinates with multiple membrane

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compartments along the secretory and endocytic systems. Of particular interest, ER–PM contacts have fascinated cell biologists for decades and recent findings have highlighted the importance of these conserved structures in membrane transport and cell signaling pathways.

Discovery and features of ER–PM contacts

ER–PM contact sites are ubiquitous structures that take on unique architectures in different cell types [4]. Junctions between peripheral ER structures and the PM were first discovered by Porter and Palade in electron microscopy studies on muscle cells [5]. These structures, termed dyads and triads, were later shown to control calcium fluxes involved in excitation–contraction coupling [6]. Subsequently, ER–PM junctions have been described in several cell types including neurons, immune cells, insect photoreceptor cells, and in plants [7-11]. While ER–PM contacts are not abundant in some cell types, large regions of the PM in yeast cells (approximately 20–45% of the PM) have an underlying network of peripheral ER, with an average distance of 30 nm between the organelles (Figure 1b) [12•,13•]. Owing to the close association of the PM and ER, ribosomes are excluded from the face of the ER adjacent to the PM [12•]. In mammalian cells, this distance can even narrow to within 10 nm (Figure 1c) [14], suggesting that the spacing of ER–PM contacts is precisely regulated.

While ER–PM contacts are ubiquitous structures, they adopt distinct shapes and architectures. Even in the same cell type, such as neurons, ER–PM junctions take on different forms. ER–PM contacts appear as discrete punctate regions at the synapse [7]. In other regions (the cell body, at bases of axons and dendrites, and the PM of cells neighboring synaptic boutons) ER–PM associations are more extensive (>1 μm) [8]. In yeast cells, the cortical ER is a meshwork of sheets and tubules shaped by the membrane-inserting reticulon proteins [12•], suggesting that ER curvature may be particularly important for some processes at ER–PM contacts. The many features (shapes and sizes) displayed by distinct ER–PM contacts also suggest multiple functions for these conserved structures. Consistent with this, ER–PM contacts have well-established roles in both calcium and lipid transport. Within the last few years, ER–PM contacts have become even more prominent in cell biology and are no longer limited to calcium and lipid homeostasis. As such, recent studies have uncovered additional roles in the control of organelle shape and morphology, inter-organelle communication, cell stress responses and signaling networks, and membrane trafficking pathways.

Calcium transport

A well-known function of ER–PM contacts is control of calcium (Ca^{2+}) dynamics. In metazoans, the ER is a major storage site for Ca^{2+} and release of this intracellular pool is triggered by various stimuli at the PM to generate cytoplasmic Ca^{2+} signals. At ER–PM junctions in muscle (termed triads, Figure 2a), voltage-gated Ca^{2+} channels (VGCCs) in PM invaginations (named T-tubules,Figure 2a) activate closely apposed ryanodine receptors (RyR) in the ER to elicit Ca^{2+} release during excitation–contraction coupling (Figure 2b) [6]. An accessory protein named junctophilin serves as a bridge to maintain the close association of the ER and PM in muscle (Figure 2b) [15]. Junctophilin is an ER-localized

integral membrane protein with several cytoplasmic MORN (membrane occupation and recognition nexus) repeats suggested to bind phosphoinositide (PIP) lipids at the PM. Interestingly, loss of the PIP 3-kinase isoforms, PI3KC1A and PI3KC1B, in mice results in mislocalization of both junctophilin and VGCCs, as well as a reduction in T-tubules [16•].

ER–PM contacts came into the spotlight in cell biology upon the discovery that ER-localized STIM (stromal interaction molecule) proteins couple with the PM Ca^{2+} channel Orai1, also named the Ca^{2+} release-activated Ca^{2+} channel (CRAC), in the store-operated Ca^{2+} entry (SOCE) pathway [17,18]. ER Ca^{2+} pools must be replenished to sustain excitation–coupling contraction, as well as for Ca^{2+} fluxes mediated by the ER-localized inositol trisphosphate (IP_3) receptor in response to activation of signaling receptors and phospholipase C isoforms at the cell surface [4]. The STIM proteins sense Ca^{2+} concentrations in the ER lumen and oligomerize at ER–PM contacts when Ca^{2+} levels are depleted (Figure 2c) [11]. Cytoplasmic regions in the STIM proteins include a polybasic domain that binds PIP lipids at the PM and the CRAC activation domain (CAD) that directly interacts with the PM-localized Ca^{2+} channel Orai1 (Figure 2c). STIM–Orai1 interaction both activates and bolsters the Ca^{2+} selectivity of the Orai1 ion channel [19]. Interestingly, the crystal structure of the Orai1 channel has been recently solved, revealing further insights into Orai1 channel activity and STIM interactions [20••].

