Endoplasmic Reticulum-Plasma Membrane Contacts Regulate Cellular Excitability

7

Eamonn J. Dickson

Abstract

Cells that have intrinsic electrical excitability utilize changes in membrane potential to communicate with neighboring cells and initiate cellular cascades. Excitable cells like neurons and myocytes have evolved highly specialized subcellular architectures to translate these electrical signals into cellular events. One such structural specialization is sarco-/endoplasmic reticulum-plasma membrane contact sites. These membrane contact sites are positioned by specific membrane-membrane tethering proteins and contain an ever-expanding list of additional proteins that organize information transfer across the junctional space (~ 15–25 nm distance) to shape membrane identity and control cellular excitability. In this chapter we discuss how contacts between the sarco-/endoplasmic reticulum and plasma membrane are essential for regulated excitation-contraction coupling in striated muscle and control of lipid-dependent ion channels.

Keywords

Membrane-membrane contact sites • ER-PM contact sites • Phosphoinositides • PI(4,5)P_2 • Ion channels • Endoplasmic reticulum • Tethering proteins • Excitation-contraction coupling

This chapter concerns the regulation of cellular excitability at endoplasmic reticulum-plasma membrane contacts (ER-PM). When referring to "excitability," we primarily mean electrical excitability. Cells that have intrinsic electrical excitability, such as neurons, muscle cells, and endocrine cells, respond to stimulation by altering their electrical properties to initiate intracellular processes, including synaptic transmitter release, muscle contraction, or hormone secretion. The basis for cellular excitability is the ability to generate a labile resting membrane potential that is dependent on electrochemical gradients and energy consumption. The

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distinctive feature of electrically excitable cells is the generation of electrical current in response to membrane depolarization. Whereas a non-excitable cell that has been slightly depolarized will return to its original resting membrane potential, an electrically excitable cell that is depolarized to the same extent will respond with an action potential and/or contraction. These responses occur due to the movement of small charged particles (ions) such as Na^+, K^+, Ca^{2+}, and Cl^- across the plasma membrane through ion channels, pumps, and transporters. Electrically excitable cells produce an action potential because of the presence of voltage-gated channels in the plasma membrane. Thus, ion channels (pumps and transporters) are the foundation of electrical excitability. These ion channels can have their properties tuned by post-translational modification, accessory subunits, phosphorylation, and also lipids.

In addition to ion channels, eukaryotic cells have evolved compartmentalization to orchestrate and separate a myriad of cellular processes. One organizing tool is the partitioning of the cytoplasm into membrane-enclosed compartments called organelles. These specialized subcellular structures have distinct functions and communicate to organize specific cellular tasks via vesicular transport and via membrane-membrane junctions or contact sites. These intimate sites (~ 10–30 nm) of information transfer can be stable or dynamic and are mediated via specific proteins (Figs. 7.1, 7.2, and 7.4). Two important functions of ER-PM contact sites are (1) the transfer of calcium ions to generate and coordinate cytoplasmic calcium signaling events, and (2) the transfer and regulation of lipids. This chapter briefly highlights the major principles and molecular identity of the proteins involved in regulating cellular excitability at ER-PM contact sites in mammalian cells.

7.1 Role of Calcium in Regulating Cellular Excitability at Membrane Contact Sites

The most widely studied and physiologically important example of how ER-PM contact sites regulate cellular excitability takes place during excitation-contraction (EC) coupling in striated muscle cells. EC coupling is the connection between the electrical action potential and mechanical muscle contraction in cardiac, skeletal, and smooth muscle myocytes. This process of converting an electrical stimulus into a mechanical response is the essential event for many fundamental physiological processes including walking (skeletal muscle), pumping blood around the body (cardiac muscle), and peristalsis (smooth muscle) and relies on the tight physical coupling between the plasma membrane (sarcolemma) and sarcoplasmic reticulum. In the next few sections, we focus our attentions on cardiac and skeletal muscle EC coupling. For cell-specific review of smooth muscle EC coupling mechanisms, see (Berridge 2008; Kotlikoff 2003; Navedo and Santana 2013; Sanders et al. 2012).

7.1.1 Cardiac Muscle Excitation-Contraction Coupling

In cardiac muscle (Fig. 7.1a), deep sarcolemma invaginations called transverse tubules (T-tubules) penetrate into the myocyte and are functionally coupled to sarcoplasmic reticulum (SR) cisterna in a 1:1 ratio (1 T-tubule/1 SR terminal cisterna (Moore and Ruska 1957)). These morphological units are often referred to as cardiac dyads (or diads). T-tubule biogenesis has been extensively characterized, and the molecular identity of specific signaling proteins responsible for initiating and regulating this event is constantly evolving. Several proteins thought to have fundamental roles in T-tubule biogenesis include caveolin 3 (CAV3), amphiphysin 2 (BIN1), and dysferlin (DYSF). Among these, amphiphysin 2 (BIN1) appears to have a particularly interesting role through its dual involvement in T-tubule formation (Lee et al. 2002) and shuttling of L-type Ca^{2+} channels to the T-tubule sarcolemma (Hong et al. 2010).

The membrane-membrane tethering protein primarily responsible for structural coupling between the sarcolemma and SR (PM-SR) appears to be junctophilin-2 (JPH2, Fig. 7.1a).

