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Abstract

Membrane contact sites (MCSs) are specialized regions where two or more organelle membranes come into close apposition, typically separated by only 10–30 nm, while remaining distinct and unfused. These sites play crucial roles in cellular homeostasis, signaling, and metabolism. This review focuses on ion channels, transporters, and receptors localized to MCSs, with particular emphasis on those associated with the plasma membrane and endoplasmic reticulum (ER). We discuss the molecular composition and functional significance of these proteins in shaping both organelle and cellular functions, highlighting their importance in excitable cells and their influence on intracellular calcium signaling. Key MCSs examined include ER–plasma membrane, ER–mitochondria, and ER–lysosome contacts. This review addresses our current knowledge of the ion channels found within these contacts, the dynamic regulation of MCSs, their importance in various physiological processes, and their potential implications in pathological conditions.

Keywords

calcium (Ca²⁺), calcium-induced calcium release (CICR), endoplasmic reticulum (ER), ion channels, IP₃ receptor, junctophilin (JPH), large conductance Ca²⁺-activated K₊ channel (BK_{Ca}), membrane contact sites (MCS), niemann-Pick type C1 (NPC1), neurodegeneration, orai, stromal interaction molecule (Stim), ryanodine receptors (RyR), transient receptor potential cation channel subfamily M member 4 (TRPM4), voltage-gated calcium channel (Ca_v), voltage-gated potassium channel (K_v)

Introduction

Eukaryotic cells contain a variety of membrane-bound organelles, each with specialized functions and distinct spatial organizations. The nucleus, housing genetic material, typically occupies a central position, while the endoplasmic reticulum (ER) extends throughout the cytoplasm, forming an intricate network. Mitochondria, Golgi apparatus, endo/lysosomes, lipid droplets, and peroxisomes are generally distributed throughout the cell, with their positioning often dynamic and responsive to cellular needs (Valm et al., 2017; Guo et al., 2018). These morphologies and spatial organizations facilitate inter-organelle communication at membrane contact sites (MCS). Historically, intracellular organelle MCS were broadly defined as regions of close apposition between the membranes of two organelles. However, recent advances in our understanding of the molecular mechanisms underlying MCS formation and function necessitate a more precise and evolving definition. Here, we define MCS as specialized cellular domains where two distinct cellular membranes are tethered in close proximity (typically within 10–30 nanometers) without fusing, allowing for communication and exchange of molecules between different cellular compartments (Figure 1A) (Eden, 2016; Phillips and Voeltz, 2016; Scorrano et al., 2019; Prinz et al., 2020; Guillén-Samander and De Camilli, 2022). These MCSs play crucial roles in processes such as lipid transfer, calcium (Ca²⁺) signaling, and metabolic

regulation. This review concentrates on Ca²⁺ transfer at MCS in excitable cells by examining the molecular tethering mechanisms, regulatory processes, maintenance of MCS architecture, and their functional roles in Ca²⁺ homeostasis and signaling.

The center of MCS formation and function is the ER, a large, multifaceted, and dynamic organelle comprised of interconnected tubules and cisternae that extend throughout the cytoplasm. Its extensive surface area, motility, and close association with other organelles make the ER an ideal platform for establishing contacts with other organelles. MCS between the ER and other organelles are not the result of stochastic interactions between adjacent lipid bilayers. Rather, they are highly regulated structures formed through the selective enrichment of specialized proteins that mediate the tethering of membranes and functionality of inter-organelle communication (Voeltz et al., 2024) (Figure 1A). This review does not focus on the numerous proteins that have been identified as bona

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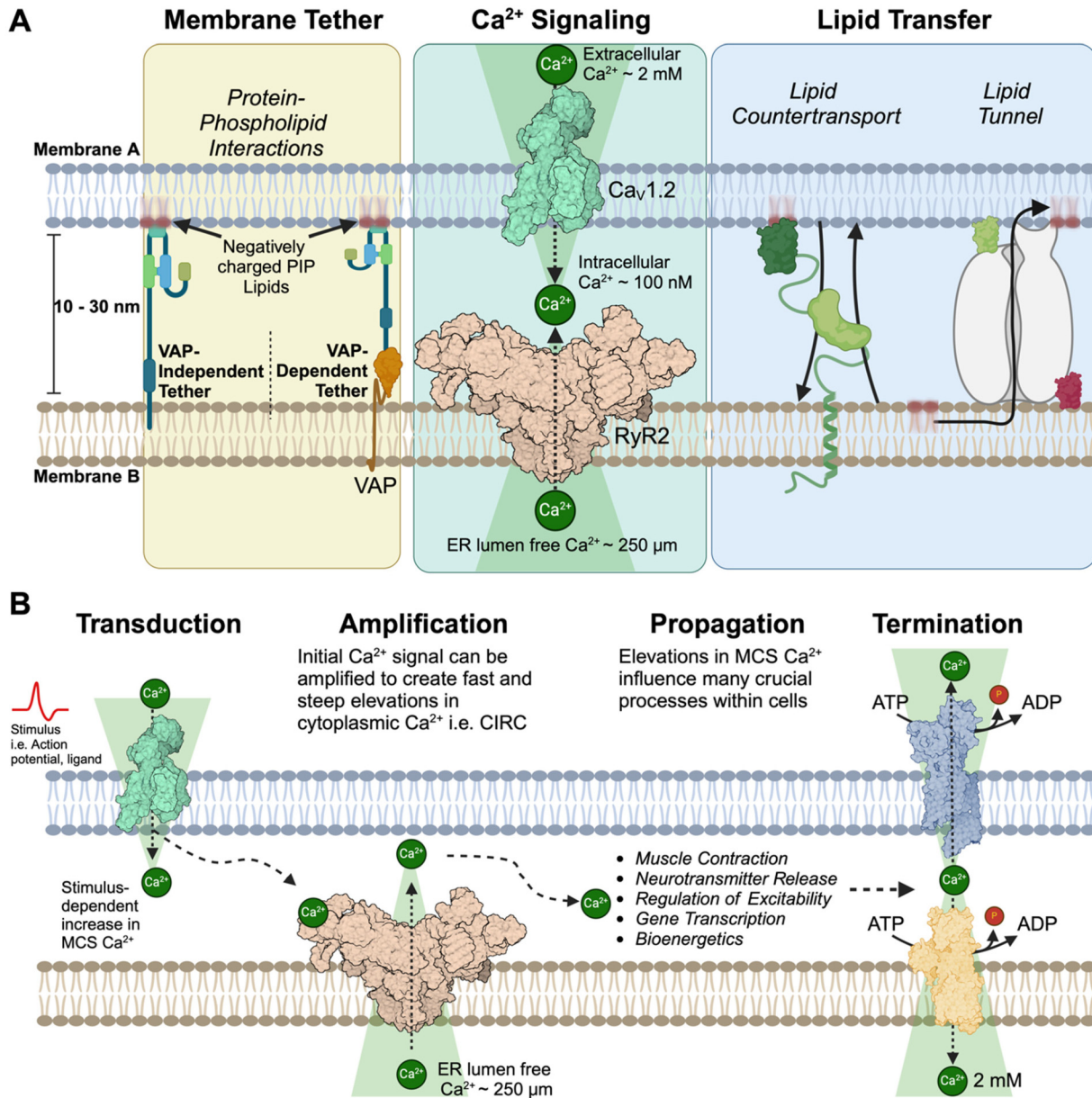


Figure 1. Molecular composition of a membrane contact site. **(A)** Typical components present within a MCS include (left to right): membrane tether, Ca²⁺-handling proteins, and/or lipid-transfer protein. Green triangles represent Ca²⁺ gradient between extracellular or luminal and intracellular Ca²⁺ (~100-200 nM) concentrations. **(B)** Role of Ca²⁺-handling proteins at MCS. Ca²⁺-handling proteins facilitate the transduction (conversion of an electrical signal or ligand into a Ca²⁺ signature), amplification, propagation (local elevations in Ca²⁺ at MCS can exert broad influence through the cell), and termination.

vide MCS tethering proteins i.e., extended-synaptotagmins (E-Syts) (Giordano et al., 2013), oxysterol-binding protein-related proteins (ORPs) (Chung et al., 2015), or, proteins essential for membrane contact site formation i.e., vesicle-associated membrane protein (VAMP)-associated proteins (VAPs) (Murphy and Levine, 2016). Instead, we focus on specific ion channels that have been identified and characterized as being present and functionally important at MCS.

At MCS, various membrane proteins—including ion channels, transporters, receptors, and pumps play pivotal

roles in orchestrating both electrical and chemical signaling cascades. These proteins function as initiators, transducers, amplifiers, and terminators of signals within specialized nanodomains (Figure 1B). Among the key signals regulated by ion channels at MCS is cytoplasmic Ca²⁺. Under resting conditions, cytoplasmic Ca²⁺ concentrations are maintained at low levels (~100–200 nM) in most cell types. Localized elevations in intracellular Ca²⁺ at MCS serve as instructive signals, modulating a diverse array of cellular processes, including signal transduction, muscle contraction, energy

production, synaptic plasticity, cell survival, and stress responses (for review please see Wei et al., 2012; Clapham, 2007; Bers, 2002; Berridge, 1998; Ghosh and Greenberg, 1995). The need for precise spatial and temporal control of Ca^{2+} signaling at MCS is underscored by the wide range of pathological conditions that result following dysregulation of Ca^{2+} signaling at these MCS nanodomains (Gomez et al., 2001; Bers and Guo, 2005; Bezprozvanny, 2010; Navedo et al., 2010; Kawamoto et al., 2012; Brini et al., 2014; Alzheimer's Association Calcium Hypothesis Workgroup and Khachaturian, Z.S., 2017; Pchitskaya et al., 2017; Zaichick et al., 2017).

This review focuses on ion channels that are found in the ER and apposing MCS membranes, we discuss the molecular composition and functional significance of these ion channels in shaping both organelle and cellular functions with a particular emphasis on MCS in excitable cells and how they influence a broad range of physiological outputs through elevations in intracellular Ca^{2+} . The importance of reviewing and understanding the molecular mechanisms governing MCSs provides insights into their roles in health and disease, potentially unveiling new therapeutic targets for conditions associated with dysfunctional organelle interactions.

Endoplasmic Reticulum—Plasma Membrane MCS

Endoplasmic reticulum–plasma membrane (ER–PM) MCS, often referred to as ER–PM junctions, have been the subject of intense study in excitable cells for more than six decades. This sustained interest stems from several key factors that have shaped our understanding of these crucial cellular structures. The initial spark in interest came from early electron microscopy studies on the sarcoplasmic reticulum (SR) (a specialized form of ER in muscle cells) in striated muscle, which revealed the close apposition between the SR and the plasma membrane (SR–PM) (Porter and Palade, 1957). This discovery laid the groundwork for further investigations into the nature and function of these junctions. Following this initial observation, researchers discovered in skeletal muscle that Ca^{2+} influx through dihydropyridine receptors (DHPRs) on the plasma membrane—also known as voltage-gated Ca^{2+} channels or Ca_v —is not necessary to activate ryanodine receptors (RyRs) in SR membranes. Instead, a direct mechanical coupling mechanism occurs across the MCS junction between Ca_v and RyRs, efficiently translating action potentials into muscle contraction (Figure 2A) (Schneider and Chandler, 1973; Rios and Brum, 1987; Block et al., 1988). This finding highlighted the crucial role of SR–PM MCS in excitation-contraction (EC) coupling, the fundamental process that facilitates contraction of muscle myocytes and enables us to breath, stand, and walk. Further facilitating investigations into SR–PM MCS is their peripheral

location within cells. This accessibility has enabled researchers to employ techniques such as patch-clamp electrophysiology and total internal reflection fluorescence (TIRF) microscopy, allowing MCS, the ion channels localized to MCS, and their downstream processes to be resolved at high temporal and spatial resolution. Another factor contributing to the extensive research in this field is the evolutionary conservation of key proteins present in ER–PM contacts. Proteins such as STIM1, Orai1, and junctophilins are found across diverse species, enabling comparative studies that have provided valuable insights into the fundamental nature and importance of these structures in cellular physiology. In the sections that follow, we will detail our current understanding of ion channels at ER–PM MCS, focusing on muscle cells (skeletal, cardiac, and smooth), and neurons. We will highlight the adaptations, molecular elements and functional significance of these structures in each cell type, providing a comprehensive overview of their role in cellular physiology and underscoring their importance in health and disease.

Muscle

As introduced above, EC coupling in muscle cells is the process that translates electrical stimuli into mechanical contraction. This mechanism critically depends on the intimate spatial relationship between ion channels in the plasma membrane (termed sarcolemma in muscle tissue) and ligand-gated receptors in the SR (referred to in this section as SR–PM MCS). In both skeletal muscle fibers and cardiac myocytes, EC coupling is initiated by the depolarization of the sarcolemma which rapidly propagates into specialized membrane invaginations called transverse tubules (T-tubules; Figure 2A). The depolarization activates voltage-gated L-type calcium channels located within these T-tubules, specifically $\text{Ca}_v1.1$ in skeletal muscle and $\text{Ca}_v1.2$ in cardiac muscle. These activated Ca_v channels then interact with RyRs situated on the adjacent SR membrane, which serves as the primary intracellular Ca^{2+} reservoir in muscle cells. The interaction between L-type calcium channels and RyRs, which occurs through either mechanical ($\text{Ca}_v1.1$) or chemical coupling ($\text{Ca}_v1.2$; to be discussed in detail in subsequent sections), triggers Ca^{2+} release from the SR into the cytoplasm. This localized elevation in cytoplasmic Ca^{2+} concentration is crucial for initiating the molecular events that ultimately lead to muscle contraction. Therefore, the process of EC coupling in different muscle types relies on precise localization and communication between specific ion channels and receptors at SR–PM MCS. In the following sections, we will detail the molecular components across various muscle tissues. These sections will highlight the specialized adaptations of EC coupling machinery, underscoring the importance of SR–PM junctions in muscle physiology and their role in facilitating the precise and rapid signal transduction necessary for muscle contractions.