In addition to Orai1, the STIM proteins interact with several other factors. A protein termed junctate serves as an accessory Ca^{2+} sensor and structural component of STIM–Orai1 assemblies at ER–PM contacts [21]. The ER resident protein SARAF associates with STIM to attenuate ER Ca^{2+} reloading, providing another regulatory layer to SOCE [22]. Interestingly, STIM proteins are known to inhibit L-type voltage gated Ca^{2+} channel (VGCC) activity [23•,24•]. Reciprocal regulation of VGCC (inhibition) and Orai1 (activation) by STIM1 ensures that SOCE predominates in cells expressing both Ca^{2+} channel types, such as immune cells. STIM proteins are linked to Ca^{2+} transporter activities as well. POST (partner of STIM) binds STIM upon depletion of Ca^{2+} ER stores, but does not regulate Orai1 channel activity [25•]. Instead, POST inhibits PM Ca^{2+} ATPase (PMCA) transporter activity at the PM and binds a SERCA (smooth ER Ca^{2+} ATPase) isoform. The effect on SERCA transporter activity is unknown, but one possibility is that POST specifically couples Orai1 activation to SERCA-mediated Ca^{2+} reloading in the ER.

Lipid transport

ER–PM contacts are also implicated in nonvesicular sterol lipid transport [1,26]. A conserved family of oxysterol-binding (OSBP)-related proteins (ORPs) is thought to carry out sterol lipid transfer. ORP family members from both yeast and mammalian cells localize to ER–PM junctions [27,28], and in addition to the conserved sterol-binding domain, specific domains or motifs target ORPs to ER–PM contacts (Figure 3a). In a subset of ORPs, a pleckstrin homology (PH) domain binds PIP lipids in the PM [29], and a motif containing two phenylalanine residues in an acidic tract (FFAT) binds to integral ER membrane proteins named VAPs (VAMP-associated proteins) [30].

The structure of an ORP family member from yeast (named Osh4/Kes1) bound to sterol has been determined [31]. The core of the sterol-binding domain forms a barrel containing hydrophobic residues critical for function. The sterol-binding domain also binds negatively charged phospholipids on the surface of the protein [28], and a crystal structure of Osh4/Kes1 bound to the PIP lipid isoform phosphatidylinositol 4-phosphate (PI4P) has been recently solved [32•]. Interestingly, interactions between basic residues at the top of the barrel and PI4P prevent sterol loading *in vitro*, suggesting a model whereby PI4P drives the directional transport of sterol from the ER to the PM (Figure 3a). At ER–PM contacts, ORPs may readily extract newly synthesized sterol lipid in the ER, where PI4P levels are relatively low. Following delivery, PI4P may prevent re-extraction of sterol from the PM. Consistent with this, loss of a PI 4-kinase (PI4KIIIα) that generates PI4P at the PM results in slightly reduced PM cholesterol levels [33•]. While this is an intriguing model, overexpression of ORPs and VAPs promotes the transport of sterol lipids from the PM to the ER [34]. Thus, additional regulatory factors (e.g. involved in PI4P metabolism) may control the directionality of sterol lipid transfer at ER–PM contacts. In addition, direct evidence for ORPs as sterol transfer proteins *in vivo* remains controversial [35], and ORPs are also suggested to function as sterol-sensing signal transduction proteins [36].

Regulation of cell signaling networks

It is becoming increasingly evident that ER–PM contacts are sites for the transmission of signals, in addition to Ca^{2+} and sterol lipids. In particular, the PIP isoform PI4P serves as an essential signaling molecule in the control of cell growth and polarity, hormone and calcium signaling, and regulated secretion and endocytosis [37•,38]. Notably, an ER-localized PIP phosphatase, named Sac1, regulates PI4P levels at ER–PM contact sites [39•]. This process is mediated by the ER-localized VAP proteins and by ORP family members localized to ER–PM junctions (Figure 3b). ORPs serve as sensors of PI4P in the PM, and in turn control PI4P levels by activating the Sac1 PIP phosphatase. While sterol binding is not required for ORP-mediated Sac1 activation, Sac1 may promote ORP sterol-binding and signaling functions. PI4P turnover by Sac1 may relieve PI4P inhibition of sterol extraction by ORP family members, and thus drive ORP-stimulated PM to ER sterol lipid transfer [34]. In addition, the founding member of the ORP family, OSBP, attenuates MAPK signaling upon binding cholesterol and downstream protein phosphatases [40]. Thus, turnover of PI4P by Sac1 at ER–PM contacts might facilitate ORP cholesterol binding and subsequent downstream signaling activities. Regulation of PI4P by Sac1 may be important for other processes at ER–PM contacts as well. A PI4K isoform (PI4KIIIα) that generates PI4P at the PM is involved in the formation of STIM-dependent ER–PM junctions and activation of the Orai1 Ca^{2+} channel [33•,41]. Consequently, Sac1 function at ER–PM contacts may be important in control of the SOCE pathway.