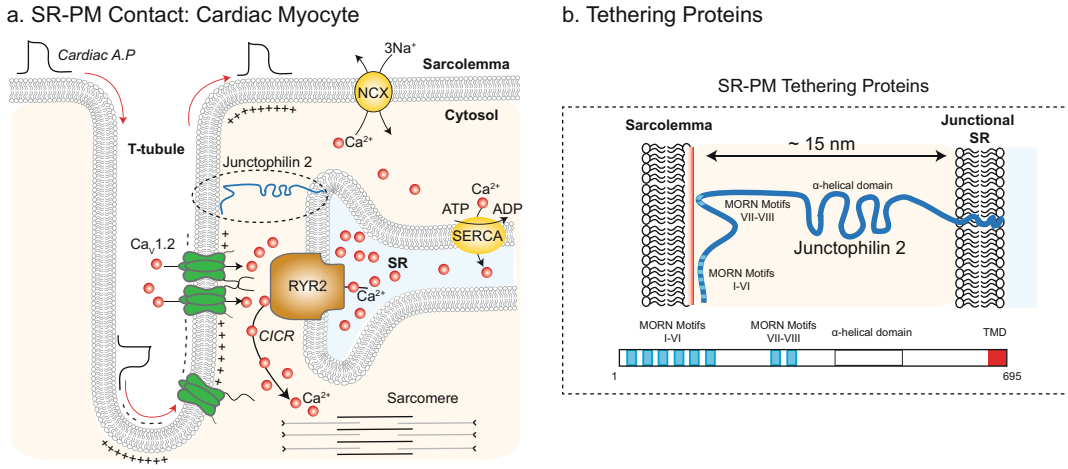
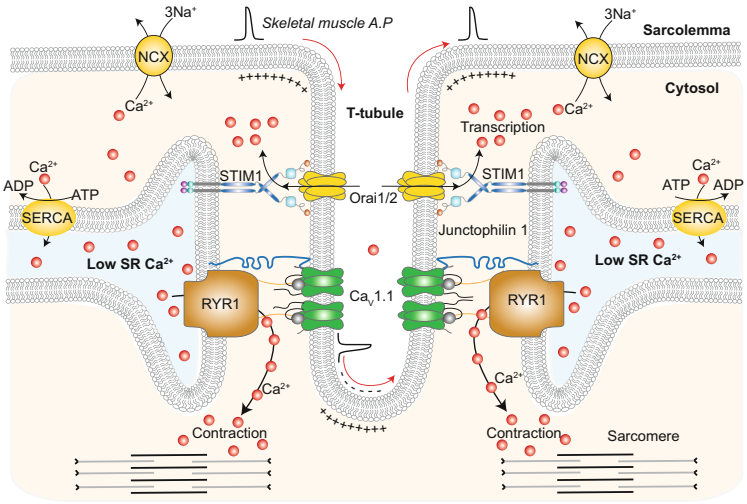


Fig. 7.1 Excitation-contraction coupling in cardiac myocytes. (**a**) Cartoon of a cardiac myocyte sarcoplasmic reticulum-plasmalemma (SR-PM) contact site. A single T-tubule is paired with a single terminal cisterna of the sarcoplasmic reticulum (dyad). Cardiac action potentials (A.P) propagating along cardiac myocyte T-tubules open clustered voltage-gated calcium channels ($Ca_V1.2$) to facilitate calcium-induced calcium release (CICR) from ryanodine receptors (RYR2) on the SR. Elevations in cytoplasmic calcium lead to myocyte contractions. (**b**) Top: organization and interactions of junctophilin-2 and the plasmalemma. Note the hypothesized interaction between the repeating MORN motifs of junctophilin-2 and the negative charges (red line) of the plasmalemma. Bottom: linear representation of junctophilin-2 protein. NCX, sodium calcium exchanger; SERCA, sarco-/endoplasmic reticulum Ca^{2+}-ATPase; CC, coiled coil; PBD, polybasic domain; TMD, transmembrane domain

JPH2 promotes coupling through its C-terminal hydrophobic segment spanning the SR membrane and its eight, N-terminus, MORN motifs (membrane occupation and recognition motifs; Fig. 7.1b) potentially interacting with sarcolemma phospholipids (Bennett et al. 2013; Lee et al. 2002) or proteins (Minamisawa et al. 2004). Further emphasizing the importance of JPH2 in mediating PM-SR contact sites, JPH2 knockout myocytes have deficient functional membrane complexes, impaired Ca^{2+} signaling, and are embryonic lethal (Takeshima et al. 2000; van Oort et al. 2011). Thus, JPH2 is a tether for the junctional membrane-membrane complex in cardiac EC coupling.

The functional coupling between PM-SR contact sites in cardiac muscle arises from the close apposition between dihydropyridine-sensitive voltage-gated calcium channels ($Ca_V1.2$) on the sarcolemma and ryanodine receptors (RYR2) on the SR membrane (Fig. 7.1a). Cardiac EC coupling is initiated when an action potential (AP) depolarizes the cell membrane of a cardiac myocyte, stimulating the brief opening of $Ca_V1.2$ channels. Influx of Ca^{2+} across a single $Ca_V1.2$ channel is known as a "Ca^{2+} sparklet" and can be visualized with Ca^{2+}-sensitive fluorescent dyes (Wang et al. 2001). These elemental calcium events allow the Ca^{2+} concentration within the junctional cleft to increase from ~100 nM to ~10 μM (Bers and Guo 2005). Recent data suggests that in order to achieve such elevations in calcium, $Ca_V1.2$ channels form clusters (average six channels per cluster), with each channel simultaneously opening (cooperative gating) during a cardiac AP (Dixon et al. 2015; Dixon et al. 2012; Inoue and Bridge 2003; Navedo et al. 2010; Sobie and Ramay 2009). This local Ca^{2+} microdomain is by itself alone not sufficient to initiate substantial contraction; rather it triggers further "Ca^{2+}-induced Ca^{2+} release" (CICR: (Fabiato 1983)) from clustered RYR2 on the SR, producing a "Ca^{2+} spark" (Cheng and Lederer 2008; Cheng et al. 1993). It is estimated that approximately 25 L-type Ca^{2+} channels and 100 RyRs closely appose one another within the