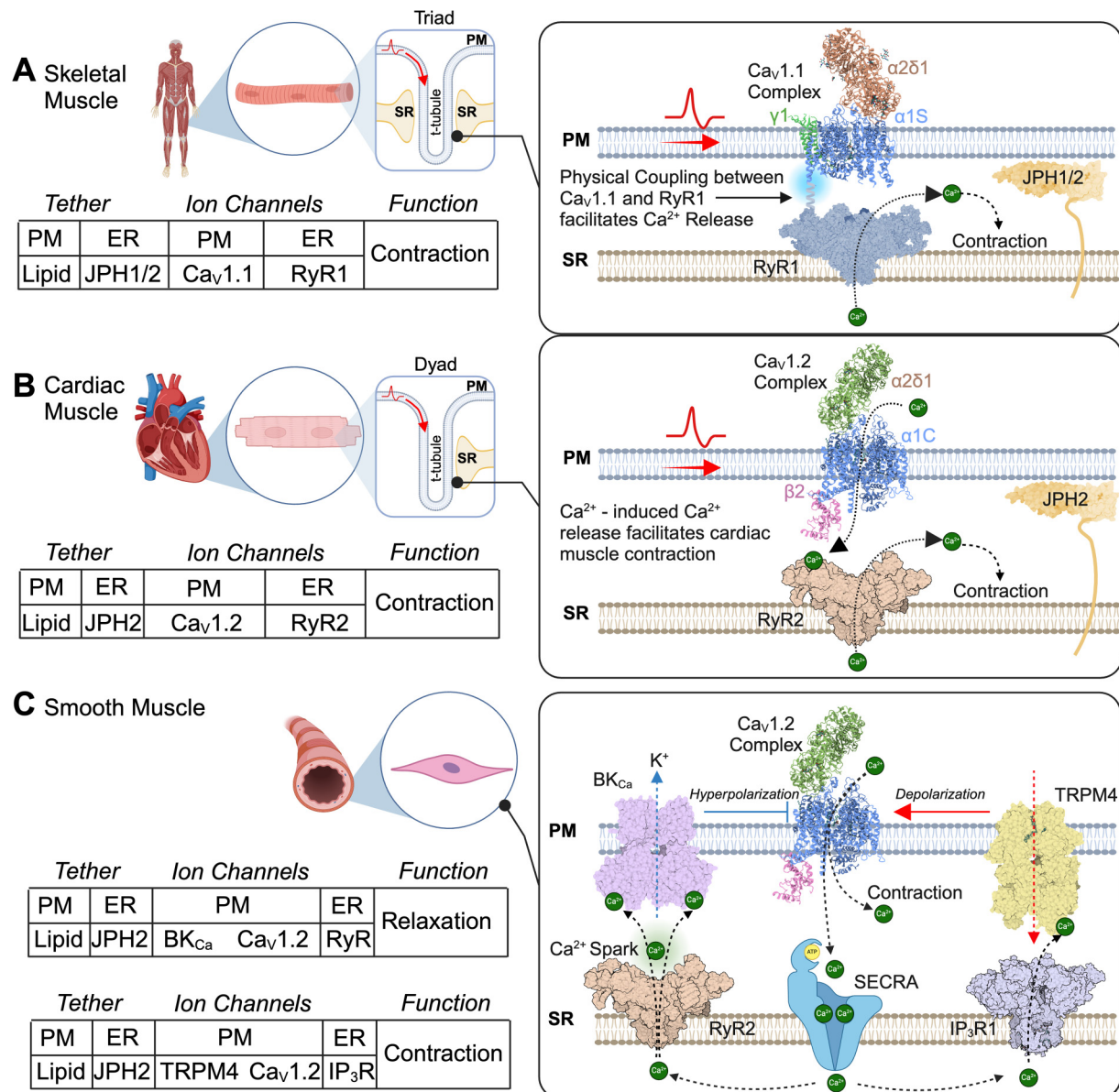


Figure 2. Ion channels in SR-PM MCS in muscle cells. **(A)** Skeletal muscle. Left: representation of triad organization in skeletal muscle cells and molecular components Right: Structural and functional organization of ion channels that facilitate CICR in skeletal muscle cells. **(B)** Same as (A) only cardiac muscle. **(C)** Same as A, B only smooth muscle.

Skeletal Muscle. Skeletal muscle triads are specialized structures crucial for EC coupling. These triads consist of three distinct elements: a central T-tubule flanked by two terminal cisternae of the SR (1:2 ratio PM:SR; Figure 2A). This unique arrangement forms two closely apposed SR-PM MCSs within each triad, typically spanning 10–15 nm.

Membrane Tethers. At skeletal muscle triads Junctophilin 1 and 2 (JPH1 and JPH2) play key roles as membrane tethers (Ito et al., 2001; Komazaki et al., 2002). Evidence supporting a role for JPH1 and JPH2 as MCS tethers in skeletal muscle

myocytes include their localization and enrichment at Triads (Rossi et al., 2019), electron microscopy analysis of JPH1 and JPH2 knockout mice showing reduced formation of triad junctions between T-tubules and SR (Ito et al., 2001; Komazaki et al., 2002), and functional studies correlating changes in MCS formation with impaired EC coupling, leading to reduced contractile force at low stimulation frequencies and abnormal sensitivity to extracellular calcium levels (Ito et al., 2001; Komazaki et al., 2002). In addition to JPH1 and JPH2, triad morphology in skeletal muscle is supported by mitsugumin29 (MG29) (Komazaki et al., 1999; Nishi et al., 1999) and bridging integrator 1 protein

(BIN1) (Lee et al., 2002). A role for MG29 in supporting triad morphology comes from electron microscopy studies characterizing its localization to the SR during muscle development and MG29 knockout mice showing abnormal ultrastructure of skeletal muscle, including swollen T-tubules, irregular SR structures, and partial alteration of triad junctions (Komazaki et al., 1999; Nishi et al., 1999). Functionally, MG29 knockout mice models also have impaired EC coupling (Nishi et al., 1999). A final component implicated in MCS formation/maintenance in skeletal muscle is BIN1 where a muscle-specific isoform of BIN1 has been shown to localize to T-tubules in skeletal muscle (Lee et al., 2002), is necessary for triad formation, and its constitutive deficiency leads to death at birth due to a skeletal muscle feeding defect (Prokic et al., 2020). While these data support a role for BIN1 in early muscle organization and position, inducible knockout of BIN1 in adult murine models had little impact (Prokic et al., 2020) on triad organization or function, presenting a model where BIN1 is necessary for triad development and function whereas other tethers contribute to the stability of triads with aging.

Molecular Organization of Skeletal Muscle ion Channels, Receptors, and Transporters. As noted above, the molecular elements responsible for EC at SR-PM MCS begins with DHPR on transverse (T) tubule membranes. DHPR are voltage-gated Ca^{2+} channels consisting of $\alpha 1$, β , γ , and $\alpha 2\delta$ subunits, with the $\alpha 1$ subunit forming the ion-conducting pore and voltage sensor (Wu et al., 2015) (Figure 2A). In skeletal muscle the predominant voltage-gated Ca^{2+} channel isoform is $\text{Ca}_v1.1$ (Catterall, 2000). Opposing DHPR channels are Ryanodine receptor 1 (RyR1) channels on SR membranes (Figure 2A). Upon membrane depolarization, $\text{Ca}_v1.1$ undergoes conformational changes that are physically communicated to RyR1, triggering the opening of RyR1 channels on the SR membrane (Schneider and Chandler, 1973; Gonzalez-Serratos et al., 1982; Rios and Brum, 1987; Dirksen and Beam, 1999). This allows Ca^{2+} ions to move down their concentration gradient and flow from the SR into the cytosol (concentration gradient $\sim 5,000 : 1$, SR : cytosol), initiating the cascade of events leading to muscle contraction. In this way, $\text{Ca}_v1.1$ functions as a voltage sensor, translating membrane potential changes in the plasma membrane into structural modifications of RyR1 in the SR membrane enabling rapid Ca^{2+} release into the cytoplasm. In skeletal muscle, Ca^{2+} released from the SR through RyR1 channels binds to troponin C, causing a conformational change that exposes myosin-binding sites on actin filaments. This allows myosin heads to interact with actin, initiating cross-bridge cycling and muscle contraction, with the force and speed of contraction directly related to the concentration of free cytosolic Ca^{2+} (Reviewed in Geeves and Holmes, 1999; Gordon et al., 2000; Rebbeck et al., 2014; Sweeney and Houdusse, 2010). Collectively, these tightly orchestrated elements

ensure efficient EC coupling in skeletal muscle. At junctional SR-PM MCS RyR1 can be regulated by various factors to tune Ca^{2+} release, including Ca^{2+} itself, ATP, Mg^{2+} , phosphorylation, and interacting proteins such as FKBP12 and calmodulin (Rodney et al., 2000; Danila and Hamilton, 2004; Lanner et al., 2010; Rebbeck et al., 2014). Skeletal muscle also contains a high concentration of sarco/endoplasmic reticulum Ca^{2+} -ATPase type 1 (SERCA1). These pumps are primarily localized to the longitudinal SR tubules rather than at junctional SR-PM contact sites (Rossi et al., 2022). SERCA1 activity in the longitudinal tubules facilitates rapid Ca^{2+} reuptake, promoting muscle relaxation and enabling subsequent contraction cycles. For more detailed information on the organization and molecular elements responsible for EC coupling in skeletal muscle please see following excellent reviews (Franzini-Armstrong, 1970; Block et al., 1988; Franzini-Armstrong and Protasi, 1997; Franzini-Armstrong et al., 1999; Protasi et al., 2002; Al-Qusairi and Laporte, 2011)

Cardiac Myocytes. In cardiac muscle cells, EC coupling is a complex process that also links electrical excitation of the sarcolemma to contraction of the myofilaments. Central to this process is the dyad, which consists of a junctional complex formed by T-tubules of the sarcolemma and a single SR membrane (1:1 ratio; Figure 2B). This arrangement ensures efficient coupling between the depolarization of the sarcolemma and the release of Ca^{2+} from the SR, essential for initiating cardiac myocyte contraction.

Membrane Tethers. In cardiac myocytes, the formation and maintenance of SR-PM MCS are primarily mediated by three key proteins: junctophilin-2 (JPH2), amphiphysin II/bridging integrator 1 protein (BIN1), and nexilin (NEXN). **JPH2:** While skeletal muscle expresses both JPH1 and JPH2, cardiomyocytes exclusively express JPH2 (Ito et al., 2001), which serves as the SR protein responsible for tethering cardiac dyads (Lehnart and Wehrens, 2022) (Figure 2B). Several lines of evidence support JPH2's critical role in dyad formation and maintenance. Structurally, transmission electron microscopy studies of purified JPH2 reveal a ~ 15 -nm long structure, approximating the width of the dyadic cleft. This structural characteristic aligns with JPH2's proposed function as a tether in dyad formation. Furthermore, JPH2 knockout is embryonic lethal between E9.5 and E11.5, coinciding with the onset of t-tubule formation and cardiac contractile activity. This timing suggests a crucial role for JPH2 in the early stages of cardiac structural development. In adult cardiac function, JPH2 continues to play a vital role with cardiac-specific silencing of JPH2 in adult mice leading to heart failure within a week. This rapid decline is characterized by reduced colocalization between $\text{Ca}_v1.2$ and RyR2, increased variation in dyadic spacing, and an aberrant increase in RyR2 activity (van Oort et al., 2011). These observations highlight JPH2's

ongoing importance in maintaining cardiac structure and function beyond the developmental stage. Beyond its structural role, JPH2 also engages in important molecular interactions. It physically interacts with $\text{Ca}_v1.2$, potentially facilitating the recruitment and retention of $\text{Ca}_v1.2$ channels at dyads (Gross et al., 2021; Poulet et al., 2021). These findings collectively underscore JPH2's significance in SR-PM MCS formation, maintenance, and organization of molecular elements in both developing and mature cardiac tissue.

BIN1: alongside JPH2, Bridging Integrator 1 (BIN1), a membrane curvature and tubule-forming protein, plays a crucial role in t-tubule biogenesis in cardiac muscle (Lee et al., 2002). BIN1's significance in cardiac physiology is underscored by several key findings. Cardiac-specific heterozygous knockout of BIN1 results in reduced cardiomyocyte capacitance, indicative of decreased t-tubule area (Hong et al., 2010), while BIN1 knockdown in adult rat ventricular myocytes leads to reduced t-tubule density (Caldwell et al., 2014). BIN1's influence extends beyond structural maintenance to functional aspects of cardiac physiology. It plays a crucial role in targeting $\text{Ca}_v1.2$ channels to t-tubules (Hong et al., 2012) and is closely associated with RyR2 (Fu et al., 2016; Westhoff et al., 2024). BIN1 knockdown results in dysfunctional Ca^{2+} signaling (Caldwell et al., 2014), highlighting its importance in EC coupling. Interestingly, BIN1 levels increase in the aging heart, leading to enhanced clustering and localization of $\text{Ca}_v1.2$ and RyR2, promotion of dysfunctional EC coupling, and reduced systolic function (Westhoff et al., 2024). Remarkably, BIN1 knockdown in aged mice restores cardiac function towards youthful levels, suggesting a potential therapeutic avenue for age-related cardiac decline. These findings collectively highlight the critical role of BIN1 in maintaining cardiac muscle structure through t-tubule formation and preservation, regulating cardiac function via its influence on Ca^{2+} handling proteins, and mediating age-related changes in cardiac performance. The multifaceted involvement of BIN1 in cardiac physiology underscores its potential as a target for therapeutic interventions in various cardiac pathologies, including age-related cardiac dysfunction.

Nexilin (NEXN): an actin-binding and z-disk-stabilizing protein (Ohtsuka et al., 1998; Hassel et al., 2009), has emerged as another crucial player in the formation and maintenance of structural and functional organization of SR-PM MCS. At cardiac dyads NEXN colocalizes with JPH2 and RyR2 (Liu et al., 2019). Mutations or genetic ablation of NEXN are linked to cardiomyopathy in various species, including humans (Hassel et al., 2009). Both global and cardiac-specific knockout of NEXN in mice result in early-onset dilated cardiomyopathy and lethality, underscoring its vital role in cardiac function (Aherrahrou et al., 2016; Liu et al., 2019). Loss of NEXN leads to a significant reduction in SR-PM junctions, impaired t-tubule formation, and reduced expression of key dyadic proteins such as $\text{Ca}_v1.2$,

RyR2, and JPH2 (Liu et al., 2019). Finally, Inducible knock-out of NEXN in adult cardiomyocytes also leads to altered Ca^{2+} handling and t-tubule network remodeling (Spinozzi et al., 2020). While these studies highlight NEXN's importance in t-tubule biogenesis, maintenance, and regulation, the precise mechanisms by which NEXN interacts with BIN1 and JPH2 to influence dyadic protein expression and cardiac function remain to be elucidated.

Molecular Organization of Cardiac Ion Channels, Receptors, and Transporters. On the sarcolemma side of the dyad, $\text{Ca}_v1.2$ voltage-gated L-type Ca^{2+} channels are the most prominent isoform that sense T-tubule membrane depolarization and undergo conformational changes allowing the pore forming alpha subunit (α_1) to open and permit Ca^{2+} to flow down its concentration gradient into the cytosol (Figure 2B). In cardiac dyads, $\text{Ca}_v1.2$ channels form distinct clusters of approximately 5–8 channels that profoundly influence their functional properties (Navedo et al., 2005; Dixon et al., 2012; Dixon et al., 2015). This clustering phenomenon facilitates cooperative gating, a mechanism that enhances channel activity. Substantial evidence demonstrates that the spatial proximity of $\text{Ca}_v1.2$ channels within these clusters promotes Ca^{2+} /calmodulin-dependent C-terminal interactions between adjacent channels, leading to an increased open channel probability (Dixon et al., 2012; Dixon et al., 2015). This cooperative behavior effectively amplifies the Ca^{2+} influx, which in turn amplifies $\text{Ca}_v1.2$ clustering to help tune EC coupling. As noted, the size and composition of $\text{Ca}_v1.2$ clusters are not static but dynamically regulated by various physiological and pathological factors. Adrenergic receptor signaling, a key modulator of cardiac function, can increase cluster size and arrangement, thereby fine-tuning Ca^{2+} influx in response to sympathetic stimulation to meet hemodynamic demands (Ito et al., 2019). Age-related increases in $\text{Ca}_v1.2$ clustering (to form “super-clusters” Westhoff et al., 2024) also impacts cardiac physiology and may contribute to altered Ca^{2+} handling and lack of sympathetic nervous system responsiveness in the aging heart (Westhoff et al., 2024). Moreover, certain disease-associated mutations can disrupt normal clustering patterns, leading to aberrant channel function and potentially contributing to cardiac pathologies (Navedo et al., 2010).