The ER-anchored protein tyrosine phosphatase PTP1B functions at ER–PM junctions as well. Several potential PM-localized substrates for PTP1B have been identified, including receptor tyrosine kinases (RTKs) such as the insulin receptor, epidermal growth factor receptor (EGFR), and ephrin (Eph) receptors (Figure 3c) [42]. Activated, internalized RTKs are dephosphorylated by PTP1B at ER-endosome membrane contact sites, and this process is necessary for RTK down-regulation and degradation in lysosomes [43]. However, new

evidence suggests that PTP1B directly dephosphorylates PM-localized RTKs at ER–PM contacts, at least in the case of Eph receptors [44•]. In addition to integral membrane RTKs, PTP1B interacts with and regulates the nonreceptor tyrosine kinase Src at ER–PM contact sites [45]. Remarkably, PTP1B-Src interactions recruit Src to cell adhesion complexes [45], and PTP1B dephosphorylates Eph receptors at ER–PM junctions associated with cell–cell contacts [44•]. These findings imply that inter-organelle signaling at ER–PM contacts may control cell–cell communication during normal cell development and disease states, such as tumor cell progression. ER–PM crosstalk at cell adhesion sites may even mediate communication between ER membrane networks between neighboring cells. PTP1B activity is regulated by reactive oxygen species (ROS) [46], and the ER is a major site for ROS production upon protein misfolding [47]. Thus, ER-mediated ROS signaling may allow a cell to convey its ER status (i.e. oxidative stress, protein misfolding, Ca^{2+} depletion, lipid misregulation) to neighboring cells by modulating PTP1B and RTK activity at ER–PM contacts. While these ideas are still speculative, inter-organelle communication between plant cells is known to occur through specialized ER–PM junctions termed plasmodesmata [48].

ER–PM tether proteins control ER organization and stress responses

The proteins primarily responsible for facilitating ER–PM contacts have remained unclear until recently. In yeast, where there are substantial ER–PM contacts (Figure 4a), three conserved protein families serve as ER–PM tethers [49•,50••,51••,52•]: the yeast VAP proteins Scs2/22, Ist2 (related to the TMEM16 channel family [53,54]), and the tricalbin proteins Tcb1/2/3 (orthologs of the extended synaptotagmin-like proteins E-Syt1/2/3 [55]). The ER–PM tethering proteins are anchored in the ER and interact with the PM via cytoplasmic lipid-binding and protein-binding domains (Figure 4b) [50••,51••]. While each of the tethering protein families is sufficient for ER–PM contacts, loss of all six ER–PM tethers results in a massive reduction in ER–PM contacts, decreasing the percentage of the PM associated with ER from ~40% to ~5% (Figure 4a) [50••]. Sequential loss of ER–PM tethers also drastically changes the morphology of the peripheral ER network and results in accumulation of internal ER structures (Figure 4a) [50••,56]. The ER is arranged into functional domains, and changes in ER architecture may cause mislocalization (i.e. mixing or dilution) of resident ER proteins resulting in ER dysfunction. Cells lacking ER–PM tethering proteins accumulate high levels of PI4P on the PM, consistent with impaired Sac1-mediated PI4P turnover at ER–PM contacts [50••]. Moreover, yeast cells lacking ER–PM contacts constitutively induce the unfolded protein response (UPR) pathway and require an intact UPR system for survival [50••]. The UPR is critical for maintaining the homeostasis of the ER [57], suggesting a role for ER–PM contacts in ER function and stress signaling pathways.