a. SR-PM Contact: Skeletal Muscle



b. SR-PM Tethering Proteins

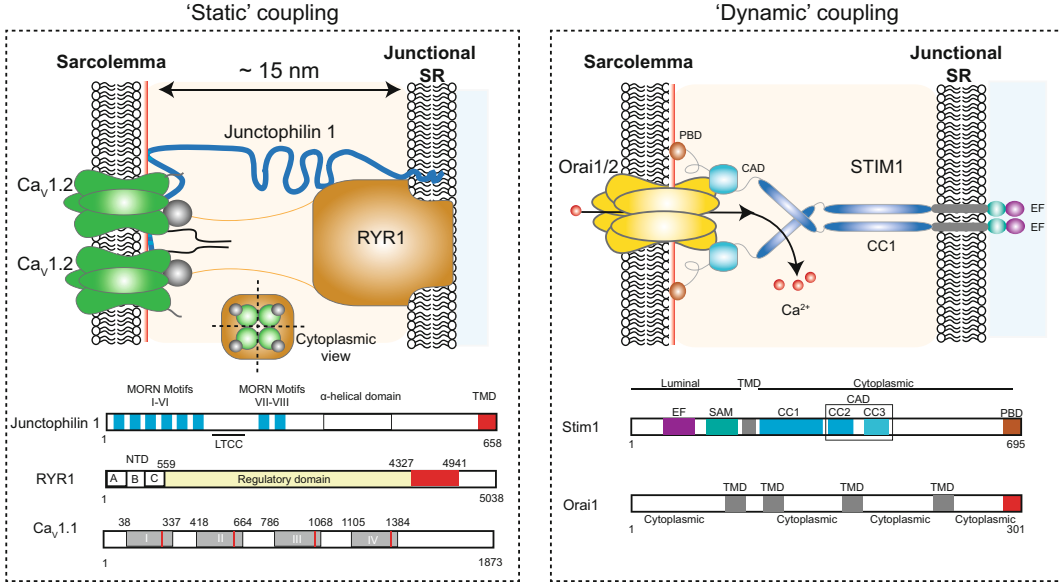


Fig. 7.2 Excitation-contraction coupling in skeletal muscle. (**a**) Schematic representation of skeletal muscle SR-PM contacts. A single T-tubule paired with two terminal cisternae of the SR (triad). Skeletal muscle action potentials (A.P) invade skeletal myocyte T-tubules to activate voltage-gated calcium channels ($Ca_V1.1$). Movement of the $Ca_V1.1$ voltage sensor physically induces release of calcium from ryanodine receptors (RYR1) to elicit muscle contraction. Note, unlike Fig. 7.1 in cardiac myocytes, this is a calcium-independent process. In a separate, voltage-independent, calcium release event, store-operated calcium entry (SOCE) is initiated following reduction of SR calcium concentration. Diagram represents the dynamic reorganization of STIM1 dimers in sarcoplasmic reticulum (SR) membranes and Orai1 hexamers in the plasmalemma. Orai1-STIM1 binding initiates calcium influx to the cytosol, the refilling of SR stores via the SERCA pump, and stimulation of transcription factors. (**b**) *Left*: "static" SR-PM contact site. *Top*: schematic representation of the organization and interactions of $Ca_V1.1$ and RYR1. *Bottom*: linear representation of junctophilin-1, RYR1, and $Ca_V1.1$. *Right*: "dynamic" SR-PM contact site. *Top*: molecular elements involved in store-operated calcium entry. *Bottom*: linear representation of STIM1 and Orai1 proteins

junctional cleft to form a "couplon" (Bers and Guo 2005). Synchronized activation of multiple sparks leads to a global elevation in intracellular Ca^{2+} sufficient to engage the contractile machinery, thereby promoting myocyte contraction and providing the force for pumping blood around the body.

$Ca_V1.2$ and RYR2 channels are both subject to regulation by multiple signaling pathways. Such signaling pathways have the ability to rapidly alter the amplitude and/or spatial properties of myocyte Ca^{2+} signaling to shape cardiac myocyte contraction. For example, $Ca_V1.2$ are subject to regulation by protein kinases, including PKA (β-adrenergic receptor stimulation, e.g., during the fight or flight response) and PKC (activated by stimulation of α_1-adrenergic, angiotensin II, and endothelin G_q-coupled receptors). RyRs are part of larger macromolecular signaling complexes and as such can have their activity modulated by one or more accessory proteins including calsequestrin, calmodulin, CaMKII-dependent phosphorylation, FK506-binding proteins, and PKA (for review, see (Lanner et al. 2010)).

Following elevations in cardiac myocyte cytosolic Ca^{2+}, and consequent activation of the cardiac contractile apparatus, Ca^{2+} must be rapidly extruded from the cytosol in preparation for the subsequent action potential. The main mechanisms for Ca^{2+} efflux in cardiac myocytes are the sarcolemma sodium/calcium exchanger and the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump on the SR (Bers 2002).

It is clear that cellular architecture, specifically the maintenance of PM-SR contacts, is critically important to the fidelity of EC coupling. Work from several groups has established that T-tubules are lost or atrophied as cardiac hypertrophy develops, resulting in the progressive orphaning of RYR2 (Gomez et al. 2001; Song et al. 2005). Deficiencies in EC coupling because of T-tubule remodeling have also been observed during the earlier stages of hypertrophy; here the width of the junctional cleft is marginally increased, altering the functional coupling between membrane depolarization and Ca^{2+} release from the SR (Xu et al. 2007). Thus, cardiac EC coupling is dependent on extracellular Ca^{2+} and functional coupling between $Ca_V1.2$ and RYR2, which relies on the close spatial proximity between the PM and SR membranes.

7.1.2 Skeletal Muscle Excitation-Contraction Coupling

Skeletal muscle cells also require precise SR-PM contact site arrangement for functional EC coupling. The highly specialized structure, responsible for translating a skeletal muscle AP into intracellular calcium release and muscle contraction, is known as the triad and consists of a single T-tubule closely (~ 15 nm) apposed to two adjacent SR terminal cisternae (1 T-tubule/2 SR terminal cisternae; Fig. 7.2a; (Block et al. 1988; Franzini-Armstrong and Protasi 1997)).

