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Regulation of calcium and phosphoinositides at endoplasmic reticulum–membrane junctions

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Abstract

Effective cellular function requires both compartmentalization of tasks in space and time, and coordination of those efforts. The endoplasmic reticulum's (ER) expansive and ramifying structure makes it ideally suited to serve as a regulatory platform for organelle–organelle communication through membrane contacts. These contact sites consist of two membranes juxtaposed at a distance less than 30 nm that mediate the exchange of lipids and ions without the need for membrane fission or fusion, a process distinct from classical vesicular transport. Membrane contact sites are positioned by organelle-specific membrane–membrane tethering proteins and contain a growing number of additional proteins that organize information transfer to shape membrane identity. Here we briefly review the role of ER-containing membrane junctions in two important cellular functions: calcium signalling and phosphoinositide processing.

Keywords

calcium signalling; endoplasmic reticulum; membrane-membrane junctions; organelle contact sites; phosphoinositide regulation

This mini-review considers the role of endoplasmic reticulum–membrane contact sites in the regulation of cellular calcium and phosphoinositide levels, with an emphasis on mammalian cells.

Eukaryotic cells have evolved compartmentalization that orchestrates and segregates a myriad of important cellular processes. One organizing tool is the partitioning of the cytoplasm into membrane-enclosed organelles each with a distinct milieu and function. Although each organelle performs important independent tasks, they are not simply autonomous platforms of regulation. The success of complicated cellular reactions such as receptor signalling or apoptosis requires coordinated action by multiple organelles. One way that organelles communicate to organize specific cellular tasks is via membrane–membrane junctions or contact sites [1]. These sites of communication between organelles can be stable or dynamic, are mediated via specific proteins (Figure 1), are closely apposed (~10–30 nm), and facilitate the transfer of ions (e.g. calcium, Figure 1), proteins or lipids (e.g. phosphoinositides, Figure 2).

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Although diverse membrane–membrane contact sites are present throughout mammalian cells, those involving the endoplasmic reticulum (ER) are the most abundant and best studied. This is perhaps not surprising considering the ER's extensive network of ramifying tubules that project throughout the cell and its roles in protein trafficking and calcium storage. Such properties allow the ER to form functional platforms of communication, both static and dynamic, with the plasma membrane (PM) [2–4], Golgi [5,6], mitochondria [7,8], secretory granules (SGs) [9] and endo/lysosomes [10–12]. Two important functions of ER–membrane junctions are (1) the transfer of calcium ions to generate and coordinate cytoplasmic calcium signals and (2) the transfer of lipids. This review briefly highlights the major principles and molecular identity of the proteins involved in both processes; for more extensive reviews on the regulation of calcium or transfer of additional lipids at ER–membrane contact sites see [13,14] and [15,16] respectively.

Calcium signalling at endoplasmic reticulum–organelle contact sites

Local or global elevations in cytoplasmic calcium concentrations are the trigger for a host of cellular events including memory, fertilization, contraction, secretion, migration and transcription. Each cell's cytoplasmic calcium concentration is tightly regulated to facilitate this array of tasks. Increases in cytoplasmic calcium typically arise from two major calcium sources: the ER [via inositol 1,4,5-trisphosphate (IP_3) receptors or ryanodine receptors (RyR)] and the extracellular space (via voltage-dependent calcium channels and store-operated channels). Calcium influx is opposed by pumps and transporters on the ER [sarcoplasmic reticulum (SR)/ER Ca^{2+} ATPases, SERCA pump], PM (plasma membrane Ca^{2+} ATPases, PMCA, NCX) and mitochondria (mitochondrial calcium uniporter, MCU). Many cell membranes maintain a steep calcium gradient at rest, facilitating rapid (millisecond) and large (micromolar) increases in local or global cytoplasmic calcium that initiate cellular signalling events. Given the importance of the ER and PM in shaping cellular calcium dynamics, understanding their communication at ER–PM contacts is key to a clear view of cellular function.