Vesicle trafficking and plasma membrane domain organization

Vesicle-mediated trafficking controls cell signaling through the regulated secretion of hormones and neuro-transmitters, and by endocytosis of signaling receptors from the cell surface. Roles for ER–PM contacts in membrane trafficking, especially PM internalization, are now being recognized. PTP1B activity at ER–PM contacts is necessary for efficient

internalization of active ligand-bound Eph receptors (Figure 3c) [58]. Interestingly, PTP1B regulates interferon receptor (IFNAR1) internalization by dephosphorylating a tyrosine residue in an endocytic motif within IFNAR1 recognized by the clathrin adaptor protein-2 (AP-2) complex [59], providing some insight into how PTP1B activity at ER–PM contacts modulates rates of PM protein internalization. In addition to regulatory roles in endocytosis, the ER-localized STIM proteins are recruited to sites of phagocytosis where they regulate Ca^{2+} and actin cytoskeletal dynamics necessary for phagosome formation [60•]. Interestingly, certain pathogens that escape the innate immune response co-opt phagosomal-ER membrane contacts for survival of the pathogen within phagosomes [61,62].

The role for ER–PM contacts in supplying local Ca^{2+} signals during phagocytosis suggests additional roles for ER–PM junctions in Ca^{2+}-regulated membrane trafficking events. The SOCE pathway provides capacitive Ca^{2+} stores necessary for protein cargo sorting in the ER and Golgi compartments [4], but it is unknown whether Orai1 regulates endocytosis or fusion of secretory vesicles at the PM. In neurons, L-type VGCCs are responsible for Ca^{2+} influx necessary for synaptic vesicle fusion and neurotransmitter release. Notably, STIM proteins interact with L-type VGCCs and promote their clearance from the PM, in addition to acutely inhibiting channel activity [23•]. Because STIM proteins inhibit VGCC activity and promote VGCC down-regulation [23•], ER–PM contacts may have important regulatory roles in neurotransmission.

The very close association of the ER and PM may preclude clathrin-coated pit (CCP) formation as well as secretory vesicle delivery at ER–PM contacts. However, the peripheral ER membrane network is a highly dynamic meshwork [3]. In yeast, constant re-organization of the peripheral ER appears to play a role in determining the distribution of CCPs at the PM [63]. Thus, the PM may be organized into ER-free zones where vesicle trafficking takes place and ER-bound regions that form productive ER–PM connections for the regulation of PM lipid composition, as well as control of PM channels, transporters, and signaling receptors (Figure 4c).

Conclusions and future directions

Organelle identity and specialization are established and maintained by membrane trafficking pathways. However, direct ER–PM crosstalk occurs at sites of close apposition between these two organelles independent of vesicle transport. The ER is a major site for protein quality control and secretion, lipid synthesis, and calcium homeostasis. Signaling between the ER and PM at membrane contact sites may regulate these important ER functions in response to changes in the cell's environment or metabolic requirements. In addition, ER–PM contacts may allow the ER to sense the state of the PM (damage, composition) and launch responses (Ca^{2+} dynamics, protein and lipid synthesis) to ensure the integrity and function of the PM even under stress conditions. As such, future studies may reveal PM proteins that bind to the recently described ER tether proteins and aid in this process.

Interestingly, the ER forms connections with several compartments in the cell (the PM, Golgi compartments, endosomes, lysosomes, mitochondria, and peroxisomes). ER-mediated

inter-organelle signaling may allow cross-talk between distinct organelles to strengthen cellular responses to various stimuli. Thus, the ER may serve as a conduit for information transfer to synergize the functions of specialized organelles. For instance, mitochondria associate with the peripheral ER network at ER–PM contacts and this aids in coupling Ca^{2+} influx to mitochondrial Ca^{2+} import [64] (see the accompanying article by B. Kornmann). The signals (e.g. Ca^{2+}, lipids, ROS, phosphorylation events) and effector proteins that transmit information along the ER network will be an important area for future studies in cell biology.