Functional coupling between these adjacent membranes arises from the direct physical contact between $Ca_V1.1$-containing heteromultimers arranged into groups of four ("tetrads") on the sarcolemma, aligned with the four subunits of every other RyR1 homotetramer on the SR membrane (Fig. 7.1b; (Block et al. 1988; Protasi et al. 2002; Protasi et al. 2000)). Accordingly, during propagation of a skeletal muscle A.P along the T-tubule, $Ca_V1.1$ channels act as voltage sensors to physically translate membrane depolarizations directly to RYR1, independently of Ca^{2+} flux (Armstrong et al. 1972), to trigger release from SR stores. Critical structural determinants required for efficient EC coupling appear to be the $Ca_V1.1\alpha_1$ II–III loop (Kugler et al. 2004; Nakai et al. 1998b; Tanabe et al. 1990), $Ca_V1.1\beta_{1a}$ carboxyl terminus (Beurg et al. 1999; Sheridan et al. 2003), and multiple regions of RyR1 (Nakai et al. 1998a; Protasi et al. 2002; Sheridan et al. 2006). A unique property of the conformational coupling between $Ca_V1.1$ and RyR1 in skeletal muscle is that it occurs bi-directionally (Nakai et al. 1996; Nakai et al. 1998a), with both an orthograde signal passing from the $Ca_V1.1$ to RyR1 and also a retrograde signal from RyR1 to the $Ca_V1.1\alpha_1$ subunit that enhances Ca^{2+} flow across the T-tubule membrane (Nakai et al. 1996).

In skeletal muscle, junctophilin-1 (JPH1) is involved in physically linking the T- tubules to the SR membrane (Fig. 7.1b; Nishi et al. 2000). Consistent with its role as a PM-SR tethering protein, ablation of JPH1 results in distinct morphological abnormalities of the junctional cleft, including deficiencies in SR-PM complexes, swollen terminal cisternae, and decreased triad number. These deficiencies result in impaired contractile force, abnormal sensitivity to extracellular Ca^{2+}, and premature death (Ito et al. 2001; Komazaki et al. 2002).

7.1.3 Store-Operated Calcium Entry

Another example of how ER/SR-PM communication can shape cellular excitability is store-operated calcium entry (SOCE). The process of store-operated SOCE, whereby Ca^{2+} influx across the PM is activated in response to depletion of intracellular ER/SR Ca^{2+} stores, plays a critical role in the regulation of gene expression, motility, immune responses, and organ development. The key molecular determinants orchestrating this calcium-signaling cascade (for a comprehensive review, see (Prakriya and Lewis 2015)) are STIM1 and STIM2 (stromal interaction molecules) in the ER/SR membrane and Orai channels (Orai1, Orai2, Orai3) in the plasma membrane (Fig. 7.2a, b). At rest, STIM molecules (typically dimers) and Orai channels (hexamers (Hou et al. 2012)) freely diffuse along the ER/SR and PM, respectively. Physiological stimuli that initiate net depletion of ER/SR Ca^{2+} stores promote loss of calcium from the luminal EF-hand domains on STIM1 dimers and induce a conformational change in their structure that favors oligomerization and translocation to ER/SR-PM contacts (Fig. 7.2b). The clustering of multiple STIM1 dimers at ER-PM junctions then traps the freely diffusing Orai hexamers and promotes formation of higher-order oligomers. This intimate molecular choreography results in the influx of calcium into the ER-PM junction (Fig. 7.2b) which (1) helps refill depleted ER/SR calcium stores and (2) activates calcineurin, which dephosphorylates the transcription factor NFAT (nuclear factor of activated T-cells), exposing a nuclear localization sequence that enables its translocation into the nucleus to help control a wide variety of genes (Feske et al. 2001). ER-PM STIM1-Orai1 puncta deoligomerize, and SOCE is terminated when ER/SR Ca^{2+} luminal calcium levels are replenished through the ATP-dependent action of the SERCA pump on ER/SR membranes.

Historically, a role for SOCE in skeletal muscle was considered unlikely since sarcolemmal Ca^{2+} entry is not directly required for contraction (see section 1.2). However recent investigations have demonstrated a key role for SOCE in the development and function of skeletal muscle fibers. For example, skeletal myocytes express high levels of both STIM1 and Orai1, with STIM1 localized to the muscle SR at triadic junctions appearing essential for myotube development (Stiber et al. 2008). Finally, loss of STIM1 or Orai1 expression in humans is associated with a congenital nonprogressive myopathy (McCarl et al. 2009). Thus, store-operated and voltage-dependent Ca^{2+} pathways may reflect two distinct molecular channel complexes within the triad junction that may enable trans-sarcolemmal Ca^{2+} entry.

7.2 Lipid Regulation of Cellular Excitability

We now consider how minority lipids, regulated at ER/SR-PM junctions, can alter cellular excitability. The original hypothesis presented following the work of Hilgemann and Ball (Hilgemann and Ball 1996) was that ion channels in the PM of mammalian cells require interactions with phosphoinositides for proper function. It is clear by now that a large and varied range of ion channels and transporters are regulated by these low-abundance negatively charged phospholipids.