Unlike skeletal muscle, opening of $\text{Ca}_v1.2$ channels on the sarcolemma in cardiac myocytes facilitates rising Ca^{2+} concentrations in the dyadic cleft which bind and activate nearby RyR2 to facilitate a process called Ca^{2+} -induced Ca^{2+} -release (CICR). Here, Ca^{2+} -binding to RyR2 sensitizes the receptor and allows Ca^{2+} release from the SR to amplify the dyad Ca^{2+} signal, resulting in a large and rapid elevation in cytosolic Ca^{2+} (Figure 2B). Like $\text{Ca}_v1.2$, RyR2 exhibit the ability to form clusters (Sun et al., 1995; Baddeley et al., 2009). The spatial arrangement of RyR2 channels within these clusters facilitates inter-channel interactions, leading to coordinated gating behavior (Dixon et al., 2022). This

cooperative activity can amplify Ca^{2+} release events, thereby fine-tuning CICR for cardiac EC coupling (Cheng et al., 1993). As with $\text{Ca}_v1.2$ channels, RyR2 clusters increase with aging to increase Ca^{2+} release (Westhoff et al., 2024).

In cardiac myocytes, elevated cytosolic Ca^{2+} binds to troponin C on the actin filaments, initiating a series of events that lead to muscle contraction (Gomez et al., 2001; Wang et al., 2001; Bers, 2002). While junctional SR-PM MCS are responsible for Ca^{2+} entry into the cytoplasm, the longitudinal SR is dedicated to the removal of Ca^{2+} from the cytosol. Following myocyte contraction, Ca^{2+} ions are rapidly removed from the cytosol by the SR Ca^{2+} -ATPase (SERCA2a), located on the SR membrane and exchanged via the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) on the sarcolemma. Therefore, SERCA2a and NCX maintain low cytosol Ca^{2+} levels and prepare myocytes for the next contraction cycle. CICR is tightly regulated by various factors, including membrane potential, Ca^{2+} concentration gradients, and the activity of regulatory proteins such as calmodulin, calsequestrin, and FKBP12 (Bers, 2002; Bers and Guo, 2005).

Smooth Muscle. Smooth muscle tissue, found in the walls of hollow organs such as blood vessels, the gastrointestinal tract, bladder, and uterus, plays a crucial role in various physiological processes including vasoconstriction, peristalsis, and the control of blood flow and pressure (Berridge, 2008). Unlike skeletal and cardiac myocytes, smooth muscle cells rely on multiple calcium (Ca^{2+}) signaling mechanisms for both contraction and relaxation. There are two primary mechanisms through which intracellular Ca^{2+} can be increased leading to a contraction: (i) electromechanical coupling, and (ii) pharmacomechanical coupling (Somlyo and Somlyo, 1968). Electromechanical coupling refers to depolarization-induced opening of voltage-gated Ca^{2+} channels ($\text{Ca}_v1.2$) leading to a contraction (Figure 2C). Whereas, pharmacomechanical coupling refers to the process by which chemical signals (typically drugs or hormones) directly cause muscle contraction or relaxation without requiring changes in membrane potential.

Molecular Organization of Smooth Muscle Ion Channels, Receptors, and Transporters. In smooth muscle, $\text{Ca}_v1.2$ channels are the primary mediators of contraction, but Ca^{2+} influx across the smooth muscle sarcolemma can come from a variety of sources including T-type calcium channels (Kuo et al., 2010; Abd El-Rahman et al., 2013), TRP channels (Gonzales et al., 2010; Mercado et al., 2014; Thakore et al., 2020), and Orai channels (Potier et al., 2009; Trebak, 2012). Like in Cardiac muscle, $\text{Ca}_v1.2$ channels in smooth muscle demonstrate the ability to cluster and cooperatively interact to increase the open probability of channels (Navedo et al., 2005; Navedo et al., 2010; Nystoriak et al., 2017). Pathologically, aberrant increases in $\text{Ca}_v1.2$ clustering in smooth muscle is observed in several conditions including diabetes (Nystoriak et al., 2017), hyperglycemia

(Prada et al., 2019), hypertension (Navedo et al., 2010), and timothy syndrome (Navedo et al., 2010). Ca^{2+} entry through $\text{Ca}_v1.2$ channels elevates intracellular Ca^{2+} which increase the probability of cytosolic Ca^{2+} -binding to calmodulin (CaM). Formation of Ca^{2+} -CaM complexes activate myosin light chain kinase (MLCK). MLCK phosphorylates myosin I regulatory light chains, enabling myosin-actin interactions. This leads to cross-bridge formation, filament sliding, and muscle contraction (Somlyo and Somlyo, 2003). The critical role of $\text{Ca}_v1.2$ channels in the development and maintenance of vascular tone and reactivity is underscored by the efficacy of L-type calcium channel blockers as potent antihypertensive drugs (Brozovich et al., 2016).

In smooth muscle, Ca^{2+} signaling exhibits a dual role, mediating both contraction and relaxation. This seemingly paradoxical function can be explained by the functional coupling between ion channels at SR-PM MCS. At SR-PM MCS Ca^{2+} release from RyRs on the SR, known as Ca^{2+} sparks (Cheng et al., 1993), occurs in close proximity to large-conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) on the plasma membrane (Figure 2C, left). These BK_{Ca} channels are uniquely positioned to respond to both membrane depolarization and localized elevations in cytoplasmic Ca^{2+} (Berkefeld et al., 2006; Fakler and Adelman, 2008; Muller et al., 2010; Irie and Trussell, 2017). Further their low affinity Ca^{2+} -binding site means that a large Ca^{2+} release is required for their activation. When activated, they facilitate K^+ efflux, resulting in membrane hyperpolarization. This hyperpolarization decreases the open probability of voltage-gated L-type Ca^{2+} channels ($\text{Ca}_v1.2$), thereby reducing Ca^{2+} entry and promoting smooth muscle relaxation (Figure 2C, left). Unlike in cardiac and skeletal muscle, where there is tight coupling between $\text{Ca}_v1.2$ channels and RyRs, smooth muscle $\text{Ca}_v1.2$ channels exhibit only loose coupling to RyR2 on SR membranes. The primary influence of $\text{Ca}_v1.2$ on RyR2 activity is thought to be indirect, primarily through the regulation of SR Ca^{2+} content (Essin et al., 2007; Takeda et al., 2011). Recent studies that optically quantified Ca^{2+} release from $\text{Ca}_v1.2$ channel clusters, termed Ca^{2+} sparklets (Navedo et al., 2005), have revealed that these events occur in proximity to both the SR and sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) pumps (Takeda et al., 2011). These Ca^{2+} sparklets can coexist within the same microenvironment as Ca^{2+} sparks, allowing for complex local Ca^{2+} signaling. In this arrangement, Ca^{2+} sparklets can indirectly modulate RyR activity through the regulation of SR Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{SR}}$), creating a sophisticated feedback mechanism for fine-tuning smooth muscle contractility. This intricate system of Ca^{2+} signaling at SR-PM MCS allows smooth muscle to precisely regulate its contractile state in response to various physiological stimuli, highlighting the importance of spatial organization in cellular signaling processes

Pharmacomechanical coupling is a crucial mechanism in smooth muscle contraction, whereby pharmacological

ligands bind to and activate specific receptors, leading to increased intracellular Ca^{2+} and subsequent muscle contraction. This process is highlighted during activation of G_q -coupled receptors by the ligand angiotensin II (AngII). In smooth muscle cells, G_q -receptor activation by AngII initiates a signaling cascade that activates phospholipase C (PLC). PLC then catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate ($\text{PI}(4,5)\text{P}_2$) into two second messengers: soluble inositol 1,4,5-trisphosphate (IP_3) and membrane-bound diacylglycerol (DAG). IP_3 rapidly diffuses through the cytoplasm and binds to IP_3 receptors (IP_3Rs) located on the SR membrane. This binding increases the open probability of IP_3Rs (Prole and Taylor, 2019), allowing Ca^{2+} to flow down its concentration gradient from the SR into the cytoplasm. The resultant localized increase in cytoplasmic Ca^{2+} near the plasma membrane has significant downstream effects. One key consequence of this IP_3R -mediated Ca^{2+} release is the activation of transient receptor potential vanilloid 4 (TRPV4) (Mercado et al., 2014) or transient receptor potential cation channel subfamily M member 4 (TRPM4) (Gonzales et al., 2010) channels located on the plasma membrane. The proximity of IP_3Rs to TRP channels creates a functional microdomain where localized Ca^{2+} increases can efficiently modulate TRP activity (Bandell et al., 2006; Gonzales et al., 2010; Mercado et al., 2014). Activation of TRP channels leads to membrane depolarization, which in turn opens voltage-gated L-type Ca^{2+} channels ($\text{Ca}_v1.2$). This further increases intracellular Ca^{2+} , ultimately causing smooth muscle contraction (Figure 2C, right). Recent research has revealed that TRPV4 channels are anchored by A-kinase anchoring protein 150 (AKAP150), which helps create a localized Ca^{2+} signaling microdomain within the plasma membrane (Mercado et al., 2014). However, it remains unclear whether AKAP150 also influences the positioning of IP_3Rs or if other anchoring proteins are involved in organizing this signaling complex. Interestingly, in addition to the G_q -mediated activation of TRP- IP_3R signaling at smooth muscle SR-PM MCS, studies on freshly isolated cerebral artery smooth muscle cells have suggested the presence of a basal level of PLC-dependent IP_3 generation and IP_3R -mediated Ca^{2+} release. This basal activity could potentially support TRP-mediated depolarizations even in the absence of explicit G_q -receptor activation.

Membrane Tethers. While substantial research has elucidated the functional coupling of ion channels at sarcoplasmic reticulum-plasma membrane (SR-PM) MCS, there has been an absence of research attention on the tethering proteins that generate and maintain these crucial junctions in smooth muscle. This gap in knowledge may be attributed to various factors, including historical perspectives, perceived functional importance, disease relevance, structural complexity, and the inherent diversity of smooth muscle types. In smooth muscle cells, several proteins have been identified as bona fide SR-PM MCS tethers, including JPH2 (Pritchard et al., 2019; Saeki et al., 2019), STIM1-Orai1 (Potier et al., 2009), and voltage-gated

potassium channel 2.1 ($\text{K}_v2.1$) (O'Dwyer et al., 2020; Matsumoto et al., 2023). Each of these proteins plays a unique role in maintaining the structural and functional integrity of SR-PM MCS. JPH2, well-known for its role in striated muscle, has been shown to be equally important in smooth muscle. Knockout studies of JPH2 in smooth muscle demonstrate a reduction in the proximity between the SR and PM (Pritchard et al., 2019). This loss of tethering significantly alters the functional coupling between ryanodine receptors (RyR2) and large-conductance Ca^{2+} -activated K^+ channels (BK_{Ca}) (Gonzales et al., 2010; Mercado et al., 2014; Mercado et al., 2014). Physiologically, the disruption of RyR2 - BK_{Ca} coupling decreases the local Ca^{2+} available to activate BK_{Ca} channels, reducing their conductance and resulting in vascular hypercontractility (Pritchard et al., 2019). These findings underscore the critical role of JPH2 in facilitating SR-PM contacts and localizing ion channels at these sites. $\text{K}_v2.1$ has emerged as another important SR-PM tethering protein in smooth muscle (O'Dwyer et al., 2020). In neurons, somatic and dendritic $\text{K}_v2.1$ serves dual roles: controlling membrane potential and generating ER-PM MCS in a phosphorylation-dependent manner (see neuron section for more details). In smooth muscle, $\text{K}_v2.1$ exhibits a similar multifaceted role, functioning both as a regulator of membrane potential and a structural organizer of $\text{Ca}_v1.2$ complexes (O'Dwyer et al., 2020; Matsumoto et al., 2023). In smooth muscle, $\text{K}_v2.1$ clusters $\text{Ca}_v1.2$ channels to enhance their function and increase contraction in a sex-specific manner (O'Dwyer et al., 2020; Matsumoto et al., 2023). In male myocytes $\text{K}_v2.1$ channels primarily function as regulators of membrane potential, whereas knockout of $\text{K}_v2.1$ or $\text{K}_v2.1_{\text{S590A}}$ mutations (non-phosphorylated mutation leading to reduced SR-PM MCS) in female myocytes reduce the clustering of $\text{Ca}_v1.2$ and Ca^{2+} influx. Thus, $\text{K}_v2.1$ could control both relaxation (by controlling the membrane potential) and contraction (by influencing $\text{Ca}_v1.2$ channel clustering) in smooth muscle, depending on sex. The potential integration of $\text{K}_v2.1$ - $\text{Ca}_v1.2$ complexes with RyR - BK_{Ca} signaling pathways at SR-PM MCS in smooth muscle remains an open question. Evidence from neuronal studies demonstrating $\text{K}_v2.1$'s capacity to interact with and cluster $\text{Ca}_v1.2$, RyR , SERCA , and PKA at ER-PM junctions (Vierra et al., 2019; Vierra et al., 2021; Vierra et al., 2023) (see below) suggests the possibility of a similar integrative signaling hub in smooth muscle cells. This hypothesis warrants further investigation, as it could reveal novel mechanisms of Ca^{2+} signaling coordination and provide insights into the fine-tuning of smooth muscle contractility.

Neurons

A typical neuron has a complex structure that includes several specialized regions, each with distinct functions. The soma, or cell body, contains the nucleus and most of the cell's organelles, serving as the metabolic center.

Extending from the soma are dendrites, which are branching projections that receive signals from other neurons and convey them towards the soma. The synaptic region, located at the end of the axon, is where neurons communicate with each other through synapses. Together, these regions enable neurons to process and transmit information efficiently within the nervous system. Given this distinct and specialized architecture, it is perhaps not surprising that ER-PM MCS, or junctions as they are often referred to in neurons, have been identified across each of these areas. Despite these findings, we still lack specific tools to clearly quantify the distribution of ER-PM MCS proteins and ion channels *in vitro* and *in vivo* across each distinct area of a neuron. Additionally, generalized characterizations of MCS in neurons should be avoided, as each brain region is unique in terms of its architecture, innervation, complement of neurons, and function. Quantitative ultrastructural analyses of distinct regions have revealed that significant portions of the plasma membrane are involved in ER-PM MCS signaling. Specifically, around 12% of the somatic PM is engaged with the cisternae of the ER, while dendritic and synaptic regions show approximately 3% engagement (Wu et al., 2017). Despite the extensive presence of ER-PM MCS in neurons, the characterization of proteins responsible for forming, regulating, and signaling at these MCS has been limited. Understanding the molecular underpinnings of these contact sites will enhance our knowledge of neuronal physiology and pathophysiology. Below, we broadly define the molecular elements of MCS in neurons, noting that each protein complex may be heterogeneously distributed within neurons (i.e., somatic, dendritic, etc.) and across regions.