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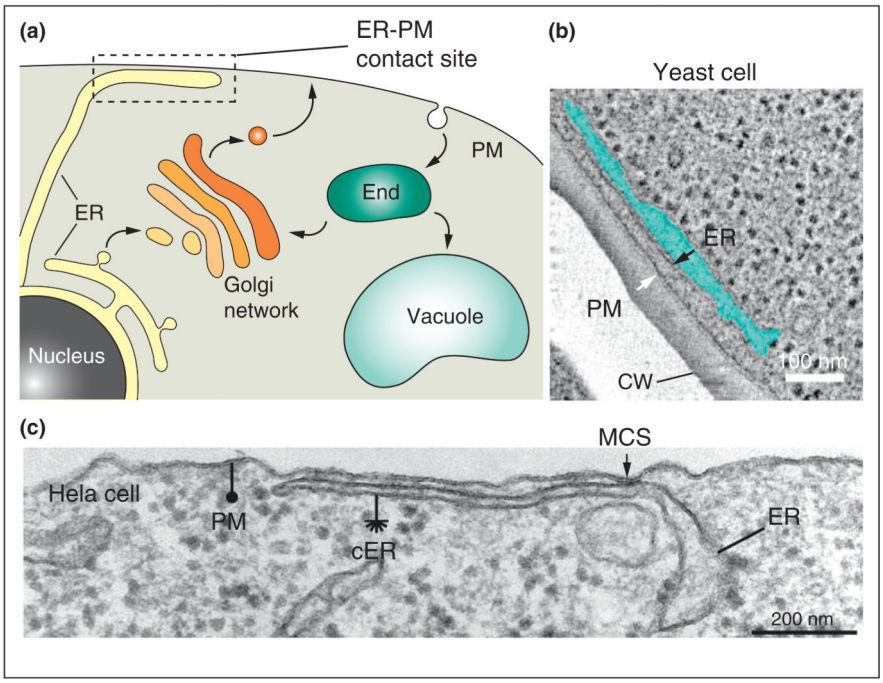

Figure 1.

Membrane contact sites between the endoplasmic reticulum (ER) and plasma membrane (PM) are conserved cellular structures. **(a)** The ER consists of a continuous membrane meshwork throughout the cell. Peripheral ER membranes form close associations with the PM without undergoing membrane fusion. These membrane contact sites allow for direct ER–PM crosstalk independently of membrane trafficking through the secretory and endocytic pathways. **(b)** In yeast cells, the PM is extensively associated with cortical ER (cER) membranes, as revealed by electron microscopy [12•], © West *et al.*, 2011. Originally published in *J Cell Biol*, **193**:333-346. The cortical ER (cER) membrane (black arrow) and PM (white arrow) are in close apposition, approximately 30 nm apart. The lumen of the cER compartment is shaded in blue. Notably, electron-dense ribosomes in the cytoplasm are excluded from the ER–PM contact zone. The cell wall (CW) and scale bar (100 nm) are indicated. ER–PM contact sites share conserved features and also display unique properties in different cell types. **(c)** Electron microscopy of a mammalian Hela cell expressing the STIM proteins [14], © Orci *et al.* and the National Academy of Sciences, 2009. Originally published in *Proc Natl Acad Sci USA* 2009, **106**:19358-19362. Similar to yeast cells, the cortical ER (cER, asterisk) is depleted of ribosomes as compared to internal ER membranes. Notably, the PM-associated cER and internal ER membranes display additional distinctions. The cER structures associated with the PM appear more flattened as compared to internal ER. Remarkably, the distance between the opposing membranes at ER–PM contacts can narrow to within 10 nm. An example of a tight membrane contact site (MCS) enriched in electron-dense material is shown (arrow). The scale bar indicates 200 nm.