7.2.1 Phosphoinositides

Phosphoinositides are a family of eight low-abundance phospholipids found on the cytoplasmic leaflet of all cellular membranes (Balla

2013; Di Paolo and De Camilli 2006). They have an inositol ring that can be phosphorylated or dephosphorylated by a variety of lipid kinases or lipid phosphatases on either the three, four, or five positions, resulting in the synthesis of seven combinatorial PIs (PI(3)P, PI(4)P, PI(5)P, $PI(3,4)P_2$, $PI(3,5)P_2$, $PI(4,5)P_2$, and $PI(3,4,5)P_3$). The consequent addition of each phosphate group to the inositol ring confers a -2 charge at the neutral pH of the cytosol. This means that the PM phosphoinositide $PI(4,5)P_2$, discussed at length in this chapter, has a -4 charge at pH 7.0. These negative charges enable phosphoinositides to act as negatively charged molecular beacons recruiting cytosolic proteins to specific lipid membranes or binding to the cytosolic domain (s) of membrane proteins. Through the ability of proteins to recognize the distinctive high negative charge and head group geometries of phosphoinositides, PIs play a major role in regulating a wide range of processes including membrane budding and fusion, actin dynamics, and ion channel activity.

Why have mammalian cells evolved lipid-dependent regulation of ion channel function? Two major hypotheses have been presented in recent years. The first is regulation in time by receptors. While integral membrane proteins like ion channels are residing in the plasma membrane, receptors coupled to PLC may become activated and transiently change key membrane lipids sufficiently to modulate channel function (Fig. 7.3 (Brown and Adams 1980)). Thus, electrical and ion-transport properties of the cell may be regulated dynamically by physiological events, such as neurotransmitter or hormone release. A second hypothesis is that phosphoinositide dependence allows control of ion channel activity as they are trafficked to targeted membranes, such as the plasma membrane. During trafficking, these important integral membrane proteins would be quiescent until they enter their favored phosphoinositide environment where they would be subsequently turned on (Hilgemann et al. 2001). Such location-specific control of ion channel and transport activity would ensure that these proteins do not perturb organelle function during trafficking to the plasma membrane.

7.2.2 Phosphoinositide-Regulated Ion Channel Function

All integral membrane proteins sit in intimate contact with a bed of diverse lipids, so it is not surprising that many proteins have evolved lipid dependence. The regulation of PM ion channel activity by phosphoinositides is now widely recognized as an integral component of electrical signaling in cells. To date around 100 heterogeneous and functionally diverse ion channels and transporters have been shown to be dependent on PM $PI(4,5)P_2$ (for comprehensive list, see (Hille et al. 2015)). In the next three sections, we describe highlights of what is known about the phosphoinositide dependence of several classes of ion channels who play important roles in controlling the electrical excitability of cells. For detailed review of $PI(4,5)P_2$-sensitive ion channels, please refer to (Hille et al. 2015; Logothetis et al. 2010).

7.2.2.1 Inwardly Rectifying Potassium (Kir) Channels

How exactly does plasma membrane $PI(4,5)P_2$ regulate the activity of so many ion channels? Two recent crystal structures of $PI(4,5)P_2$ in complex with members of the inwardly rectifying potassium (Kir) channel family, Kir2.2 (Hansen et al. 2011), and G protein-coupled inwardly rectifying potassium channel GIRK2 (Kir3.2; (Whorton and MacKinnon 2011)) show how specific protein-lipid interactions facilitate conformational changes in ion channel structure. The x-ray crystallographic structure of Kir2.2 in complex with a short-chain analog of $PI(4,5)P_2$ reveals a homotetrameric channel composed of a transmembrane domain that forms the potassium-selective pore and a large cytoplasmic domain (Fig. 7.3a). Each of the four channel subunits is in complex with one $PI(4,5)P_2$ molecule (1:1 ratio) at the interface between the transmembrane and cytoplasmic domains. The positioning of the negatively charged 4- and 5-phosphates of the inositol head group of $PI(4,5)P_2$ promotes an electrostatic interaction with positively charged residues of the cytoplasmic domain (Fig. 7.3b) and induces