K_v2.1-Mediated MCS. At somatodendritic regions of neurons, the voltage-gated potassium channel K_v2.1 is the best-characterized ion channel/MCS tether (Figure 3A). Historically, research has focused on K_v2.1's non-clustering role in regulating the resting membrane potential (Figure 3A, left). Here, following membrane depolarization, K_v2.1 opens, allowing K⁺ ion efflux to repolarize the neuronal membrane potential (Du et al., 2000). Beyond this essential role, K_v2.1 is regulated by protein kinases such as cyclin-dependent kinase-5 (CDK5) and Ca²⁺/calmodulin-dependent protein kinase II (CAMK2) (Misonou et al., 2006; Cerda and Trimmer, 2011). Channel phosphorylation acts as a molecular switch, converting K_v2.1 from a non-clustering (Figure 3A, left), conducting state (electrical dampener) to a clustering, non-conducting state (ER-PM MCS tether; see Figure 3A, right). The dynamic equilibrium between clustered and non-clustered K_v2.1 channels is further regulated by the Ca²⁺-dependent protein phosphatase calcineurin (Figure 3) (Misonou et al., 2004). Phosphorylation of K_v2.1's proximal restriction and clustering (PRC) domain creates a noncanonical FFAT motif enabling the cytoplasmic MSP domain of ER-localized VAP proteins to bind K_v2.1, forming ER-PM MCSs (Johnson et al., 2018; Kirmiz et al., 2018). Thus, the net

phosphorylation status of the PRC domain determines the proportion of K_v2.1 available for electrical (K⁺ efflux) versus chemical signaling (Ca²⁺ nanodomain formation, see below). This dynamic regulation underscores K_v2.1's critical role in modulating neuronal signaling and maintaining cellular homeostasis. K_v2.1-mediated ER-PM MCSs have been characterized in hippocampal, cortical, substantia nigra, and Purkinje neurons of the cerebellum (Lim et al., 2000; Kirmiz et al., 2018; Lebowitz et al., 2019; Casas et al., 2023). Given K_v2.1's extensive distribution throughout the brain, it is likely that K_v2.1 MCSs are present in most neurons and brain regions. At MCS, K_v2.1 serves dual functions: it acts as an MCS tether and recruits other ion channels to generate MCS Ca²⁺ nanodomains (Figure 3A). K_v2.1 physically and functionally interacts with several Ca²⁺-signaling proteins including Ca_v1.2 and Ca_v1.3 on the PM, and RyRs, and SERCA on ER membranes (Figure 3A, right) (Vierra et al., 2019) to create Ca²⁺ nanodomains that are essential for depolarization-induced activation of transcription factors CREB and c-Fos, and protein kinase A (PKA) signaling (Vierra et al., 2021; Vierra et al., 2023). The molecular motif that nucleates K_v2.1-mediated Ca²⁺-signaling complexes is called the Ca²⁺ channel association domain (CAD) located at K_v2.1's C-terminus. This domain is necessary for the recruitment of Ca_v1.2 to K_v2.1-containing ER-PM MCSs. Mutations or loss of function in K_v2.1 reduce channel clustering, MCS formation, Ca²⁺-signaling, PKA signaling, and transcription factor activation, and lead to hyperexcitability (Specia et al., 2014; Thiffault et al., 2015). Increases in K_v2.1 MCS formation have also been observed in neurodegenerative diseases, including Niemann-Pick type C1 (NPC1) disease (Casas et al., 2023). In NPC1 disease, the loss of the lysosomal cholesterol transporter NPC1 leads to CDK5-dependent increases in K_v2.1 MCS, which increases Ca²⁺ influx to drives Ca²⁺ entry into mitochondria and results in neuronal toxicity. Collectively, these data suggest that K_v2.1-containing ER-PM MCS play an important role in healthy neurons by facilitating compartmentalized Ca²⁺-dependent excitation-transcription coupling and PKA signaling. While excessive K_v2.1 MCS formation could be a trigger for neuronal degeneration.

Ca_v-RyR-K_{Ca} Crosstalk. The interactions between voltage-gated Ca²⁺ channels (Ca_v1.2, 1.3, 2.1), RyRs, and Ca²⁺-activated potassium channels (both I_{K_{Ca}} and B_{K_{Ca}}) forms a critical signaling axis in neurons, essential for various neurophysiological processes including learning and memory, regulation of excitability, integration of sensory inputs, and fine-tuning of motor signals (Berkefeld et al., 2006; Fakler and Adelman, 2008; Muller et al., 2010; Irie and Trussell, 2017). This Ca_v-RyR-K_{Ca} complex orchestrates Ca²⁺-dependent bi-directional control of membrane potential. The signaling cascade begins with Ca_v channels on the plasma membrane, which are activated by membrane depolarization, allowing Ca²⁺ influx into the cell. This initial Ca²⁺

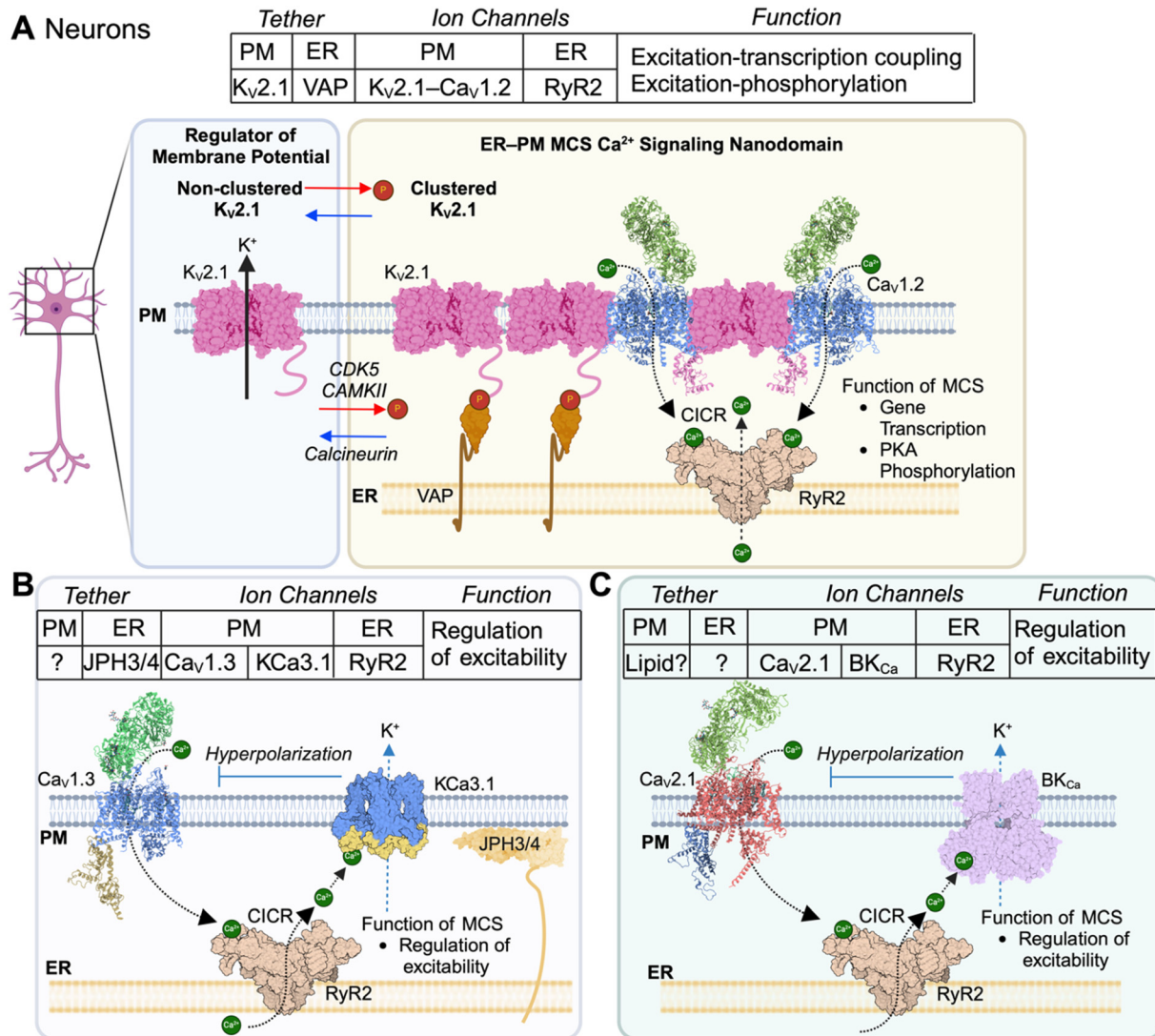


Figure 3. Ion channels in SR-PM MCS in neurons. **(A)** Left: absence of phosphorylation in K_v2.1s PCR domain results in non-clustered K_v2.1 channels that regulate PM electrical excitability. Right: phosphorylation of K_v2.1 facilitates interactions with ER VAP proteins, recruitment of Ca_v1.2 channels and RyR2, and an increase in Ca²⁺ entry into neurons leading to CICR at neuronal MCSs. **(B)** Activity-dependent influx of Ca²⁺ through Ca_v1.3 channels induces CICR from RyR2 which activates KCa3.1 and hyperpolarizes the membrane potential. **(C)** Similar mechanism as (B) only with Ca_v2.1–RyR–BKCa signaling axis.

elevation then activates RyRs positioned on the ER membrane, triggering CICR. This amplification of intracellular Ca²⁺ subsequently activates intermediate/small conductance Ca²⁺-activated K⁺ channels (Sahu et al., 2019) (IK_{Ca}3.1; Figure 3B) and large conductance Ca²⁺-activated K₊ channels (BK_{Ca}, Figure 3C). Both channel types, located on the plasma membrane, mediate K⁺ efflux upon activation, resulting in membrane hyperpolarization. This process serves as a crucial feedback mechanism to regulate neuronal excitability.

Recent studies have shed light on the structural components that enable the functional coupling within Ca_v–RyR–K_{Ca} complexes (Figure 3B). Junctophilins, particularly JPH3 and JPH4, have emerged as critical tethering components. These proteins are instrumental in generating and maintaining

the functional coupling between ion channels at this membrane contact site (MCS). Evidence for this comes from studies on hippocampal neurons from JPH3/4 double knockout animals, which exhibit a lack of apamin-sensitive currents, completely abolished after-hyperpolarizations (AHP), impaired hippocampal plasticity, and deficits in memory formation (Moriguchi et al., 2006). Further structural and functional evidence defining the key role of Ca_v–RyR–K_{Ca} complexes in regulating neuronal excitability has been obtained through super-resolution imaging, Förster Resonance Energy Transfer (FRET), and electrophysiology experiments in CA1 pyramidal cells. These studies have revealed that Ca_v1.3, RyR2, KCa3.1, and JPH3 are all located within 10–20 nm of one another, highlighting the nanodomain organization of this signaling complex (Sahu

et al., 2019). Moreover, knockdown experiments targeting JPH3 and JPH4 have demonstrated alterations in the AHP and spike frequency accommodation, further emphasizing the functional importance of these structural components.

In addition to IK_{Ca} channels, BK_{Ca} channels have been extensively studied for their functional crosstalk with Ca_V channels (Berkefeld et al., 2006; Fakler and Adelman, 2008; Muller et al., 2010; Irie and Trussell, 2017). Significant evidence supports the localization of this channel complex with RyR on the ER (Figure 3C), particularly in specialized neuronal subtypes. In cartwheel interneurons of the dorsal cochlear nucleus, triggered opening of plasma membrane $Ca_V2.1$ channels facilitates CICR from RyR on ER membranes to activate somatic BK_{Ca} channels (Irie and Trussell, 2017). This functional unit controls burst firing in the neuron population and demonstrates tight spatial and functional coupling between these channels. Further evidence for a tripartite arrangement comes from cerebellar Purkinje cells, where $Ca_V2.1$ Ca^{2+} channels and BK_{Ca} channels colocalize over somatic subsurface cisternae (Indriati et al., 2013). This colocalization indicates that $Ca_V2.1$ -RyR- BK_{Ca} triads are strategically positioned at ER-PM junctions to ensure rapid and efficient Ca^{2+} signaling to low affinity BK_{Ca} channels. While the molecular tether that anchors this complex is unknown, spatial proteomic analysis of $Ca_V2.1$ interactors revealed the presence of BK_{Ca} alongside JPH3 suggesting it may help facilitate the organization of this channel complex at ER-PM MCS.

Collectively, these findings underscore the importance of spatial organization in ion channel function and highlight the dynamic nature of ER-PM junctions in neuronal signaling. The precise arrangement of Ca_V , RyR, and BK channels within these nanodomains allows for highly localized and efficient Ca^{2+} signaling, enabling fine-tuned control of neuronal excitability. Despite the progress made in identifying some of the key ion channel and tethering proteins at MCS in neurons, there are likely many more involved in neuronal MCS formation and regulation. Advanced proteomic approaches, such as mass spectrometry and proximity labeling, could help identify novel neuronal MCS proteins. Once identified, their specific roles and interactions within MCSs need to be characterized for neurons across multiple brain regions.

Store-Operated Ca^{2+} Entry (SOCE)

The STIM1–ORAI1 complex at ER–PM MCS is the most well-characterized ion channel complex mediating store-operated calcium entry (SOCE) (for comprehensive reviews, see Majewski and Kuznicki, 2015; Wegierski and Kuznicki, 2018; Tiscione et al., 2019). This complex plays a critical role in immune cell function, particularly in T-cell activation, proliferation, and cytokine production, as well as mast cell degranulation and B cell function. While SOCE has been described in various cell types, including cardiac, skeletal, and smooth

muscles, as well as neurons, we briefly focus on its role in immune cells. James Putney first proposed SOCE in 1986 as a crucial mechanism for ER store refilling following receptor activation (Putney, 1986). In this model, depletion of intracellular Ca^{2+} stores triggers Ca^{2+} influx across the plasma membrane. The field advanced significantly with the identification of STIM1 as the ER Ca^{2+} sensor (Liou et al., 2005; Roos et al., 2005) and Orai1 as the pore-forming subunit of the Ca^{2+} release-activated Ca^{2+} (CRAC) channel in the plasma membrane (Feske et al., 2006; Vig et al., 2006). These discoveries enabled precise characterization of the localization, kinetics, regulatory mechanisms, and functional roles of this ER–PM MCS complex. SOCE activation is initiated when ER Ca^{2+} stores are depleted, typically through Gq-receptor activation of phospholipase C, leading to IP_3 -mediated release. The resulting decrease in ER Ca^{2+} reduces Ca^{2+} binding to STIM1's EF-hand domain, causing STIM1 to oligomerize and translocate to ER-plasma membrane junctions. There, it interacts with and activates Orai1 channels, allowing Ca^{2+} influx (Hogan et al., 2010). Multiple experimental approaches have demonstrated SOCE's importance in immune cell function. Patch-clamp electrophysiology studies revealed the biophysical properties of CRAC channels (Zweifach and Lewis, 1993), while Ca^{2+} imaging techniques showed sustained Ca^{2+} elevations in T cells upon antigen stimulation, later linked to SOCE (Lewis and Cahalan, 1995). Genetic approaches, including knockouts and mutations of STIM1 and Orai1, revealed their critical roles in T cell activation, proliferation, and cytokine production (Oh-hora et al., 2008). Clinical evidence further underscores SOCE's significance as patients with mutations in STIM1 or Orai1 genes exhibit severe combined immunodeficiency, demonstrating SOCE's essential role in immune function (Feske et al., 2006; McCarl et al., 2009; Feske et al., 2010). Functionally, SOCE plays a crucial role in lymphocyte activation following antigen recognition. Sustained SOCE activates the phosphatase calcineurin, which dephosphorylates NFAT (Nuclear Factor of Activated T cells). This allows NFAT to translocate to the nucleus and initiate transcription of genes crucial for T cell activation (Yazbeck et al., 2017). By mediating these diverse functions, SOCE helps coordinate and fine-tune the immune response, enabling immune cells to respond effectively to pathogens and other threats.