Excitation-contraction coupling

A compelling and historically significant example of ER–PM contact sites is the regulation of cytoplasmic calcium during excitation–contraction (EC) coupling in striated muscle cells. In both cardiac (Figure 1A) and skeletal muscle (Figure 1B) deep PM (sarcolemma) invaginations called transverse-tubules (T-tubules) penetrate into the myocyte and are functionally coupled to the SR. These SR–sarcolemma contacts form dyads (1 T-tubule:1 SR terminal cisterna; Figure 1A) in cardiac muscle [17] and triads (1 T-tubule:2 SR terminal cisternae; Figure 1B) in skeletal muscle [18,19]. In both cases the functional coupling arises from the close apposition between dihydropyridine-sensitive voltage-gated calcium channels (DHPR) on the sarcolemma and RyR on the SR membrane (Figures 1A and 1B). The DHPR channels are activated by action potential propagation depolarizing the sarcolemma in both muscle types. However, the mode of SR coupling is muscle-specific: for skeletal muscle there is direct physical coupling between DHPR channels and RyR1 [19], whereas cardiac muscle relies on cooperative gating of clustered $Ca_V1.2$ calcium channels [20,21] to increase calcium in the cleft between membranes and thereby activate RyR2 via calcium-induced

calcium release [22]. In both cases, the release of SR calcium rapidly elevates cytoplasmic calcium to initiate muscle contraction. These stereotyped interactions between the SR and PM in myocytes are long lasting and essential for regulated EC-coupling.

Store-operated calcium entry

Another example of ER–PM cooperation is store-operated calcium entry (SOCE), wherein Ca^{2+} decrease in the ER lumen triggers Ca^{2+} influx through the PM. SOCE is a major calcium signalling pathway in excitable and non-excitable cells often initiated through activation of G_q protein-coupled receptors. The resulting hydrolysis of PM phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$] produces diffusible IP_3 which binds to IP_3 receptors on the ER membrane to stimulate release of ER calcium into the cytoplasm. It is this loss of calcium from the ER lumen that triggers SOCE and thus influx of calcium into the cytoplasm from the extracellular space.

The key determinants orchestrating this calcium-signalling cascade (for a comprehensive review see [23]) are stromal interaction molecules (STIM1 and STIM2 in mammals) in the ER membrane and Orai channels (Orai1, Orai2, Orai3 in mammals) in the PM (Figure 1C). How do these two proteins interact to facilitate the influx of calcium into the cytoplasm? At rest, STIM1 molecules (typically dimers) and Orai channels (reportedly hexamers) can freely diffuse along the ER and PM respectively. Depletion of ER calcium causes loss of calcium from the luminal EF hand domains on STIM1 dimers and a conformational change in their structure that promotes oligomerization and subsequent translocation to regions of the ER closely apposed to the PM. The clustering of multiple STIM1 dimers at ER–PM junctions then traps diffusing Orai hexamers. Higher-order oligomers form after activation. This intimate molecular choreography results in the influx of calcium into the ER–PM junction (Figure 1C) that aids refilling of ER stores and controls gene expression, proliferation, cell migration and cell excitability. SOCE is terminated by refilling of ER calcium via the SERCA pump (Figure 1C).

Endoplasmic reticulum–endo/lysosome

Dynamic membrane–membrane junctions that mediate calcium-signalling cascades are not restricted to ER–PM junctions. Recently, junctions between the ER and acidic organelles have been identified as platforms for calcium regulation at these non-canonical calcium stores. For example, G_q-coupled receptor-stimulated release of calcium from SG (free calcium concentration ~200 μM) is mediated by STIM1 molecules in ER membranes and Orai1 channels in SG membranes (Figure 1D; [9]). In addition, ER cisternae make junctions with phagosomes via STIM1 and junctate, opening phagosomal Ca^{2+} channels to generate localized Ca^{2+} elevations that promote phagocytosis [24,25]. Finally, recent work suggests that junctions between the ER and lysosomes can help shape receptor signalling mediated by IP_3 [26,27] or nicotinic acid adenine dinucleotide phosphate (NAADP; [11,28]). NAADP activates two-pore channels in lysosomal membranes, increasing calcium at sites close to the ER. These calcium microdomains are proposed to initiate calcium-induced calcium release via IP_3R or RyR on ER membranes to increase cytosolic calcium throughout the cell. Thus, ER–membrane interactions underlie calcium release from a diverse pool of acidic granules.