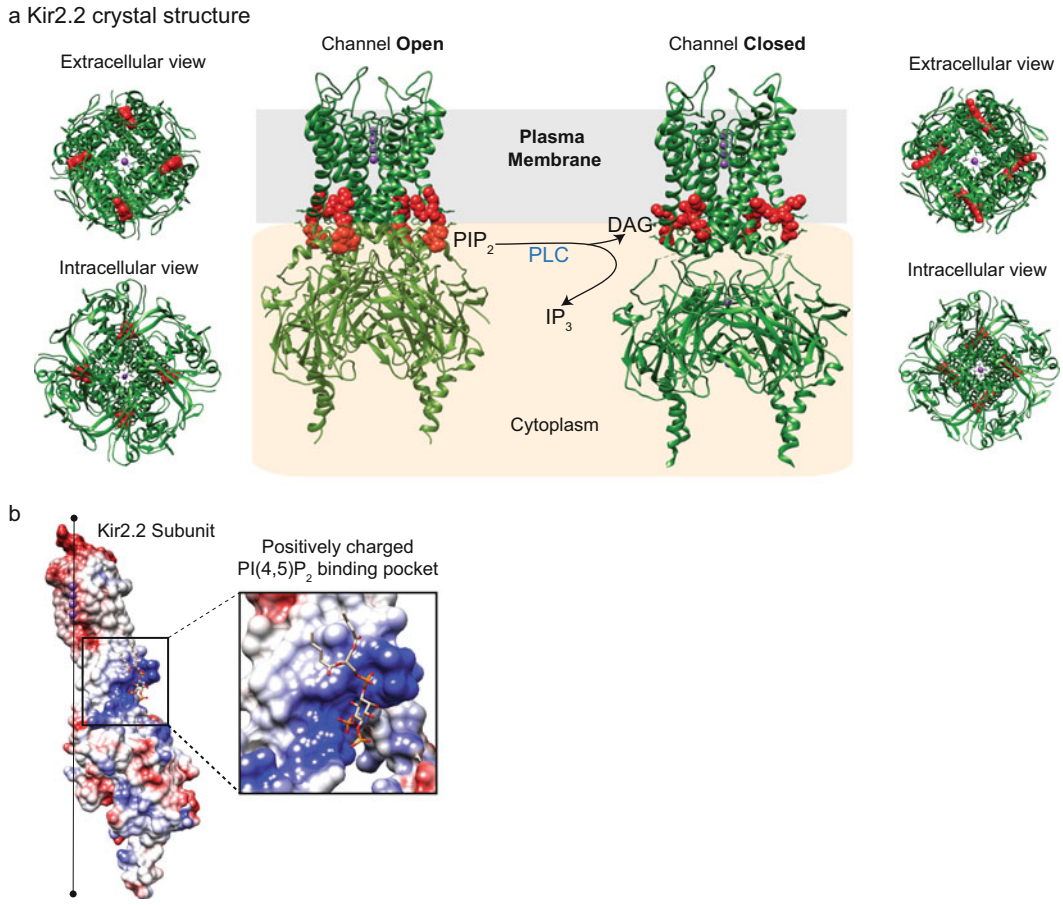


Fig. 7.3 Regulation of phosphoinositide-dependent ion channels at ER/SR-PM junctions. (**a**) Hypothetical model of Kir2.2 channel closure following hydrolysis of PM $PI(4,5)P_2$ by activated phospholipase C (PLC). Left: open configuration. Crystal structure of four Kir2.2 channel subunits in complex with one $PI(4,5)P_2$ molecule (1:1 ratio; pdb 3SPI). Closed configuration. Hydrolysis of PM $PI(4,5)P_2$ (pdb 3SPC) induces a translation (~ 6 Å) of the cytoplasmic domain away from the plasma membrane and closure of the inner helices. Note this is a hypothetical scenario. The channel structure in the "closed" configuration is Kir2.2 in complex with pyrophosphatidic acid (PPA), which is similar in structure to DAG. (**b**) Electrostatic map of a single Kir2.2 subunit in complex with a single $PI(4,5)P_2$ molecule (*blue* is positive; *red* is negative; *white* is neutral). The *vertical line* is the pore axis with four K^+ ions (*purple*) visible. The $PI(4,5)P_2$-binding site comprises numerous basic residues (*blue*) that interact electrostatically with the negatively charged phosphates of $PI(4,5)P_2$

a translation (~ 6 Å) of the cytoplasmic domain upward toward the membrane domain and promotes rotation of the inner helices (Fig. 7.3a). This lipid-mediated rearrangement of channel structure favors the active conformation of the channel (Fig. 7.2a) and provides evidence for lipid binding being a condition for channel activation. In the structure of a constitutively active GIRK2 channel (Kir3.2 R201A) with $PI(4,5)P_2$, there are similar striking changes in channel conformation, with a comparable rotation of the cytoplasmic domains and splaying apart of the inner helices, promoting gate opening (Whorton and MacKinnon 2011). Given that all Kir channels appear to be $PI(4,5)P_2$ dependent (Rohács et al. 2003; Zhang et al. 1999), and have conserved residues within their $PI(4,5)P_2$ binding pockets (Hansen et al. 2011), it is reasonable to suggest that such lipid-dependent, electrostatically-mediated conformational changes in

protein structure may present a conserved gating mechanism for Kir's and possibly other ion channels.

7.2.2.2 Voltage-Gated Potassium Channels

Voltage-gated potassium channels are critical determinants in setting (resting membrane potential) and shaping (duration/repolarization of the action potential) the membrane potential in excitable cells. Despite the large number of voltage-gated potassium channels (K_v1-K_v11), the clearest and most prominent examples of regulation by $PI(4,5)P_2$ are members of the slowly activating, non-inactivating K_v7 ($K_v7.1$–7.5) potassium channel family. All members of the K_v7 channel family are $PI(4,5)P_2$ dependent (Kruse et al. 2012; Suh et al. 2004; Zaydman and Cui 2014; Zaydman et al. 2013). In particular it is accepted for the neuronal $K_v7.2/K_v7.3$ heterotetrameric potassium channels that $PI(4,5)P_2$ is essential for their voltage-gated activity and that depletion of $PI(4,5)P_2$ renders them inactive so the neuron becomes more excitable until the lipid is resynthesized (Hille et al. 2014; Vivas et al. 2014; Zhang et al. 2003).

For the other tested members of the K_V channel family, $PI(4,5)P_2$ is apparently not essential, and whether it has modulatory effects under physiological conditions is controversial in several examples (Kruse and Hille 2013). Why then, in contrast to all Kir channels, do only a subpopulation of K_V channels appear to be regulated by PM $PI(4,5)P_2$? One hypothesis is that despite the observed increase in excitability during $PI(4,5)P_2$ depletion, a reserve of potassium channel activity is needed to repolarize the membrane potential (Kruse and Hille 2013). For that reason, the $PI(4,5)P_2$ insensitivity of many other important voltage-gated potassium channels (such as $K_V1.1/K_V\beta1.1$, $K_V1.5/K_V\beta1.3$, K_V3, and K_V4) might be a necessity to initiate action potential repolarization during periods of receptor-mediated $PI(4,5)P_2$ depletion.

7.2.2.3 Voltage-Gated Calcium Channels

Voltage-gated calcium (Ca_V) channels mediate calcium influxes upon membrane depolarization that initiate the effector actions of electrically excitable cells (see sections 1.2, 1.3). Over the past few years, several voltage-activated Ca_V channels have been shown to have partial (30–50%) $PI(4,5)P_2$ dependence (Suh et al. 2012; Wu et al. 2002). Intriguingly, this phosphoinositide dependence of Ca_V channels changes with coexpression of different Ca_V β-subunits (Suh et al. 2012). Ca_V channels that interact with β_{2a} (lipidated) have little $PI(4,5)P_2$ sensitivity, whereas Ca_V β_3 subunits (non-lipidated) show greater $PI(4,5)P_2$ sensitivity (Suh et al. 2012). These findings raise the interesting concept that excitable cells may be able to "tune" the lipid dependence of specific ion channels by regulating the expression of not only the lipid or pore-forming α-subunit but also auxiliary β-subunits.