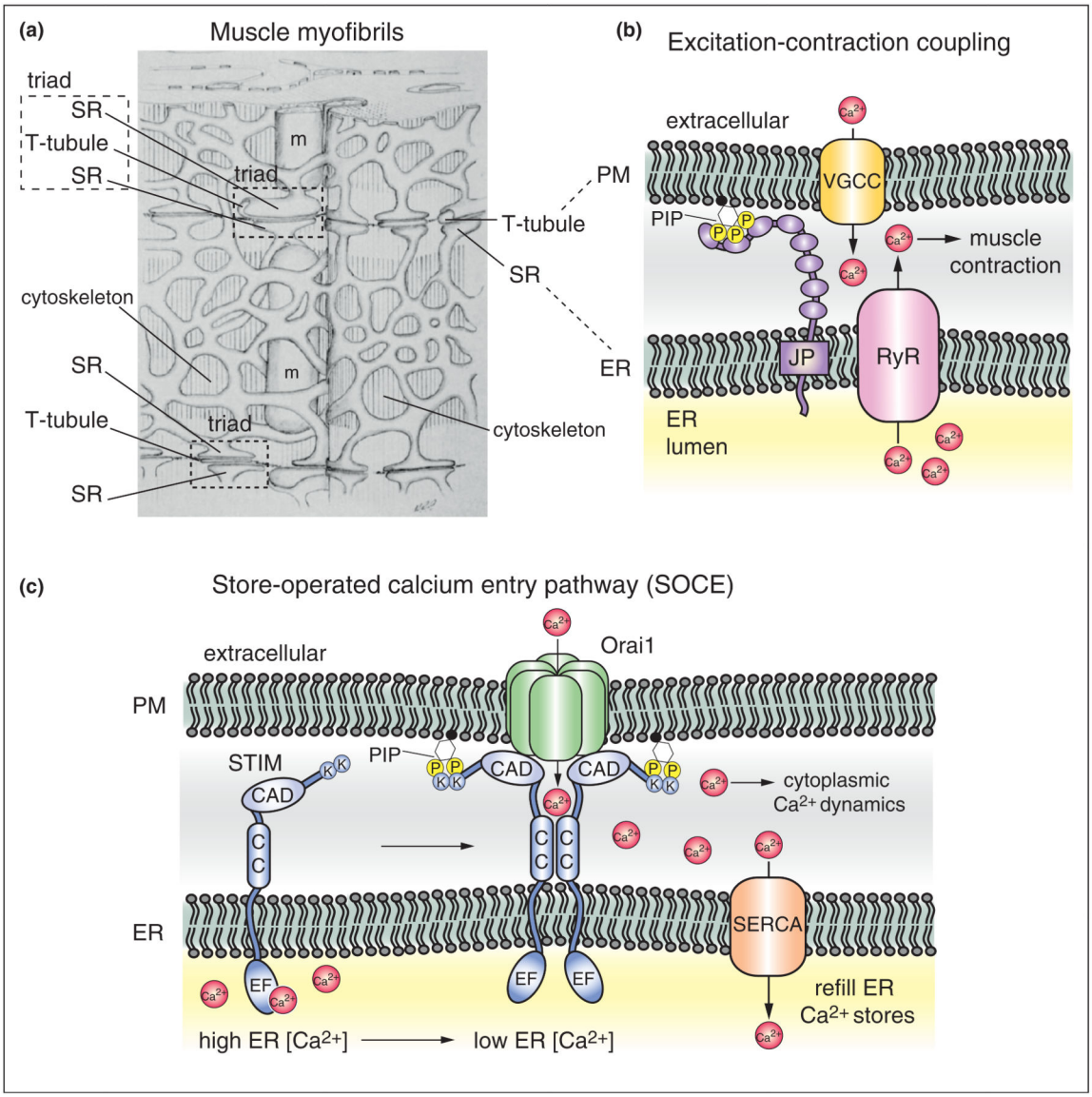

Figure 2.

ER–PM membrane contact sites regulate calcium (Ca^{2+}) dynamics. Associations between the ER and other organelles, such as the PM and mitochondria (m), were first observed by Keith Porter and George Palade in electron microscopy experiments on muscle cells [5]. **(a)** An illustration of cardiac muscle from this study is shown, © Rockefeller University Press, 1957. Originally published in *J Biophys Biochem Cytol*, **3**:269-300. Large invaginations of the sarcolemma (the PM), termed transverse tubules (T-tubules), were found to be closely associated with membranes of the sarcoplasmic reticulum (SR, the ER in muscle cells). These structures were termed triads (a T-tubule closely apposed by two terminal SR compartments) and were later shown to be involved in Ca^{2+}-mediated and cytoskeletal-mediated muscle contraction, known as excitation–coupled contraction. **(b)** Molecular components responsible for excitation-coupled contraction have been identified. At ER–PM contacts, voltage-gated Ca^{2+} channels (VGCC) localized in T-tubules interact with and

activate ryanodine receptors (RyR) in the ER [6]. Upon activation, the RyR releases Ca^{2+} stores from the ER lumen to generate Ca^{2+} signals in the cytoplasm necessary for cytoskeletal-mediated muscle cell contraction. The protein junctophilin (JP) is involved in ER–PM contact formation in muscle cells [15]. Junctophilin is an integral ER membrane protein with several cytoplasmic MORN repeats proposed to bind PIP lipids in the PM. **(c)** Following release, luminal ER Ca^{2+} stores must be replenished for normal ER function (e.g. lipid synthesis, protein folding and secretion). In many cell types, the STIM proteins function in the store-operated Ca^{2+} entry (SOCE) pathway to maintain Ca^{2+} homeostasis in the ER lumen [4]. The ER-localized STIM protein senses Ca^{2+} levels in the ER lumen via a Ca^{2+}-binding EF hand domain. Upon depletion of Ca^{2+} in the ER lumen, STIM proteins oligomerize via cytoplasmic coiled-coil domains (CC) and translocate to ER–PM contact sites. A polybasic region rich in lysine residues (K) within the cytoplasmic tail of STIM binds to PIP lipids in the PM. The STIM proteins subsequently interact with and recruit PM-localized Orai1 Ca^{2+} channels (also known as Ca^{2+} release-activated channels, CRAC) to ER–PM junctions. The CRAC-activation domain (CAD) within the STIM proteins directly binds and activates Orai1 channels, resulting in Ca^{2+} influx necessary for cytoplasmic Ca^{2+} signals and for reloading Ca^{2+} stores into the ER lumen through SERCA (smooth ER Ca^{2+} ATPase) transporters.