Endoplasmic Reticulum—Mitochondria MCS

ER—mitochondria contact sites, often referred to as mitochondria-associated membranes (MAMs), have been extensively characterized given their ubiquity and importance for cellular signaling and homeostasis. These specialized nanodomains facilitate the transfer of ions and lipids between the ER and mitochondria, impacting a range of cellular functions.

Membrane Tethers

Several membrane tethers have been implicated in forming ER–Mito MCS (Figure 4A) including: (i) Mitofusins 1/2 (Mfn): ER and mitochondrial residing low-affinity GTPase's that form homo- and hetero- complexes with Mfn1 or Mfn2, respectively, localized on the mitochondrial membrane, likely through dimerization in trans of the Heptated Repeated 2 (HR2) domains (de Brito and Scorrano, 2008; Filadi et al., 2018), (ii) Glucose-regulated Protein 75 (GRP75): a chaperone protein that links the inositol 1,4,5-trisphosphate receptor (IP₃R) on the ER to the voltage-dependent anion channel 1 (VDAC1) on the mitochondria (Szabadkai et al., 2006), and (iii) VAPB-PTPIP51: interactions between VAPB on the ER and protein tyrosine phosphatase-interacting protein 51 (PTPIP51) on mitochondria (Gomez-Suaga et al., 2017). These tethering complexes ensure efficient transfer of Ca²⁺ and lipids between ER and mitochondria which is crucial for energy metabolism, calcium buffering (both in cytoplasm and ensuring sufficient Ca²⁺ in the mitochondrial matrix), and regulation of apoptosis.

Molecular Organization of ion Channels, Receptors, and Transporters

At ER–Mito MCS multiple ion channels are located including the IP₃R and RyR on ER membranes and the voltage-dependent anion channel (VDAC) on mitochondrial membranes. IP₃R–VDAC (Rizzuto et al., 1998; Szabadkai et al., 2006; Bartok et al., 2019) and RyR–VDAC (Hajnóczky et al., 2002; Min et al., 2012) complexes are anchored through the actions of GRP75. When Ca²⁺ is released from the ER (Giacomello et al., 2010) following cytoplasmic IP₃ binding to IP₃R or when elevations in Ca²⁺ facilitate CICR from RyR, causing a Ca²⁺ increase into the micromolar range, VDAC enables Ca²⁺ entry across the outer mitochondrial membrane (Colombini, 1979; De Pinto et al., 1987; Blachly-Dyson et al., 1990). This Ca²⁺ is subsequently taken up into the mitochondrial matrix by the mitochondrial Ca²⁺ uniporter (MCU; Figure 4A). The constitutive release of IP₃R Ca²⁺ at ER–Mito MCS is essential for ATP production and suppression of autophagy, while the loss of this Ca²⁺ transfer results in decreased ATP production and activation of AMP kinase to promote autophagy (Cárdenas et al., 2010). In neurons (Wu et al., 2017), these contacts are especially critical due to the high energy demands and complex Ca²⁺ signaling required for neuronal function. For example, ER–mitochondria MCS help maintain mitochondrial health, ensuring adequate ATP supply necessary for sustained neuronal activity. Further, efficient Ca²⁺ signaling at ER–mitochondria contacts support synaptic activity and plasticity (Hirabayashi et al., 2017; Lee et al., 2018; Fowler et al., 2019; Gao et al., 2022), essential for learning and memory. Finally, proper functioning of MAMs protects neurons from Ca²⁺-induced excitotoxicity, which is implicated in neurodegenerative diseases (Toglia et al., 2016; Ludtmann and Abramov, 2018; Verma

et al., 2018; Lin et al., 2019; Calvo-Rodriguez et al., 2020; Apicco et al., 2021; Casas et al., 2023). Thus, ER–mitochondria contact sites are integral to cellular and neuronal function. Understanding the molecular intricacies of these contacts, including the roles of ion channels and tethers, is vital for unraveling their contributions to both normal physiology and disease states.

Endoplasmic Reticulum –Endo/Lysosome MCS

ER–lysosome contact sites are critical for maintaining cellular homeostasis and facilitating various signaling pathways. These contacts, enable direct communication between the ER and lysosomes, impacting ion homeostasis, lipid metabolism, and autophagy.

Member Tethers

At ER–lysosome MCS, several tethers have been identified in bringing each organelle membrane into close apposition, including: (i) VAP and ORPs (Du et al., 2011; Zhao and Ridgway; Lim et al., 2019): here, the ER-resident VAP proteins recruit through FFAT interactions different ORP proteins (i.e., OSBP, ORP1L, ORP5, ORP9, ORP10) which in turn bind to negatively charged phosphoinositide's on the lysosomal membrane to generate ER–lysosome MCS (Kutchukian et al., 2021), and (ii) Protrudin-Rab7-GTPase–PDZD8 (Raiborg et al., 2015): association of the integral ER protein protrudin and PDZD8 contact the endo/lysosomal membrane through binding to Rab7-GTP together with PI(3)P, or to only the Rab7-GTPase through the Rab-Binding Domain (RBD) (Guillén-Samander et al., 2019; Elbaz-Alon et al., 2020; Khan et al., 2021; Gao et al., 2022). Other ER-localized proteins that have been reported to also interact with endo/lysosome membranes include STARD3 (Wilhelm et al., 2017) and VPS13C (Kumar et al., 2018). The majority of current information on ER–lysosome tethers come from experiments in expression system cells, although extremely informative, there is a clear need to extend these datasets into primary cells like neurons or myocytes.

Molecular Organization of ion Channels, Receptors, and Transporters

At ER-lysosomal MCS multiple ion channels and receptors have been identified including the transient receptor potential mucolipin 1 (Dong et al., 2008; Dong et al., 2010) (TRPML1) and two-pore channels (TPC1 and TPC2 Cang et al., 2014; Wang et al., 2012) on the lysosomal membrane and IP₃R and RyR on ER membranes (Atakpa et al., 2018). On the ER side of the MCS, it has been reported that endogenous IP₃R form small clusters within ER membranes with the majority of IP₃R clusters within the ER being mobile (~75%) however it is a small fraction of immobile IP₃R clusters that are responsible for Ca²⁺ signals (called Ca²⁺ “puffs”) (Thillaiappan et al.,

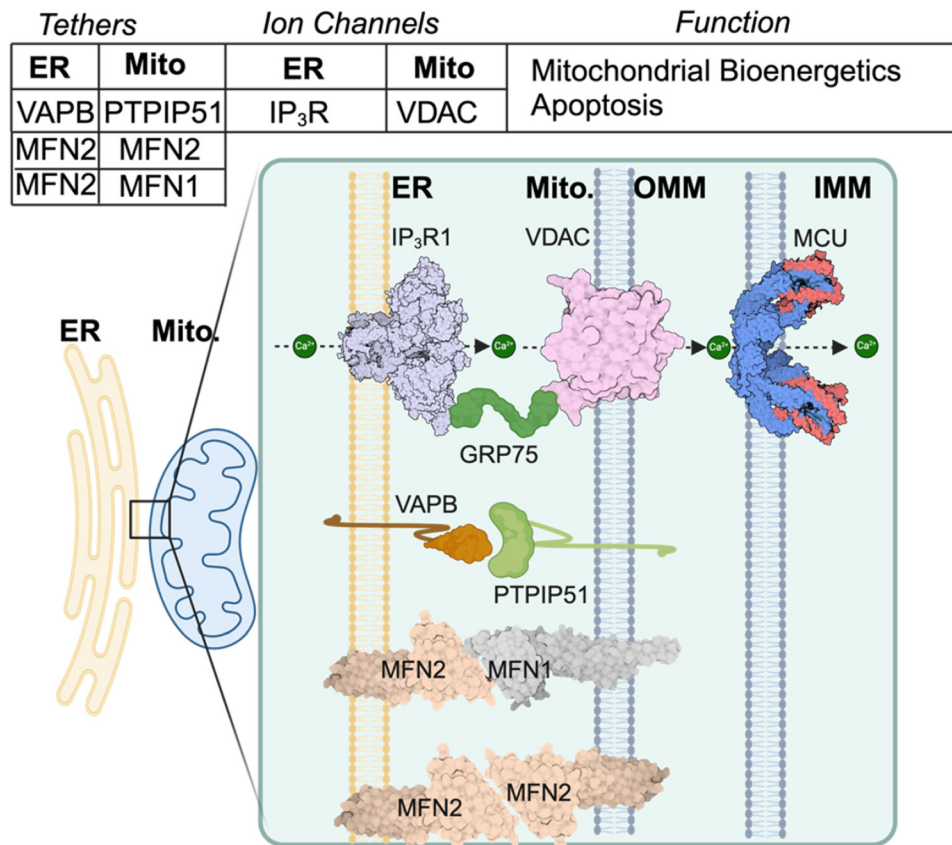


Figure 4. Ion channels in ER–mitochondrial MCS. Tethers and ion channels present at ER–mitochondrial MCS influence mitochondrial Ca^{2+} levels to regulate bioenergetics and apoptosis.

2017). At ER–lysosome MCS IP_3Rs are not required for MCS formation but rather selectively deliver large Ca^{2+} signals to lysosomes (Atakpa et al., 2018). On the Endosome site of the MCS, TPC1 localizes to ER–endosome contact sites and appear essential for MCS formation as knocking down its expression, or reducing its ligand NAADP reduces the formation of these contact sites (Kilpatrick et al., 2017). At ER–endosome contacts release of Ca^{2+} from IP_3R on ER membranes has been reported to amplify TPC Ca^{2+} release (Ruas et al., 2010). While there is evidence that stimulating Ca^{2+} release from acidic endocytic vesicles can stimulate ER Ca^{2+} mobilization and vice versa (Lopez-Sanjurjo et al., 2013; Morgan et al., 2013), more investigations are required to disentangle the substantial mechanisms of crosstalk at ER–endosome MCS and how they influence organelle function and position within cells. In addition to TPC1 , endo/lysosome membranes also have TRPML1 Ca^{2+} release channels. TRPML1 Ca^{2+} release is important for regulating a host of different pathways including: (i) Ca^{2+} dependent activation of the protein phosphatase, calcineurin, which dephosphorylates the transcription factor TFEB, allowing it to enter the nucleus and switch on the expression of autophagy regulating proteins (Medina et al., 2015). An additional layer of regulation of TRPML1 complex comes in the

form of the endo/lysosomal phosphoinositide, $\text{PI}(3,5)\text{P}_2$, which acts as a positive cofactor for channel function (Dong et al., 2010; Zhang et al., 2012). Loss of function of TRPML1 function is associated with mucopolipidosis type IV (Dong et al., 2008). While the specific localization of TRPML1 at ER–endosome contacts is open for debate, it is worth noting that activation of TRPML1 channels reduces cholesterol accumulation in NPC1 disease (Shen et al., 2012). Given that cholesterol egress at lysosomes occurs at MCS presents good evidence that TRPML1 may also be present at these contacts. While many ion channels have been detailed to be important and regulated at ER–Lysosome MCSs, further research is needed to better understand how these complexes collaborate to shape organelle function and how lipid transport at ER–lysosomal contacts interfaces with these Ca^{2+} signaling nanodomains. Understanding these interactions is crucial for elucidating the roles of ER–lysosome contact sites in both normal physiology and disease states.

Non-ER MCS

The majority of MCS have been identified as being between the ER and another closely apposed organelle. That said,

other non-ER MCS have been identified. It should be noted that given the importance of Ca^{2+} signaling for MCS, the extensive and dynamic nature of the ER, and reports of 3-way MCSs (Wang et al., 2016), it seems that the ER could very well also be closely apposed and add a further layer of regulation to these MCS.

Mitochondria-Lysosome

Dynamic contacts between mitochondria and lysosomes have been reported as key regulators of mitochondrial positioning, dynamics and fission (Aston et al., 2017; Valm et al., 2017; Wong et al., 2018). The molecular tether facilitating contact between these two organelles appears to at least involve the small guanosine triphosphatase (GTPase), RAB7 (Wong et al., 2018). In the GTP-bound form, lysosomal RAB7-GTP promotes MCS formation with mitochondria which positions lysosomal TRPML1 and mitochondrial VDAC1 to coordinate to transfer Ca^{2+} into mitochondria (Peng et al., 2020). Loss of function or disease mutations in TRPML1 decrease lysosome-mitochondrial Ca^{2+} transfer (Peng et al., 2020). The mechanism that facilitates interaction between RAB7-GTP and mitochondria to enable Ca^{2+} transfer has yet to be fully elucidated. The uncoupling of lysosomes and mitochondria MCS is regulated by the mitochondrial protein FIS1 which recruits the GTPase-activating protein (GAP) TBC1D15 to hydrolyze RAB7-GTP and release the contacts. Formation of mitochondrial-lysosome MCS spatially organizes lysosomal TRPML1 and mitochondrial VDAC1 to coordinate to transfer Ca^{2+} into mitochondria (Peng et al., 2020). Functionally, these contacts appear to mark sites of mitochondrial fission, allowing regulation of mitochondrial networks by lysosomes. The role of TRPML and VDAC in mitochondrial division, or bioenergetics requires further investigation.

Plasma Membrane—Mitochondrial Contacts

Mitochondria can also form associations with the plasma membrane, called plasma membrane-associated mitochondria (PAM) (Kakizawa et al., 2007; Giacomello et al., 2010; Suski et al., 2014; Montes de Oca Balderas, 2021). In mammalian cells, the complete molecular identity of the tethering complex responsible for ER-mitochondria association remains elusive. In contrast, work in yeast has identified the Num1/Mdm36 complex as a key player in this process (Lackner et al., 2013). While the exact spatial arrangement of ion channels and transporters at these contact sites in mammalian cells is not fully characterized, the strategic positioning of mitochondrial Ca^{2+} channels near other Ca^{2+} handling proteins would facilitate localized Ca^{2+} signaling and regulation of cellular Ca^{2+} dynamics. Further research is needed to elucidate the precise molecular architecture and functional implications of these putative arrangements.