Endoplasmic reticulum–mitochondrion

Membrane junctions between the ER and mitochondria, often referred to as mitochondrial-associated membranes are essential for the transfer of Ca^{2+} from the ER lumen to the mitochondrial matrix (Figure 1E). The main molecular components involved are the ER–mitochondrial membrane tethering proteins mitofusin 1 and 2 [29], IP_3 receptor channels in the ER membrane and a non-specific 'voltage-dependent anion channel' (VDAC1) in the outer mitochondrial membrane (OMM) (Figure 1E; [30]). The IP_3R and VDAC1 are functionally coupled at the ER–mitochondrial membrane interface via a chaperone protein, glucose-regulated protein GRP75 (Figure 1E). Upon release of calcium from the ER by IP_3R, the calcium is transported rapidly across the OMM by VDAC1 resulting in elevated calcium concentrations within the mitochondrial intermembrane space that are sufficient to trigger uptake by the low-affinity MCU. Consequently, when intermembrane calcium is elevated to micromolar concentrations, calcium is transferred down the steep electrical gradient into the mitochondrial matrix (Figure 1E). Although the fluidity of ER–mitochondrial contacts remains to be fully established, the physiological functions of calcium transferred from the ER into the mitochondrial matrix appear to include buffering cytoplasmic calcium, facilitating calcium-dependent respiration and regulating cell death (apoptosis).

Regulation of phosphoinositides at membrane–membrane junctions

Phosphoinositides are a family of eight minority phospholipids found on the cytoplasmic leaflet of all endomembranes [31,32]. They serve as negatively charged molecular beacons recruiting cytosolic proteins to lipid membranes or binding to the cytosolic domain(s) of membrane proteins. The parent lipid, phosphatidylinositol (PI), is generated and synthesized on ER membranes via PI synthase enzymes conjugating ER-derived CDP-diacylglycerol and cytoplasmic *myo*-inositol. Following the synthesis of PI, specific lipid kinases and phosphatases add or remove phosphate groups at the 3′, 4′ or 5′ position on the inositol ring to generate seven additional phosphoinositides (PI(3)P, PI(4)P, PI(5)P, $PI(3,4)P_2$, $PI(4,5)P_2$, $PI(3,5)P_2$, $PI(3,4,5)P_3$). The localization and activity of phosphoinositide metabolizing enzymes generates a heterogeneous distribution of phosphoinositide species throughout cells, providing each membrane an identifying phosphoinositide signature despite membrane traffic between organelles (e.g. ER: PI; Golgi: PI(4)P; PM: $PI(4,5)P_2$ etc.). Recently, non-vesicular lipid transfer at ER–membrane junctions has been implicated as a mechanism through which cells maintain lipid compositions despite constant membrane recycling [33]. The functional importance of phosphoinositides is underscored by the large number of diseases and developmental malformations that result from mutations in phosphoinositide metabolizing enzymes [32,34–36] and by the variety of cellular activities they regulate [31,32,37–40].

Phosphoinositide regulation at ER–PM junctions

The signature phosphoinositide of the PM is $PI(4,5)P_2$. Here it serves as an essential substrate for many biological activities, including endocytosis, exocytosis and ion channel function [32,37]. In mammalian cells, $PI(4,5)P_2$ is generated in two steps: PI is phosphorylated by a PI 4-kinase to generate PI(4)P, which is subsequently phosphorylated

by PI(4)P 5-kinase to generate PI(4,5)P_2. Both the PM and the Golgi contain pools of PI(4)P that supply PM PI(4,5)P_2 and PI(4,5)P_2 itself helps organize contact sites between the ER and the PM via the extended synaptotagmin (E-Syt) proteins [32,37].