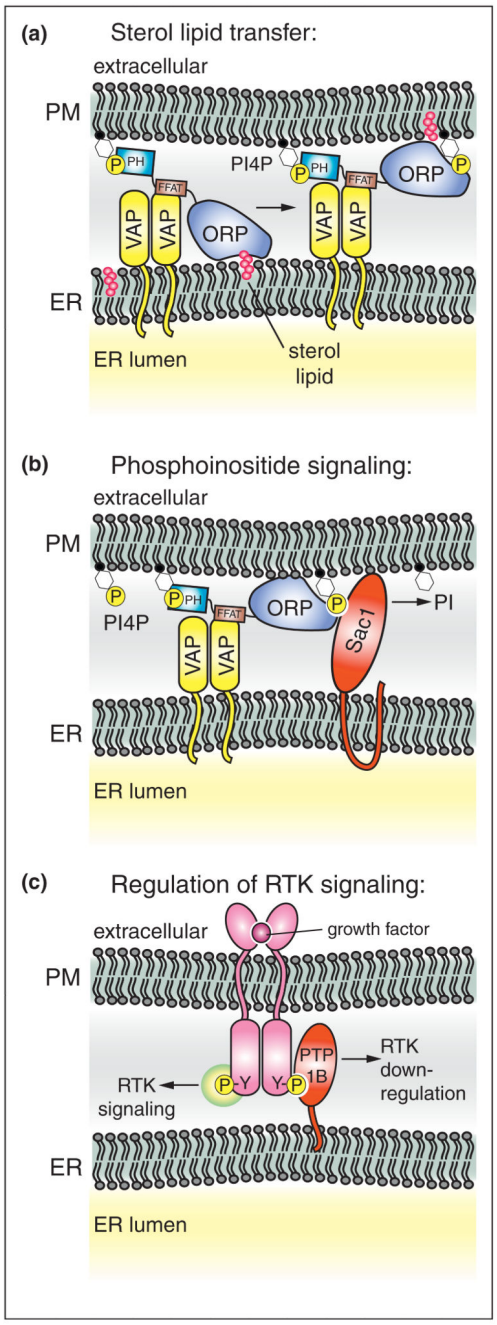

Figure 3.

Essential cellular processes occur at ER–PM contact sites. **(a)** Non-vesicular sterol lipid transfer is facilitated at ER–PM junctions. Oxysterol-binding protein related proteins (ORPs) are recruited to ER–PM junctions by protein–protein and protein–lipid interactions [28]. ORP family members contain a FFAT motif that binds integral ER membrane proteins termed VAPs and a PH domain that binds the PIP isoform PI4P in the PM [29,30]. The conserved ORP sterol-binding domain extracts newly synthesized sterol lipids (magenta) in the ER. Upon subsequent delivery of sterol to the PM, interactions between the mouth of the ORP sterol-binding domain and PI4P may inhibit extraction of sterol from the PM [32•]. **(b)**

ER–PM contacts are also sites for regulation of PI4P levels in the PM [39•,50••]. The ER-localized PIP phosphatase Sac1 is activated by ORPs family members at ER–PM contacts, resulting in PI4P turnover at the PM. This process is facilitated by VAPs in the ER. **(c)** In addition to lipid transfer and metabolism, growth factor receptor signaling is regulated at ER–PM junctions. The ER-anchored protein tyrosine phosphatase isoform PTP1B interacts with and dephosphorylates ligand-bound receptor tyrosine kinases (RTKs) at ER–PM contacts. Dephosphorylation of active RTKs terminates signaling events at the PM and promotes RTK endocytosis and down-regulation [42,43,44•,58].