Discussion and Future Directions

Over the past 70 years, our understanding of ion channel and transporter activity at MCSs has advanced significantly. We now possess considerable knowledge about the tethering proteins that establish these contacts, the lipid and Ca^{2+} handling proteins within them, and their critical importance for cellular homeostasis. However, numerous questions remain in this rapidly evolving field of cell biology. Here, we outline key areas for future research:

Identification and Characterization of Molecular Tethers

While progress has been made in identifying key MCS-forming proteins, many more likely remain undiscovered. Advanced proteomic approaches, such as quantitative mass spectrometry and proximity labeling techniques (e.g., BioID, APEX), will be crucial in identifying novel MCS proteins. Once identified, their specific roles, interactions, and regulatory mechanisms within MCSs across different cell types need to be elucidated. This includes investigating their binding partners, post-translational modifications, and how they respond to various cellular stimuli. Uncovering and characterizing novel member tethering proteins will undoubtedly expand our understanding of ion channels within MCSs.

Mechanisms of Protein Recruitment and Localization

A critical question is how proteins, particularly ion channels, are recruited to specific MCSs across distinctive cellular subdomains (e.g., soma, dendrites, synaptic regions). What molecular signals govern the recruitment of ion channels to MCSs, and how is this process regulated? Insights from lipid transport at MCSs suggest the need for recruiting signals at each membrane, such as VAPs on ER membranes and complementary lipid or protein effectors on the apposing membrane. This mechanism may be conserved for ion channels, as exemplified by $\text{K}_v2.1$ -VAP interactions at somatic ER-PM junctions and IP_3R -GRP75-VDAC complexes at ER-mitochondria contacts. Future studies should focus on identifying these recruiting signals and elucidating their regulatory pathways.

Detailed Analysis of MCS Proteins Across Cell Types

Most studies identifying MCS proteins have been conducted in immortalized cell lines like HEK293 and HeLa. While this work is invaluable, it is evident that not all MCS proteins are present in primary human cells, especially those with specialized functions such as neurons or myocytes. Detailed characterization of MCS organization and function in primary cells is essential, as differences in cellular architecture and function suggest that the repertoire and roles of MCS might vary significantly across cell types. This research should

employ a combination of proteomics, high-resolution imaging, and functional assays to provide a comprehensive understanding of cell type-specific MCS composition and function.

Structural and Functional Plasticity of MCSs

Further high-resolution structural analysis, such as cryo-electron microscopy (cryo-EM), super-resolution imaging techniques (e.g., STORM, PALM), or correlative light and electron microscopy (CLEM) is essential to characterize MCSs at the molecular level. These approaches will help elucidate how dynamic interactions between different proteins change in response to post-translational modifications (e.g., phosphorylation, ubiquitination) and cellular perturbations.

Impact of MCSs on Physiology and Pathophysiology

Exploring how different MCSs integrate their functions to contribute to cellular signaling will enable a more comprehensive understanding of their physiological roles. This includes investigating how MCS coordinate responses to various stimuli, such as changes in metabolic state or cellular stress. Additionally, studying how disruptions in MCS proteins contribute to pathophysiological conditions, such as neurodegenerative disorders or metabolic diseases, could provide insights into disease mechanisms and potential therapeutic targets.

Role of Lipid Composition in MCS Formation and Function

Investigating how the lipid composition of apposing membranes within an MCS influences its formation and function is crucial. Given that common membrane tethers form both lipid and Ca^{2+} -signaling nanodomains at MCSs, and ion channels are regulated by lipids (Hille et al., 2015) means these two instructional signals may be intimately related and shape each other's transfer abilities. Key questions include: could elevations in Ca^{2+} at MCSs drive or halt lipid signaling? Conversely, could lipid transfer at MCSs enhance or diminish the release of Ca^{2+} into the MCS space? Understanding the interplay between these two exchange processes is important for comprehending how MCS signaling may be integrated and controlled. Future studies should employ lipidomics approaches, coupled with Ca^{2+} imaging and functional assays, to dissect the relationship between lipid composition and calcium signaling at MCS.

Longitudinal Mapping of MCS Dynamics

Conducting longitudinal studies to monitor changes in MCS during development, aging, and in response to various physiological and pathological stimuli will provide insights into their roles over time. This could involve the use of long-term live-cell imaging techniques, coupled with genetic

manipulations, to track MCS formation, dissolution, and remodeling in various cellular contexts. For example, studying how MCSs between the ER and PM change during neuronal development and synaptic plasticity could reveal new insights into the role of these structures in brain function and cognition.

With the development of new technologies, such as improved super-resolution microscopy techniques, more sensitive mass spectrometry approaches, and advanced genetic tools for manipulating MCS proteins, researchers can gain a more comprehensive understanding of the molecular underpinnings of MCSs in different cell types, their physiological significance, and their potential as targets for therapeutic intervention in disease. By addressing these key areas of research, we can expect significant advances in our understanding of MCS biology and its implications for human health and disease in the coming years

Conclusion

The study of MCS and their associated ion channels, transporters, and receptors has significantly advanced our understanding of cellular organization and function. These specialized regions facilitate critical processes such as Ca^{2+} signaling, lipid transfer, and organelle communication. The intricate interplay between various ion channels at MCSs, particularly those involving the ER, highlights the complexity and importance of these structures in maintaining cellular homeostasis and responding to diverse stimuli. The field of MCS, particularly concerning tethers and ion channels at these sites, represents a vibrant and rapidly evolving area of cell biology. Continued research in this field promises to yield valuable insights into fundamental cellular processes and opportunities to treat a wide range of diseases.

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References

- Abd El-Rahman RR, Harraz OF, Brett SE, Anfinogenova Y, Mufti RE, Goldman D, Welsh DG (2013). Identification of L- and T-type Ca²⁺ channels in rat cerebral arteries: Role in myogenic tone development. *Am J Physiol Heart Circ Physiol* *304*, H58–H71. doi: 10.1152/ajpheart.00476.2012
- Aherrahrou Z, Schlossarek S, Stoelting S, Klinger M, Geertz B, Weinberger F, Kessler T, Aherrahrou R, Moreth K, Bekeredjian R, et al. (2016). Knock-out of nexilin in mice leads to dilated cardiomyopathy and endomyocardial fibroelastosis. *Basic Res Cardiol* *111*, 6. doi: 10.1007/s00395-015-0522-5.
- Al-Qusairi L, Laporte J (2011). T-tubule biogenesis and triad formation in skeletal muscle and implication in human diseases. *Skeletal Muscle* *1*, 26. doi: 10.1186/2044-5040-1-26
- Alzheimer's Association Calcium Hypothesis Workgroup and Khachaturian, Z.S (2017). Calcium Hypothesis of Alzheimer's disease and brain aging: A framework for integrating new evidence into a comprehensive theory of pathogenesis. *Alzheimer's Dementia: J Alzheimer's Assoc* *13*, 178–182 e117. doi: 10.1016/j.jalz.2016.12.006
- Apicco DJ, Shlevkov E, Nezich CL, Tran DT, Guilmette E, Nicholatos JW, Bantle CM, Chen Y, Glajch KE, Abraham NA, et al. (2021). The Parkinson's disease-associated gene ITPKB protects against α -synuclein aggregation by regulating ER-to-mitochondria calcium release. *Proc Natl Acad Sci* *118*, e2006476118. doi: 10.1073/pnas.2006476118
- Aston D, Capel RA, Ford KL, Christian HC, Mirams GR, Rog-Zielinska EA, Kohl P, Galione A, Burton RAB, Terrar DA (2017). High resolution structural evidence suggests the Sarcoplasmic Reticulum forms microdomains with Acidic Stores (lysosomes) in the heart. *Sci Rep* *7*, 40620. doi: 10.1038/srep40620
- Atakpa P, Thillaiappan NB, Mataragka S, Prole DL, Taylor CW (2018). IP₃ Receptors preferentially associate with ER-lysosome contact sites and selectively deliver Ca(2+) to lysosomes. *Cell Rep* *25*, 3180–3193.e3187. doi: 10.1016/j.celrep.2018.11.064
- Baddeley D, Jayasinghe I, Lam L, Rossberger S, Cannell MB, Soeller C (2009). Optical single-channel resolution imaging of the ryanodine receptor distribution in rat cardiac myocytes. *Proc Natl Acad Sci* *106*, 22275–22280. doi: 10.1073/pnas.0908971106
- Bandell M, Dubin AE, Petrus MJ, Orth A, Mathur J, Hwang SW, Patapoutian A (2006). High-throughput random mutagenesis screen reveals TRPM8 residues specifically required for activation by menthol. *Nat Neurosci* *9*, 493–500. doi: 10.1038/nn1665
- Bartok A, Weaver D, Golenár T, Nichtova Z, Katona M, Bánsághi S, Alzayady KJ, Thomas VK, Ando H, Mikoshiba K, et al. (2019). IP₃ Receptor isoforms differently regulate ER-mitochondrial contacts and local calcium transfer. *Nat Commun* *10*, 3726. doi: 10.1038/s41467-019-11646-3
- Berkefeld H, Sailer CA, Bildl W, Rohde V, Thumfart JO, Eble S, Klugbauer N, Reisinger E, Bischofberger J, Oliver D, et al. (2006). BKCa-Cav channel complexes mediate rapid and localized Ca²⁺-activated K⁺ signaling. *Science* *314*, 615–620. doi: 10.1126/science.1132915
- Berridge MJ (1998). Neuronal calcium signaling. *Neuron* *21*, 13–26. doi: 10.1016/S0896-6273(00)80510-3
- Berridge MJ (2008). Smooth muscle cell calcium activation mechanisms. *J Physiol* *586*, 5047–5061. doi: 10.1113/jphysiol.2008.160440
- Bers DM (2002). Cardiac excitation-contraction coupling. *Nature* *415*, 198–205. doi: 10.1038/415198a
- Bers DM, Guo T (2005). Calcium signaling in cardiac ventricular myocytes. *Ann NY Acad Sci* *1047*, 86–98. doi: 10.1196/annals.1341.008
- Bezprozvanny IB (2010). Calcium signaling and neurodegeneration. *Acta Naturae* *2*, 72–82. doi: 10.32607/20758251-2010-2-1-72-80
- Blachly-Dyson E, Peng S, Colombini M, Forte M (1990). Selectivity changes in site-directed mutants of the VDAC ion channel: Structural implications. *Science* *247*, 1233–1236. doi: 10.1126/science.1690454
- Block BA, Imagawa T, Campbell KP, Franzini-Armstrong C (1988). Structural evidence for direct interaction between the molecular components of the transverse tubule/sarcoplasmic reticulum junction in skeletal muscle. *J Cell Biol* *107*, 2587–2600. doi: 10.1083/jcb.107.6.2587
- Brini M, Cali T, Ottolini D, Carafoli E (2014). Neuronal calcium signaling: Function and dysfunction. *Cell Mol Life Sci* *71*, 2787–2814. doi: 10.1007/s00018-013-1550-7
- Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG (2016). Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. *Pharmacol Rev* *68*, 476–532. doi: 10.1124/pr.115.010652
- Caldwell JL, Smith CE, Taylor RF, Kitmitto A, Eisner DA, Dibb KM, Trafford AW (2014). Dependence of cardiac transverse tubules on the BAR domain protein amphiphysin II (BIN-1). *Circ Res* *115*, 986–996. doi: 10.1161/circresaha.116.303448
- Calvo-Rodriguez M, Hou SS, Snyder AC, Kharitonova EK, Russ AN, Das S, Fan Z, Muzikansky A, Garcia-Allojo M, Serrano-Pozo A, et al. (2020). Increased mitochondrial calcium levels associated with neuronal death in a mouse model of Alzheimer's disease. *Nat Commun* *11*, 2146. doi: 10.1038/s41467-020-16074-2
- Cang C, Bekele B, Ren D (2014). The voltage-gated sodium channel TPC1 confers endolysosomal excitability. *Nat Chem Biol* *10*, 463–469. doi: 10.1038/nchembio.1522
- Cárdenas C, Miller RA, Smith I, Bui T, Molgó J, Müller M, Vais H, Cheung KH, Yang J, Parker I, et al. (2010). Essential regulation of cell bioenergetics by constitutive InsP₃ receptor Ca²⁺ transfer to mitochondria. *Cell* *142*, 270–283. doi: 10.1016/j.cell.2010.06.007
- Casas M, Murray KD, Hino K, Vierra NC, Simó S, Trimmer JS, Dixon RE, Dickson EJ (2023). NPC1-dependent Alterations in K(V)2.1-Ca(V)1.2 nanodomains drive neuronal death in models of Niemann-Pick Type C disease. *Nat Commun* *14*, 4553. doi: 10.1038/s41467-023-39937-w
- Catterall WA (2000). Structure and regulation of voltage-gated Ca²⁺ channels. *Annu Rev Cell Dev Biol* *16*, 521–555. doi: 10.1146/annurev.cellbio.16.1.521.
- Cerda O, Trimmer JS (2011). Activity-dependent phosphorylation of neuronal Kv2.1 potassium channels by CDK5. *J Biol Chem* *286*, 28738–28748. doi: 10.1074/jbc.M111.251942
- Cheng H, Lederer WJ, Cannell MB (1993). Calcium sparks: Elementary events underlying excitation-contraction coupling