Transfer of PI at ER–PM junctions—Following G_q-receptor stimulation, PM PI(4,5)P_2 is hydrolysed and proteins containing phosphoinositide transfer domains (phosphoinositide transfer proteins, PITP), called Nir2 and Nir3, are recruited to ER–PM junctions [Figure 2A(i)] [33,41,42]. This recruitment is triggered through the combined actions of phosphatidic acid and diacylglycerol [41], and mediated through an interaction with the FFAT motif on the ER vesicle-associated membrane protein (VAMP)-associated proteins A and B (VAP-A and VAP-B) [43] [Figure 2A(i)]. The stimulated recruitment of Nir2 and Nir3 at ER–PM junctions delivers PI to the PM to replenish PM phosphoinositides, and concurrently delivers phosphatidic acid to the ER for future PI synthesis [41].

Regulation of PI(4)P at ER–PM junctions—The majority of PM PI(4)P seems to be generated by the activity of the lipid kinase PI4KIIIα and its associated factors [44,45]. This pool of PM PI(4)P is important for the maintenance of PM PI(4,5)P_2 [45,46]. Recently, two integral ER membrane proteins, oxysterol-binding protein (OSBP)-related protein 5 (ORP5) and ORP8 have been identified as key regulators of PM PI(4)P [45]. Through the action of a hydrophobic tail sequence that anchors it in the ER membrane and a pleckstrin homology (PH) domain that interacts with PM PI(4)P, ORP5/8 tethers ER–PM junctions [Figure 2A(ii)] and facilitates the exchange of phosphatidylserine (PS) for PI(4)P at ER–PM junctions [2,47]. Evidence for this includes: (i) the purified OSBP-related domain (ORD) of ORP8 binds PI(4)P and PS, (ii) overexpression of ORP5 reduces resting PM PI(4)P whereas increasing PM PS signals, (iii) rapamycin-induced recruitment of ORP5 lacking its PH domain (ΔPH-ORP5) or ΔPH-ORP8 to the PM reduces PI(4)P and (iv) rapamycin-induced recruitment of ΔPH-ORP5 to the PM increases PM PS following treatment with a PI4KIII inhibitor [2,47]. It remains to be seen if endogenous ORP5/8 is sufficient to induce ER–PM junctions in mammalian cells or if it stabilizes pre-existing ER–PM contacts. In addition to the ORP proteins, in yeast an ER PI(4)P phosphatase, Sac1, is found in ER–PM junctions and has been proposed to regulate PM PI(4)P. There is evidence for Sac1 dephosphorylating PI(4)P *in cis* on ER membrane (Figure 2A(iii); [48]) and *in trans* on PM (Figure 2A(iv); [49,50]). Although Sac1 has been shown to decrease PM PI(4)P in mammalian cells [51], its presence, localization and role in regulating phosphoinositides at ER–PM junctions remain to be fully determined. This is the subject of our ongoing work.

Phosphoinositide regulation at endoplasmic reticulum–Golgi junctions

The predominant phosphoinositide found on Golgi membranes is PI(4)P. Here it binds to specific effector proteins to orchestrate constitutive membrane trafficking and maintain the structural integrity of the Golgi complex.

Transfer of PI at ER–Golgi junctions—As at ER–PM junctions, PI transfer proteins (Nir) are thought to transfer PI down its concentration gradient from the ER to Golgi. This transfer is facilitated through an interaction of Nir2 with VAP-A and -B on ER membranes [6] [Figure 2B(i)], and mediated via the N-terminal PI-transfer domain of Nir2. Upon being

transferred to Golgi membranes, PI is subsequently phosphorylated by Golgi-localized PI4K enzymes to produce PI(4)P [52].