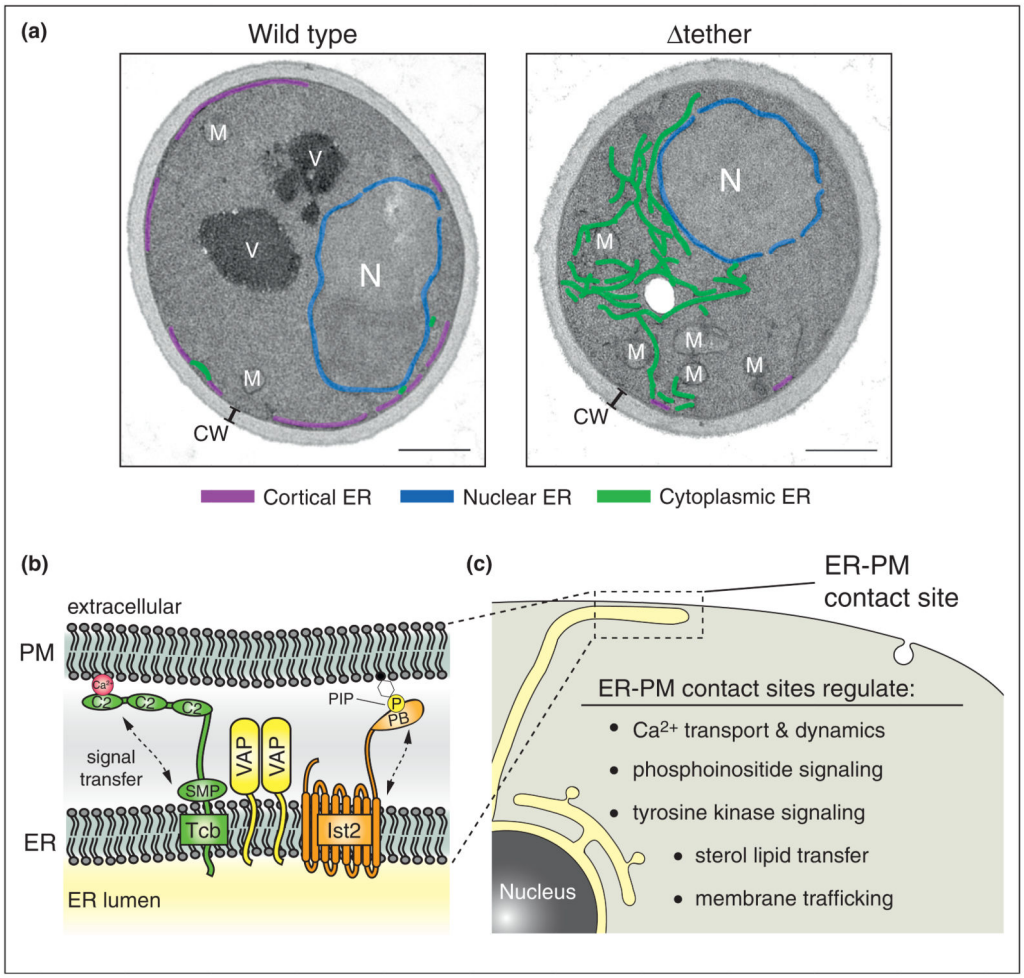

Figure 4.

Three conserved protein families are involved in ER–PM contact site formation and function. The tricalbin proteins (Tcb, orthologs of the extended synaptotagmin-like proteins), VAP family members, and Ist2 (related to the TMEM16 channel family) tether the ER to the PM in yeast cells [49•,50••,51••,52•]. **(a)** Electron microscopy of wild type and Δtether yeast cells [50••]. Originally published in *Dev Cell*, 2012, **23**: 1129–1140. In wild type cells, large regions of the PM are associated with cortical ER (purple). In cells lacking the ER–PM tether proteins (Δtether), cortical ER structures are depleted, and a meshwork of cytoplasmic ER accumulates (green). The nuclear ER (blue), mitochondria (M), nucleus (N), cell wall (CW) and vacuole (V) are also labeled. Scale bars, 500 nm. **(b)** All of the ER–PM tether proteins are integral ER membrane proteins that contain conserved cytoplasmic lipid-binding and protein-binding domains. The tricalbin proteins possess C2 domains that display Ca^{2+}-stimulated lipid binding activity and a conserved SMP domain that is sufficient for lipid binding and ER targeting. The Ist2 protein contains a carboxyl terminal polybasic domain (PB) proposed to bind PIP lipids at the PM. The lipid binding activities in the tether proteins may facilitate the transmission of signals between the PM and ER. The VAP proteins recruit lipid-binding ORP family members to ER–PM contact sites; additional interactions with PM proteins and lipids may be involved in VAP tethering function. **(c)**

ER–PM contact sites serve key roles in Ca^{2+} dynamics, sterol and phosphoinositide lipid signaling, growth factor receptor signaling, and membrane trafficking pathways. In addition to specific signaling roles, the ER–PM tether proteins may have general housekeeping functions in several of these essential cellular processes.