- in heart muscle. *Science* 262, 740–744. doi: 10.1126/science.8235594
- Chung J, Torta F, Masai K, Lucast L, Czapla H, Tanner LB (2015). PI4P/phosphatidylserine countertransport at ORP5- and ORP8-mediated ER-plasma membrane contacts. *Science* 349, 428–432. doi: 10.1126/science.aab1370
- Clapham DE (2007). Calcium signaling. *Cell* 131, 1047–1058. doi: 10.1016/j.cell.2007.11.028
- Colombini M (1979). A candidate for the permeability pathway of the outer mitochondrial membrane. *Nature* 279, 643–645. doi: 10.1038/279643a0
- Danila CI, Hamilton SL (2004). Phosphorylation of ryanodine receptors. *Biol Res* 37, 521–525. doi: 10.4067/s0716-97602004000400005
- de Brito OM, Scorrano L (2008). Mitofusin 2 tethers endoplasmic reticulum to mitochondria. *Nature* 456, 605–610. doi: 10.1038/nature07534
- De Pinto V, Ludwig O, Krause J, Benz R, Palmieri F (1987). Porin pores of mitochondrial outer membranes from high and low eukaryotic cells: Biochemical and biophysical characterization. *Biochim Biophys Acta* 894, 109–119. doi: 10.1016/0005-2728(87)90180-0
- Dirksen RT, Beam KG (1999). Role of calcium permeation in dihydropyridine receptor function. Insights into channel gating and excitation-contraction coupling. *J Gen Physiol* 114, 393–403. doi: 10.1085/jgp.114.3.393
- Dixon RE, Moreno CM, Yuan C, Opitz-Araya X, Binder MD, Navedo MF, Santana LF (2015). Graded Ca^{2+} /calmodulin-dependent coupling of voltage-gated $CaV1.2$ channels. *Elife* 4. doi: 10.7554/eLife.05608
- Dixon RE, Navedo MF, Binder MD, Santana LF (2022). Mechanisms and physiological implications of cooperative gating of clustered ion channels. *Physiol Rev* 102, 1159–1210. doi: 10.1152/physrev.00022.2021
- Dixon RE, Yuan C, Cheng EP, Navedo MF, Santana LF (2012). Ca^{2+} signaling amplification by oligomerization of L-type $CaV1.2$ channels. *Proc Natl Acad Sci U S A* 109, 1749–1754. doi: 10.1073/pnas.1116731109
- Dong XP, Cheng X, Mills E, Delling M, Wang F, Kurz T, Xu H (2008). The type IV mucopolidosis-associated protein TRPML1 is an endolysosomal iron release channel. *Nature* 455, 992–996. doi: 10.1038/nature07311
- Dong XP, Shen D, Wang X, Dawson T, Li X, Zhang Q, Cheng X, Zhang Y, Weisman LS, Delling M, Xu H (2010). $PI(3,5)P_2$ controls membrane trafficking by direct activation of mucolipin Ca^{2+} release channels in the endolysosome. *Nat Commun* 1, 38. doi: 10.1038/ncomms1037
- Du J, Haak LL, Phillips-Tansey E, Russell JT, McBain CJ (2000). Frequency-dependent regulation of rat hippocampal somatodendritic excitability by the K^+ channel subunit $Kv2.1$. *J Physiol* 522(Pt 1), 19–31. doi: 10.1111/j.1469-7793.2000.t012-00019.xm
- Du X, Kumar J, Ferguson C, Schulz TA, Ong YS, Hong W, Prinz WA, Parton RG, Brown AJ, Yang H (2011). A role for oxysterol-binding protein-related protein 5 in endosomal cholesterol trafficking. *J Cell Biol* 192, 121–135. doi: 10.1083/jcb.201004142
- Eden ER (2016). The formation and function of ER-endosome membrane contact sites. *Biochim Biophys Acta* 1861, 874–879. doi: 10.1016/j.bbali.2016.01.020
- Elbaz-Alon Y, Guo Y, Segev N, Harel M, Quinell DE, Geiger T, Avinoam O, Li D, Nunnari J (2020). PDZD8 Interacts with Protrudin and Rab7 at ER-late endosome membrane contact sites associated with mitochondria. *Nat Commun* 11, 3645. doi: 10.1038/s41467-020-17451-7
- Essin K, Welling A, Hofmann F, Luft FC, Gollasch M, Moosmang S (2007). Indirect coupling between $CaV1.2$ channels and ryanodine receptors to generate Ca^{2+} sparks in murine arterial smooth muscle cells. *J Physiol* 584, 205–219. doi: 10.1113/jphysiol.2007.138982
- Fakler B, Adelman JP (2008). Control of $K(Ca)$ channels by calcium nano/microdomains. *Neuron* 59, 873–881. doi: 10.1016/j.neuron.2008.09.001
- Feske S, Gwack Y, Prakriya M, Srikanth S, Puppel SH, Tanasa B, Hogan PG, Lewis RS, Daly M, Rao A (2006). A mutation in $ORAI1$ causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179–185. doi: 10.1038/nature04702
- Feske S, Picard C, Fischer A (2010). Immunodeficiency due to mutations in $ORAI1$ and $STIM1$. *Clin Immunol* 135, 169–182. doi: 10.1016/j.clim.2010.01.011
- Filadi R, Pendin D, Pizzo P (2018). Mitofusin 2: From functions to disease. *Cell Death Dis* 9, 330. doi: 10.1038/s41419-017-0023-6
- Fowler PC, Garcia-Pardo ME, Simpson JC, O’Sullivan NC (2019). Neurodegeneration: The central role for ER contacts in neuronal function and axonopathy, lessons from hereditary spastic paraplegias and related diseases. *Front Neurosci* 13, 1051. doi: 10.3389/fnins.2019.01051
- Franzini-Armstrong C (1970). Studies of the triad: I. Structure of the junction in frog twitch fibers. *J Cell Biol* 47, 488–499. doi: 10.1083/jcb.47.2.488
- Franzini-Armstrong C, Protasi F (1997). Ryanodine receptors of striated muscles: A complex channel capable of multiple interactions. *Physiol Rev* 77, 699–729. doi: 10.1152/physrev.1997.77.3.699
- Franzini-Armstrong C, Protasi F, Ramesh V (1999). Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles. *Biophys J* 77, 1528–1539. doi: 10.1016/S0006-3495(99)77000-1
- Fu Y, Shaw SA, Naami R, Vuong CL, Basheer WA, Guo X, Hong T (2016). Isoproterenol promotes rapid ryanodine receptor movement to bridging integrator 1 (BIN1)-organized dyads. *Circulation* 133, 388–397. doi: 10.1161/circulationaha.115.018535
- Gao Y, Xiong J, Chu QZ, Ji WK (2022). PDZD8-mediated lipid transfer at contacts between the ER and late endosomes/lysosomes is required for neurite outgrowth. *J Cell Sci* 135. doi: 10.1242/jcs.255026
- Geeves MA, Holmes KC (1999). Structural mechanism of muscle contraction. *Annu Rev Biochem* 68, 687–728. doi: 10.1146/annurev.biochem.68.1.687
- Ghosh A, Greenberg M (1995). Calcium signaling in neurons: Molecular mechanisms and cellular consequences. *Science* 268, 239–247. doi: 10.1126/science.7716515
- Giacomello M, Drago I, Bortolozzi M, Scorzeto M, Gianelle A, Pizzo P, Pozzan T (2010). Ca^{2+} hot spots on the mitochondrial surface are generated by Ca^{2+} mobilization from stores, but not by activation of store-operated Ca^{2+} channels. *Mol Cell* 38, 280–290. doi: 10.1016/j.molcel.2010.04.003
- Giordano F, Saheki Y, Idevall-Hagren O, Colombo SF, Pirruccello M, Milosevic I, Gracheva EO, Bagriantsev SN, Borgese N, De Camilli P (2013). $PI(4,5)P_2$ -dependent and Ca^{2+} -regulated

- ER-PM interactions mediated by the extended synaptotagmins. *Cell* 153, 1494–1509. doi: 10.1016/j.cell.2013.05.026
- Gomez-Suaga P, Paillusson S, Stoica R, Noble W, Hanger DP, Miller CCJ (2017). The ER-mitochondria tethering complex VAPB-PTPIP51 regulates autophagy. *Curr Biol* 27, 371–385. doi: 10.1016/j.cub.2016.12.038
- Gomez AM, Guatimosim S, Dilly KW, Vassort G, Lederer WJ (2001). Heart failure after myocardial infarction: Altered excitation-contraction coupling. *Circulation* 104, 688–693. doi: 10.1161/hc3201.092285
- Gonzales AL, Amberg GC, Earley S (2010). Ca²⁺ release from the sarcoplasmic reticulum is required for sustained TRPM4 activity in cerebral artery smooth muscle cells. *Am J Physiol Cell Physiol* 299, C279–C288. doi: 10.1152/ajpcell.00550.2009
- Gonzalez-Serratos H, Valle-Aguilera R, Lathrop DA, Garcia MC (1982). Slow inward calcium currents have no obvious role in muscle excitation-contraction coupling. *Nature* 298, 292–294. doi: 10.1038/298292a0
- Gordon AM, Homsher E, Regnier M (2000). Regulation of contraction in striated muscle. *Physiol Rev* 80, 853–924. doi: 10.1152/physrev.2000.80.2.853
- Gross P, Johnson J, Romero CM, Eaton DM, Poulet C, Sanchez-Alonso J, Lucarelli C, Ross J, Gibb AA, Garbincius JF, et al. (2021). Interaction of the joining region in junctophilin-2 with the L-type Ca(2+) channel is pivotal for cardiac dyad assembly and intracellular Ca(2+) dynamics. *Circ Res* 128, 92–114. doi: 10.1161/circresaha.119.315715
- Guillén-Samander A, Bian X, De Camilli P (2019). PDZD8 Mediates a Rab7-dependent interaction of the ER with late endosomes and lysosomes. *Proc Natl Acad Sci U S A* 116, 22619–22623. doi: 10.1073/pnas.1913509116
- Guillén-Samander A, De Camilli P (2022). Endoplasmic reticulum membrane contact sites, lipid transport, and neurodegeneration. *Cold Spring Harb Perspect Biol* 15, a041257. doi: 10.1101/cshperspect.a041257
- Guo Y, Li D, Zhang S, Yang Y, Liu JJ, Wang X, Liu C, Milkie DE, Moore RP, Tulu US, et al. (2018). Visualizing intracellular organelle and cytoskeletal interactions at nanoscale resolution on millisecond timescales. *Cell* 175, 1430–1442.e1417. doi: 10.1016/j.cell.2018.09.057
- Hajnoczky G, Csordás G, Yi M (2002). Old players in a new role: Mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria. *Cell Calcium* 32, 363–377. doi: 10.1016/S0143416002001872
- Hassel D, Dahme T, Erdmann J, Meder B, Hüge A, Stoll M, Just S, Hess A, Ehlermann P, Weichenhan D, et al. (2009). Nexilin mutations destabilize cardiac Z-disks and lead to dilated cardiomyopathy. *Nat Med* 15, 1281–1288. doi: 10.1038/nm.2037
- Hille B, Dickson EJ, Kruse M, Vivas O, Suh B-C (2015). Phosphoinositides regulate ion channels. *Biochim Biophys Acta-Mol Cell Biol Lipids* 1851, 844–856. doi: 10.1016/j.bbalip.2014.09.010
- Hirabayashi Y, Kwon S-K, Paek H, Pernice WM, Paul MA, Lee J, Erfani P, Raczkowski A, Petrey DS, Pon LA, Polleux F (2017). ER-mitochondria tethering by PDZD8 regulates Ca²⁺ dynamics in mammalian neurons. *Science* 358, 623–630. doi: 10.1126/science.aan6009
- Hogan PG, Lewis RS, Rao A (2010). Molecular basis of calcium signaling in lymphocytes: STIM and ORAI. *Annu Rev Immunol* 28, 491–533. doi: 10.1146/annurev.immunol.021908.132550
- Hong TT, Smyth JW, Chu KY, Vogan JM, Fong TS, Jensen BC, Fang K, Halushka MK, Russell SD, Colecraft H, et al. (2012). BIN1 Is reduced and Cav1.2 trafficking is impaired in human failing cardiomyocytes. *Heart Rhythm: Off J Heart Rhythm Soc* 9, 812–820. doi: 10.1016/j.hrthm.2011.11.055
- Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, Fong TS, Jensen BC, Colecraft HM, Shaw RM (2010). BIN1 Localizes the L-type calcium channel to cardiac T-tubules. *PLoS Biol* 8, e1000312. doi: 10.1371/journal.pbio.1000312
- Indriati DW, Kamasawa N, Matsui K, Meredith AL, Watanabe M, Shigemoto R (2013). Quantitative localization of Cav2.1 (P/Q-type) voltage-dependent calcium channels in Purkinje cells: Somatodendritic gradient and distinct somatic coclustering with calcium-activated potassium channels. *J Neurosci* 33, 3668–3678. doi: 10.1523/jneurosci.2921-12.2013
- Irie T, Trussell LO (2017). Double-Nanodomain coupling of calcium channels, ryanodine receptors, and BK channels controls the generation of burst firing. *Neuron* 96, 856–870.e854. doi: 10.1016/j.neuron.2017.10.014
- Ito DW, Hannigan KI, Ghosh D, Xu B, Villar SGD, Xiang YK, Dickson EJ, Navedo MF, Dixon RE (2019). beta-adrenergic-mediated dynamic augmentation of sarcolemmal CaV 1.2 clustering and cooperativity in ventricular myocytes. *J Physiol* 597, 2139–2162. doi: 10.1113/jp277283
- Ito K, Komazaki S, Sasamoto K, Yoshida M, Nishi M, Kitamura K, Takeshima H (2001). Deficiency of triad junction and contraction in mutant skeletal muscle lacking junctophilin type 1. *J Cell Biol* 154, 1059–1067. doi: 10.1083/jcb.200105040
- Johnson B, Leek AN, Solé L, Maverick EE, Levine TP, Tamkun MM (2018). Kv2 potassium channels form endoplasmic reticulum/plasma membrane junctions via interaction with VAPA and VAPB. *Proc Natl Acad Sci U S A* 115, E7331–E7340. doi: 10.1073/pnas.1805757115
- Kakizawa S, Kishimoto Y, Hashimoto K, Miyazaki T, Furutani K, Shimizu H, Fukaya M, Nishi M, Sakagami H, Ikeda A, et al. (2007). Junctophilin-mediated channel crosstalk essential for cerebellar synaptic plasticity. *EMBO J* 26, 1924–1933. doi: 10.1038/sj.emboj.7601639
- Kawamoto EM, Vivar C, Camandola S (2012). Physiology and pathology of calcium signaling in the brain. *Front Pharmacol* 3, 61–61. doi: 10.3389/fphar.2012.00061
- Khan H, Chen L, Tan L, Im YJ (2021). Structural basis of human PDZD8–Rab7 interaction for the ER-late endosome tethering. *Sci Rep* 11, 18859. doi: 10.1038/s41598-021-98419-5
- Kilpatrick BS, Eden ER, Hockey LN, Yates E, Futter CE, Patel S (2017). An endosomal NAADP-sensitive two-pore Ca²⁺ channel regulates ER-endosome membrane contact sites to control growth factor signaling. *Cell Rep* 18, 1636–1645. doi: 10.1016/j.celrep.2017.01.052
- Kirmiz M, Vierra NC, Palacio S, Trimmer JS (2018). Identification of VAPA and VAPB as Kv2 channel-interacting proteins defining endoplasmic Reticulum-plasma membrane junctions in mammalian brain neurons. *J Neurosci* 38, 7562–7584. doi: 10.1523/jneurosci.0893-18.2018
- Komazaki S, Ito K, Takeshima H, Nakamura H (2002). Deficiency of triad formation in developing skeletal muscle cells lacking junctophilin type 1. *FEBS Lett* 524, 225–229. doi: 10.1016/S0014-5793(02)03042-9
- Komazaki S, Nishi M, Kangawa K, Takeshima H (1999). Immunolocalization of mitsugumin29 in developing skeletal