Regulation of PI(4)P at ER–Golgi junctions—The accumulation of PI(4)P on the Golgi membrane, coupled with the presence of VAP-A and -B on ER membranes allows the cytosolic protein OSBP to be recruited to and subsequently to tether ER–Golgi junctions. The molecular components facilitating such membrane tethering are VAP proteins on the ER membrane and interactions between PI(4)P and the PH domain of OSBP on Golgi membranes [Figure 2B(ii)–(iv)]. Specifically, the FFAT motif of OSBP binds a highly conserved positive patch on the VAP protein surface, in a 2:2 stoichiometry, to anchor OSBP at the ER–cytosol interface (Figure 2B(ii); [53]). This positions OSBP and allows its N-terminal PH domain to detect two determinants of the *trans*-Golgi: Golgi PI(4)P and the small G protein Arf1-GTP [Figure 2B(iii)], thereby promoting ER–Golgi tethering. Consequently, the lipid transfer domain (ORD) of OSBP is positioned to facilitate sterol transfer to Golgi membranes, for the synthesis of sphingomyelin [54] and glycosphingolipids [55] and the transfer of PI(4)P to ER membranes [Figure 2B(iv)] for dephosphorylation by the ER lipid phosphatase Sac1 (Figure 2B(v)); [5,56]).

Regulation of phosphoinositides at other ER–membrane junctions—ER–endosome and ER–lysosome membrane contact sites have also been reported. They may regulate phosphoinositides. Recent evidence has implicated ER–endosome membrane junctions as regulators of microtubule-dependent endosomal transport. These junctions, mediated via VAP-A and protrudin on ER membranes binding to Rab7-GTP and PI(3)P on endosomal membranes [57], promote endosome translocation and neurite outgrowth. Given that several OSBP and ORP proteins may also localize to endosome membranes [58–61], it is possible that mechanisms at ER–PM or ER–Golgi junctions could be conserved at ER–endosome junctions to regulate endosomal PI(3)P or PI(4)P, for instance via Sac1 on ER membranes or recruitment of Sac2 via Rab5-GTP [62]. Finally, although ER–mitochondrial contacts are clearly important for calcium handling and mitochondrial division, they are not known to participate in phosphoinositide regulation.

Conclusions and perspectives

Current research on organelle–organelle junctions is advancing at a rapid pace despite technological limitations to monitor membrane–lipid composition in real time and to identify the full repertoire of participating proteins. As protein/lipid imaging and screening technologies advance we will better understand the role of membrane–membrane junctions in regulating membrane identity and shaping cellular responses. For example, improvements in imaging single molecules [63] and in fluorescent labelling of endogenous lipids and proteins [64–66] will enable us to monitor native membrane–membrane junctions in living cells. Future experiments must address not just the nanoscopic structural organization and function of membrane–membrane junctions, but also their role in coordinating macroscopic cellular events. We have summarized the role of membrane–membrane junctions in the exchange and regulation of calcium and phospholipids and the implications for cellular function, but many questions remain. Are other molecules exchanged at membrane–membrane junctions? In addition to acting as a portal for metabolite exchange, do these

junctions serve other regulatory tasks? How does membrane–membrane communication interface with vesicular transport? Do various tethering molecules work synergistically or independently? Is there cooperative action between different membrane–membrane junctions? For example, certain cellular events, such as activation of PM G_q protein-coupled receptors would be expected to initiate a cellular cascade that impacts at least three ER–membrane junctions (ER–PM, ER–Golgi and ER–mitochondrial junctions). A more comprehensive view of membrane–membrane junctions will consider their combined effects across the cell.

Membrane–membrane junctions serve important functions in regulating membrane proteins, lipids and ion composition. We anticipate that future studies will uncover additional proteins involved in information transfer at membrane–membrane junctions and elucidate their impact on cellular pathways and organismal function and health.