7.3 Regulation of Phosphoinositide Metabolism at Membrane Contact Sites

In this section we discuss how membrane contact sites represent platforms for regulating PM phosphoinositides and thus ion channel function and cellular excitability. As partially discussed in section 1, and in other chapters in this book, membrane contact sites are regions of close proximity (< 20 nm) between adjacent membranes in mammalian cells (Fig. 7.4a). They should be considered portals of information transfer, distinct from classic vesicular transport, and are essential for regulating cellular calcium dynamics and membrane lipid identity. Membrane contact sites are established by organelle-specific membrane-membrane tethering proteins and contain a growing number of additional proteins that organize information transfer to shape membrane phosphoinositide composition.

7.3.1 Receptor-Mediated $PI(4,5)P_2$ Depletion

In excitable cells a major mechanism by which PM $PI(4,5)P_2$ levels are reduced is through activation of the G protein-coupled receptor

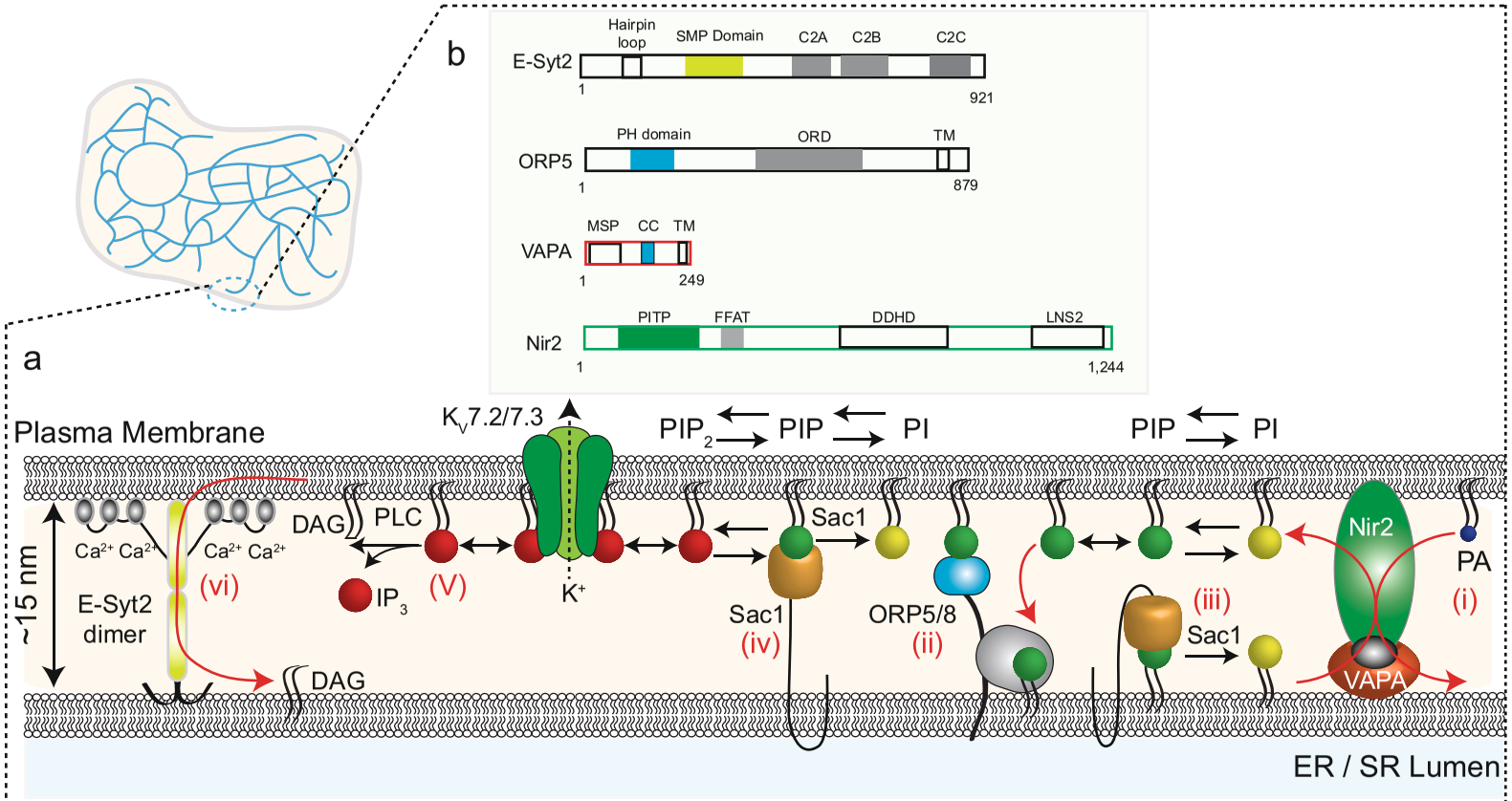


Fig. 7.4 Phosphoinositide metabolism at membrane contact sites. (**a**) Endoplasmic reticulum-plasma membrane junction (ER-PM). *(i)* During periods of net PI(4,5)P_2 depletion, Nir2 translocates and binds to VAPA/B on ER membranes to mediate the transfer of PI. *(ii)* ORP5/8 proteins tether ER-PM junctions via interactions between their PH domain and facilitate the countertransport of PM PI(4)P for ER PS. (*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*) During periods of PM PI(4,5)P_2 depletion, PI(4,5)P_2-dependent ion channels like K_V7.2/7.3 will have their activities suppressed to modulate cellular electrical excitability. (*vi*) Extended synaptotagmin (E-Syt) proteins tether ER-PM junctions via interactions with PM PI(4,5)P_2 via their C2 domains. The SMP domain of E-Syt2 is also responsible for clearing PM DAG. The distance between ER and PM membrane junctions is approximately 15 nm. (**b**) Linear representation of the tethering proteins present at ER-PM contacts