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Abbreviations

DHPR	dihydropyridine-sensitive voltage-gated calcium channel
EC	excitation–contraction
ER	endoplasmic reticulum
E-Syt	extended synaptotagmin
IP_3	inositol 1,4,5-trisphosphate
MCU	mitochondrial calcium uniporter
NAADP	nicotinic acid adenine dinucleotide phosphate
OMM	outer mitochondrial membrane
ORD	oxysterol-binding protein-related domain
ORP5	oxysterol-binding protein-related protein 5
OSBP	oxysterol-binding protein
PH	pleckstrin homology
PI	phosphatidylinositol
PI(4)P	phosphatidylinositol 4-phosphate
$PI(4,5)P_2$	phosphatidylinositol 4,5-bisphosphate
PM	plasma membrane
PS	phosphatidylserine

RyR	ryanodine receptor
SERCA	sarcoplasmic reticulum/endoplasmic reticulum ATPase
SG	secretory granule
SOCE	store-operated calcium entry
SR	sarcoplasmic reticulum
STIM	stromal interaction molecule
VAMP	vesicle-associated membrane protein
VAP	vesicle-associated membrane protein-associated protein
VDAC	voltage-dependent anion channel

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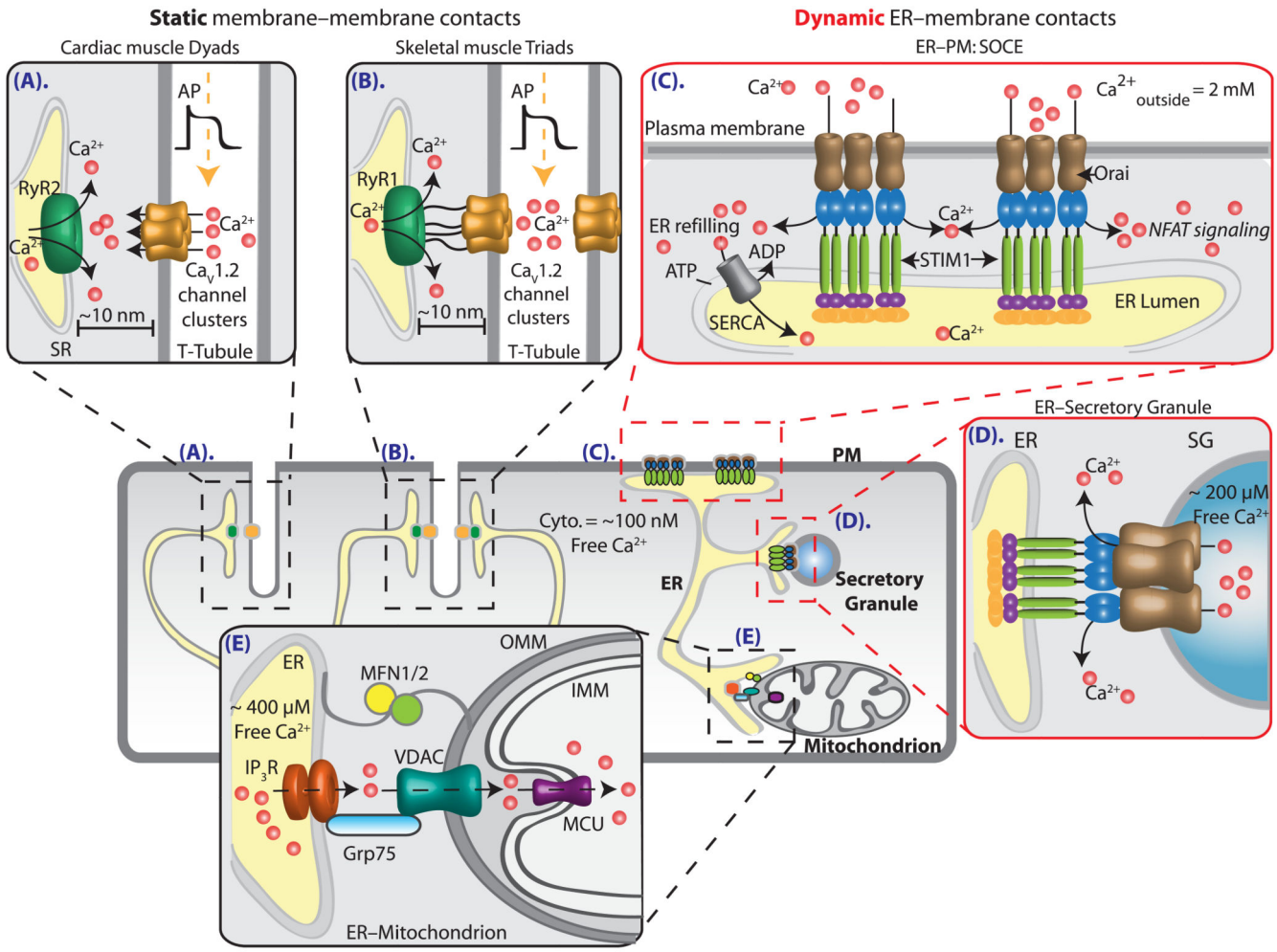

Figure 1. Static and dynamic ER–membrane junctions regulate cellular calcium concentrations

(**A**) Cardiac muscle dyad. A static single T-tubule paired with a terminal cisterna of the SR. Action potentials propagating along cardiac myocyte T-tubules open clustered voltage-gated calcium channels ($Ca_V1.2$) to facilitate calcium-induced calcium release from the SR and elicit muscle contraction. (**B**) Skeletal muscle triad. Two static terminal cisternae of the SR paired with a T-tubule. Physical coupling between $Ca_V1.2$ channels and RyR1 gates the release of calcium from SR stores to produce muscle contraction. (**C**) SOCE. Diagram represents the dynamic reorganization of STIM1 dimers in ER membranes and Orai1 channels in the PM following depletion of ER calcium. Orai1–STIM1 binding initiates calcium influx to the cytoplasm, the refilling of ER stores via the SERCA pump and stimulation of transcription factors via nuclear factor of activated T-cells (NFAT). (**D**) Dynamic SG–ER membrane junction. G protein-coupled receptor activation promotes interaction between ER STIM1 and SG Orai channels, releasing SG calcium to the cytoplasm. (**E**) ER–mitochondrion junction. Interactions between IP_3R and VDAC facilitate the flux of calcium ions across ER–mitochondrial junctions.