signaling cascade. Activation of G_q subsequently activates PLC, which cleaves PM PI(4,5)P_2 into the second messengers, cytosolic messenger inositol 1,4,5-trisphosphate (IP_3), and membrane-bound diacylglycerol (DAG; see Fig. 7.4). IP_3 binding to IP_3 receptors (IP_3Rs) at the ER triggers the release of Ca^{2+} into the cytosol. Calcium and DAG activate PKC. Net depletion of PI(4,5)P_2 will also result in the closure of PI(4,5)P_2-dependent ion channels, like K_v7.2/7.3 (Fig. 7.4). Following G_q-receptor stimulation and subsequent PM PI(4,5)P_2 hydrolysis, there is an immediate need for cells to replenish their depleted PM PI(4,5)P_2 pools. 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. One role for ER-PM contact sites is to help regulate the resynthesis of PM PI(4,5)P_2 following its depletion.

7.3.2 Transfer of PI to the Plasma Membrane

Following G_q-receptor-mediated PM PI(4,5)P_2 depletion, phosphoinositide transfer domains, called Nir2 and Nir3, are recruited to ER-PM junctions (Fig. 7.4 a(i)) (Chang et al. 2013; Kim et al. 2013; Kim et al. 2015). This recruitment is triggered through the combined actions of DAG and phosphatidic acid (Kim et al. 2015) 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) (Amarilio et al. 2005) (Fig. 7.4a, b). The stimulated recruitment of Nir2 and Nir3 to contact sites serves to deliver PI to the PM to replenish PM PI(4)P. The same process also delivers phosphatidic acid to the ER for future PI synthesis (Fig. 7.4a) (Kim et al. 2015).

7.3.3 Transfer and Regulation of PI(4)P at ER-PM Junctions

The majority of PM PI(4)P, the precursor to PM PI(4,5)P_2, seems to be generated by the PM-targeted lipid kinase PI4KIIIα (Chung et al. 2015a; Nakatsu et al. 2012) and delivery of PI(4)P from the Golgi (Dickson et al. 2014; Szentpetery et al. 2010). Recently, several integral ER proteins have been identified as key regulators of PM PI(4)P. The first of these are the oxysterol-binding protein (OSBP)-related protein 5 (ORP5) and ORP8 (Chung et al. 2015b). 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 serves dual functions at ER-PM contact sites (Fig. 7.4a (ii)). Not only do these proteins facilitate contact between the two membranes, i.e., acts as a tether, but they also facilitate the exchange of phosphatidylserine (PS) for PI(4)P at these junctions (Chung et al. 2015b; Maeda et al. 2013). In addition to the ORP proteins, an ER PI(4)P phosphatase, Sac1, is present at ER-PM junctions (Fig. 7.4 (iii and iv)) and can regulate PM PI(4)P (Dickson et al. 2016; Stefan et al. 2011) directly and PM PI(4,5)P_2 indirectly (Dickson et al. 2016). The precise mode of action of Sac1, i.e., acting in trans (Fig. 7.4 (iv)), across the junctional cleft to directly dephosphorylate PM PI(4)P, or in cis (Fig. 7.4 (iii)) to dephosphorylate transferred ER PI(4)P, remains to be fully determined.

7.3.4 PI(4,5)P_2 at ER-PM Contact Sites

Many ER-PM tethering proteins share a unifying architecture with an ER-anchoring domain and lipid-interacting domain (Bennett et al. 2013; Giordano et al. 2013). For example, interactions between the ER and PM can be enhanced following increases in intracellular calcium (E-Syt1) (Idevall-Hagren et al. 2015) or dissolved following receptor-mediated PI(4,5)P_2 depletion (Chung et al. 2015b; Dickson et al. 2016; Giordano et al. 2013). Such dynamic lipid-protein interactions mean that PI(4,5)P_2-dependent contact sites are inherently dynamic and also self-regulating (Fig. 7.4). At steady-state net depletion of PM PI(4,5)P_2 reduces the number of ER-PM contacts and reduces the population of Sac1 proximate to the PM. Loss of both

Sac1 and ORP5 would release an inhibitory brake on the PM PI(4)P pool, allowing PM PI(4)P and $PI(4,5)P_2$ levels to accumulate. Increases in PM $PI(4,5)P_2$ help reestablish ER-PM contacts and, after PI(4)P accumulation, bring ORP5 and also Sac1 back into close proximity of the PM, reapplying the brake on PI(4)P and $PI(4,5)P_2$ accumulation. Physiologically, the dynamic correlation between phosphoinositides and ER-PM contacts would be beneficial for the recovery of each of the PM phosphoinositide species following periods of net depletion and provide a mechanism for the temporal and spatial positioning of ER-PM contacts depending on the requirements of the cell.

7.4 Perspectives

The concept that ER and PM contacts represent "intracellular synapses" for the transfer of information to shape membrane identity and excitability has been discussed for 60 years. With the advancement of new technologies to image at higher resolution in living cells, we are beginning to understand the molecular identity and activities of many more proteins and effectors that exist within these intimate regions of membrane-membrane contact. There is still much information to determine. Future advances must address not only the nanoscopic structural organization and function of ER-PM contacts in excitable cells but also their role(s) in coordinating macroscopic cellular events. It remains to be seen if single endogenous tethering proteins work independently to create their own distinct signaling nano-domains or if multiple tethers work synergistically/cooperatively in primary cells, like myocytes or neurons.

7.5 Conclusion

Endoplasmic reticulum-plasma membrane contact sites orchestrate, segregate, and organize specific cellular tasks in a spatially defined, compartmentalized manner to shape cellular excitability. These contact sites 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 role in cellular pathways and physiological events.

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