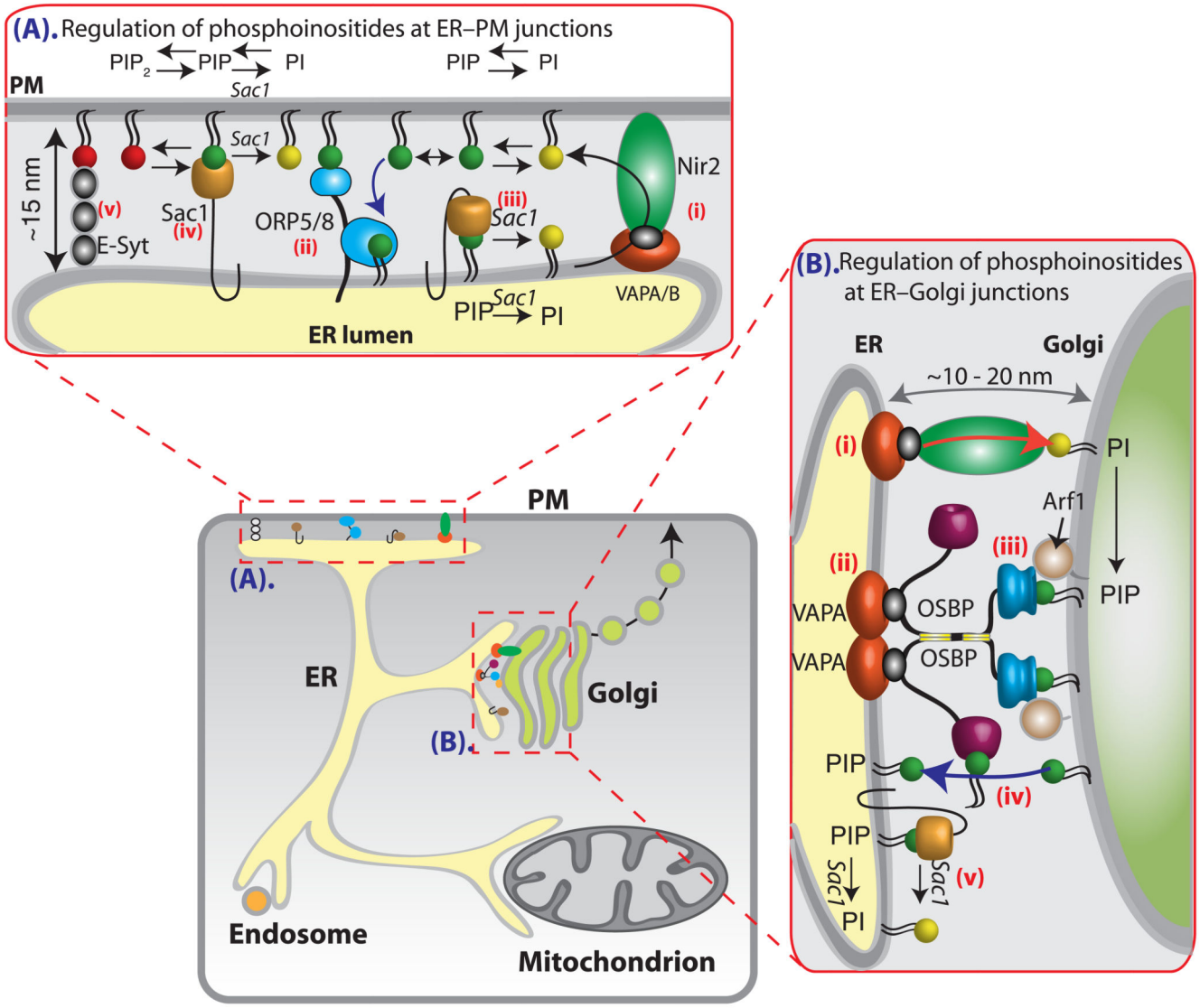

Figure 2. Phosphoinositide regulation at membrane–membrane junctions

(**A**) ER–PM junctions. (i) Following G_q protein-coupled receptor activation, Nir2 translocates and binds to VAPA/B on ER membranes to transfer PI to the PM. (ii) ORP5/8 complexes tether ER–PM junctions via interactions between their PH domain and PM PI(4)P to transfer PI(4)P to the ER membrane. (iii) ER PI(4)P is dephosphorylated to PI by the ER-resident lipid phosphatase Sac1 acting in *cis*. (iv) PM PI(4)P is dephosphorylated to PI via Sac1 acting in *trans*. (v) E-Syt proteins tether ER–PM junctions via a hair-pin insertion into the ER membrane and interactions with PM $PI(4,5)P_2$ via their C2 domains. (**B**) ER–Golgi junctions (ER–Golgi). (i) Nir2 or Nir3 bind to VAPA/B on ER membranes and transfer PI to Golgi membranes. (ii) OSBP tethers ER–Golgi junctions through interactions with VAPA/B on ER membranes and (iii) PI(4)P and Arf1 on Golgi membranes. (iv) The tethering of ER–Golgi membranes by OSBP allows its oxysterol-binding domain (ORD) to transfer PI(4)P to ER membranes and sterol to Golgi membranes. (v) PI(4)P on

ER membranes is dephosphorylated by Sac1 to PI. The distance between ER and Golgi membrane junctions is approximately 10–20 nm.