- muscle and effects of the protein expressed in amphibian embryonic cells. *Dev Dyn* 215, 87–95. doi: 10.1002/(sici)1097-0177(199906)215:2%3C87::Aid-dvdy1%3E3.0.Co;2-y
- Kumar N, Leonzino M, Hancock-Cerutti W, Horenkamp FA, Li P, Lees JA, Wheeler H, Reinisch KM, De Camilli P (2018). VPS13A And VPS13C are lipid transport proteins differentially localized at ER contact sites. *J Cell Biol* 217, 3625–3639. doi: 10.1083/jcb.201807019
- Kuo IY, Ellis A, Seymour VA, Sandow SL, Hill CE (2010). Dihydropyridine-insensitive calcium currents contribute to function of small cerebral arteries. *J Cereb Blood Flow Metab* 30, 1226–1239. doi: 10.1038/jcbfm.2010.11
- Kutchukian C, Vivas O, Casas M, Jones JG, Tiscione SA, Simó S, Ory DS, Dixon RE, Dickson EJ (2021). NPC1 Regulates the distribution of phosphatidylinositol 4-kinases at Golgi and lysosomal membranes. *EMBO J*, 40, e105990. doi: 10.15252/embj.2020105990
- Lackner LL, Ping H, Graef M, Murley A, Nunnari J (2013). Endoplasmic reticulum-associated mitochondria-cortex tether functions in the distribution and inheritance of mitochondria. *Proc Natl Acad Sci U S A* 110, E458–E467. doi: 10.1073/pnas.1215232110
- Lanner JT, Georgiou DK, Joshi AD, Hamilton SL (2010). Ryanodine receptors: Structure, expression, molecular details, and function in calcium release. *Cold Spring Harb Perspect Biol* 2, a003996. doi: 10.1101/cshperspect.a003996
- Lebowitz JJ, Pino JA, Mackie PM, Lin M, Hurst C, Divita K, Collins AT, Koutzoumis DN, Torres GE, Khoshbouei H (2019). Clustered Kv2.1 decreases dopamine transporter activity and internalization. *J Biol Chem* 294, 6957–6971. doi: 10.1074/jbc.RA119.007441
- Lee A, Hirabayashi Y, Kwon S-K, Lewis TL, Polleux F (2018). Emerging roles of mitochondria in synaptic transmission and neurodegeneration. *Curr Opin Physiol* 3, 82–93. doi: 10.1016/j.cophys.2018.03.009
- Lee E, Marcucci M, Daniell L, Pypaert M, Weisz OA, Ochoa GC, Farsad K, Wenk MR, De Camilli P (2002). Amphiphysin 2 (Bin1) and T-tubule biogenesis in muscle. *Science* 297, 1193–1196. doi: 10.1126/science.1071362
- Lehnart SE, Wehrens XHT (2022). The role of junctophilin proteins in cellular function. *Physiol Rev* 102, 1211–1261. doi: 10.1152/physrev.00024.2021
- Lewis RS, Cahalan MD (1995). Potassium and calcium channels in lymphocytes. *Annu Rev Immunol* 13, 623–653. doi: 10.1146/annurev.iy.13.040195.003203
- Lim ST, Antonucci DE, Scannevin RH, Trimmer JS (2000). A novel targeting signal for proximal clustering of the Kv2.1 K⁺ channel in hippocampal neurons. *Neuron* 25, 385–397. doi: 10.1016/S0896-6273(00)80902-2
- Lim C-Y, Davis OB, Shin HR, Zhang J, Berdan CA, Jiang X, Counihan JL, Ory DS, Nomura DK, Zoncu R (2019). ER–Lysosome contacts enable cholesterol sensing by mTORC1 and drive aberrant growth signalling in Niemann–Pick type C. *Nat Cell Biol* 21, 1206–1218. doi: 10.1038/s41556-019-0391-5
- Lin KJ, Lin KL, Chen SD, Liou CW, Chuang YC, Lin HY, Lin TK (2019). The overcrowded crossroads: Mitochondria, alpha-synuclein, and the endo-lysosomal system interaction in Parkinson's disease. *Int J Mol Sci* 20, 5312. doi: 10.3390/ijms20215312
- Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrell JE Jr, Meyer T (2005). STIM Is a Ca²⁺ sensor essential for Ca²⁺-store-depletion-triggered Ca²⁺ influx. *Curr Biol* 15, 1235–1241. doi: 10.1016/j.cub.2005.05.055
- Liu C, Spinozzi S, Chen J-Y, Fang X, Feng W, Perkins G, Cattaneo P, Guimarães-Camboa N, Dalton ND, Peterson KL, et al. (2019). Nexilin is a new component of junctional membrane complexes required for cardiac T-tubule formation. *Circulation* 140, 55–66. doi: 10.1161/CIRCULATIONAHA.119.039751
- Lopez-Sanjurjo CI, Tovey SC, Prole DL, Taylor CW (2013). Lysosomes shape Ins(1,4,5)P₃-evoked Ca²⁺ signals by selectively sequestering Ca²⁺ released from the endoplasmic reticulum. *J Cell Sci* 126, 289–300. doi: 10.1242/jcs.116103
- Ludtmann MHR, Abramov AY (2018). Mitochondrial calcium imbalance in Parkinson's disease. *Neurosci Lett* 663, 86–90. doi: 10.1016/j.neulet.2017.08.044
- Majewski L, Kuznicki J (2015). SOCE In neurons: Signaling or just refilling? *Biochim Biophys Acta (BBA) - Mol Cell Res* 1853, 1940–1952. doi: 10.1016/j.bbamer.2015.01.019
- Matsumoto C, O'Dwyer SC, Manning D, Hernandez-Hernandez G, Rhana P, Fong Z, Sato D, Clancy CE, Vierra NC, Trimmer JS, Fernando Santana L (2023). The formation of K(V)2.1 macroclusters is required for sex-specific differences in L-type ca(V)1.2 clustering and function in arterial myocytes. *Commun Biol* 6, 1165. doi: 10.1038/s42003-023-05527-1
- McCarl CA, Picard C, Khalil S, Kawasaki T, Rother J, Papolos A, Kutok J, Hivroz C, Ledest F, Plogmann K, et al. (2009). ORAI1 Deficiency and lack of store-operated Ca²⁺ entry cause immunodeficiency, myopathy, and ectodermal dysplasia. *J Allergy Clin Immunol* 124, 1311–1318.e1317. doi: 10.1016/j.jaci.2009.10.007
- Medina DL, Di Paola S, Peluso I, Armani A, De Stefani D, Venditti R, Montefusco S, Scotto-Rosato A, Prezioso C, Forrester A, et al. (2015). Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat Cell Biol* 17, 288–299. doi: 10.1038/ncb3114
- Mercado J, Baylie R, Navedo MF, Yuan C, Scott JD, Nelson MT, Brayden JE, Santana LF (2014). Local control of TRPV4 channels by AKAP150-targeted PKC in arterial smooth muscle. *J Gen Physiol* 143, 559–575. doi: 10.1085/jgp.201311050
- Min CK, Yeom DR, Lee KE, Kwon HK, Kang M, Kim YS, Park ZY, Jeon H, Kim DH (2012). Coupling of ryanodine receptor 2 and voltage-dependent anion channel 2 is essential for Ca²⁺ transfer from the sarcoplasmic reticulum to the mitochondria in the heart. *Biochem J* 447, 371–379. doi: 10.1042/bj20120705
- Misonou H, Menegola M, Mohapatra DP, Guy LK, Park K-S, Trimmer JS (2006). Bidirectional activity-dependent regulation of neuronal ion channel phosphorylation. *J Neurosci* 26, 13505–13514. doi: 10.1523/jneurosci.3970-06.2006
- Misonou H, Mohapatra DP, Park EW, Leung V, Zhen D, Misonou K, Anderson AE, Trimmer JS (2004). Regulation of ion channel localization and phosphorylation by neuronal activity. *Nat Neurosci* 7, 711–718. doi: 10.1038/nn1260
- Montes de Oca Balderas P (2021). Mitochondria-plasma membrane interactions and communication. *J Biol Chem* 297, 101164. doi: 10.1016/j.jbc.2021.101164
- Morgan AJ, Davis LC, Wagner SK, Lewis AM, Parrington J, Churchill GC (2013). Bidirectional Ca(2)(+) signaling occurs

- between the endoplasmic reticulum and acidic organelles. *J Cell Biol* 200, 789–805. doi: 10.1083/jcb.201204078
- Moriguchi S, Nishi M, Komazaki S, Sakagami H, Miyazaki T, Masumiya H, Saito SY, Watanabe M, Kondo H, Yawo H, et al. (2006). Functional uncoupling between Ca²⁺ release and afterhyperpolarization in mutant hippocampal neurons lacking junctophilins. *Proc Natl Acad Sci U S A* 103, 10811–10816. doi: 10.1073/pnas.0509863103
- Muller CS, Haupt A, Bildl W, Schindler J, Knaus HG, Meissner M, Rammner B, Striessnig J, Flockerzi V, Fakler B, Schulte U (2010). Quantitative proteomics of the Cav2 channel nano-environments in the mammalian brain. *Proc Natl Acad Sci U S A* 107, 14950–14957. doi: 10.1073/pnas.1005940107
- Murphy SE, Levine TP (2016). VAP, a Versatile access point for the endoplasmic Reticulum: Review and analysis of FFAT-like motifs in the VAPome. *Biochim Biophys Acta* 1861, 952–961. doi: 10.1016/j.bbali.2016.02.009
- Navedo MF, Amberg GC, Votaw VS, Santana LF (2005). Constitutively active L-type Ca²⁺ channels. *Proc Natl Acad Sci U S A* 102, 11112–11117. doi: 10.1073/pnas.0500360102
- Navedo MF, Cheng EP, Yuan C, Votaw S, Molkenin JD, Scott JD, Santana LF (2010). Increased coupled gating of L-type Ca²⁺ channels during hypertension and Timothy syndrome. *Circ Res* 106, 748–756. doi: 10.1161/circresaha.109.213363
- Nishi M, Komazaki S, Kurebayashi N, Ogawa Y, Noda T, Iino M, Takeshima H (1999). Abnormal features in skeletal muscle from mice lacking mitsugumin29. *J Cell Biol* 147, 1473–1480. doi: 10.1083/jcb.147.7.1473
- Nystoriak MA, Nieves-Cintrón M, Patriarchi T, Buonarati OR, Prada MP, Morotti S, Grandi E, Fernandes JD, Forbush K, Hofmann F, et al. (2017). Ser1928 phosphorylation by PKA stimulates the L-type Ca²⁺ channel CaV1.2 and vasoconstriction during acute hyperglycemia and diabetes. *Sci Signal* 10. doi: 10.1126/scisignal.aaf9647
- O'Dwyer SC, Palacio S, Matsumoto C, Guarina L, Klug NR, Tajada S, Rosati B, McKinnon D, Trimmer JS, Santana LF (2020). Kv2.1 channels play opposing roles in regulating membrane potential, Ca(2+) channel function, and myogenic tone in arterial smooth muscle. *Proc Natl Acad Sci U S A* 117, 3858–3866. doi: 10.1073/pnas.1917879117
- Oh-hora M, Yamashita M, Hogan PG, Sharma S, Lamperti E, Chung W, Prakriya M, Feske S, Rao A (2008). Dual functions for the endoplasmic reticulum calcium sensors STIM1 and STIM2 in T cell activation and tolerance. *Nat Immunol* 9, 432–443. doi: 10.1038/ni1574
- Ohtsuka T, Nakanishi H, Ikeda W, Satoh A, Momose Y, Nishioka H, Takai Y (1998). Nexilin: A novel actin filament-binding protein localized at cell-matrix adherens junction. *J Cell Biol* 143, 1227–1238. doi: 10.1083/jcb.143.5.1227
- Pchitskaya E, Popugayeva E, Bezprozvanny I (2017). Calcium signaling and molecular mechanisms underlying neurodegenerative diseases. *Cell Calcium*, 70. doi: 10.1016/j.ceca.2017.06.008
- Peng W, Wong YC, Krainc D (2020). Mitochondria-lysosome contacts regulate mitochondrial Ca(2+) dynamics via lysosomal TRPML1. *Proc Natl Acad Sci U S A* 117, 19266–19275. doi: 10.1073/pnas.2003236117
- Phillips MJ, Voeltz GK (2016). Structure and function of ER membrane contact sites with other organelles. *Nat Rev Mol Cell Biol* 17, 69–82. doi: 10.1038/nrm.2015.8
- Porter KR, Palade GE (1957). Studies on the endoplasmic reticulum. III. Its form and distribution in striated muscle cells. *Jo Biophys Biochem Cytol* 3, 269–300. doi: 10.1083/jcb.3.2.269
- Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisailon JM, Singer HA, Trebak M (2009). Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: Role in proliferation and migration. *FASEB J* 23, 2425–2437. doi: 10.1096/fj.09-131128
- Poulet C, Sanchez-Alonso J, Swiatlowska P, Mouy F, Lucarelli C, Alvarez-Laviada A, Gross P, Terracciano C, Houser S, Gorelik J (2021). Junctophilin-2 tethers T-tubules and recruits functional L-type calcium channels to lipid rafts in adult cardiomyocytes. *Cardiovasc Res* 117, 149–161. doi: 10.1093/cvr/cvaa033
- Prada MP, Syed AU, Buonarati OR, Reddy GR, Nystoriak MA, Ghosh D, Simó S, Sato D, Sasse KC, Ward SM, et al. (2019). A G(s)-coupled purinergic receptor boosts Ca(2+) influx and vascular contractility during diabetic hyperglycemia. *Elife* 8. doi: 10.7554/eLife.42214
- Prinz WA, Toulmay A, Balla T (2020). The functional universe of membrane contact sites. *Nat Rev Mol Cell Biol* 21, 7–24. doi: 10.1038/s41580-019-0180-9
- Pritchard HAT, Griffin CS, Yamasaki E, Thakore P, Lane C, Greenstein AS, Earley S (2019). Nanoscale coupling of junctophilin-2 and ryanodine receptors regulates vascular smooth muscle cell contractility. *Proc Natl Acad Sci U S A* 116, 21874–21881. doi: 10.1073/pnas.1911304116
- Prokic I, Cowling BS, Kutchukian C, Kretz C, Tasfaout H, Gache V, Hergueux J, Wendling O, Ferry A, Toussaint A, et al. (2020). Differential physiological roles for BIN1 isoforms in skeletal muscle development, function and regeneration. *Dis Models Mech* 13. doi: 10.1242/dmm.044354
- Prole DL, Taylor CW (2019). Structure and function of IP3 receptors. *Cold Spring Harb Perspect Biol* 11, a035063. doi: 10.1101/cshperspect.a035063
- Protasi F, Paolini C, Nakai J, Beam KG, Franzini-Armstrong C, Allen PD (2002). Multiple regions of RyR1 mediate functional and structural interactions with alpha(1S)-dihydropyridine receptors in skeletal muscle. *Biophys J* 83, 3230–3244. doi: 10.1016/s0006-3495(02)75325-3
- Putney JW Jr (1986). A model for receptor-regulated calcium entry. *Cell Calcium* 7, 1–12. doi: 10.1016/0143-4160(86)90026-6
- Raiborg C, Wenzel EM, Pedersen NM, Olsvik H, Schink KO, Schultz SW, Vietri M, Nisi V, Bucci C, Brech A, et al. (2015). Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth. *Nature* 520, 234–238. doi: 10.1038/nature14359
- Rebeck RT, Karunasekara Y, Board PG, Beard NA, Casarotto MG, Dulhunty AF (2014). Skeletal muscle excitation-contraction coupling: Who are the dancing partners? *Int J Biochem Cell Biol* 48, 28–38. doi: 10.1016/j.biocel.2013.12.001
- Rios E, Brum G (1987). Involvement of dihydropyridine receptors in excitation-contraction coupling in skeletal muscle. *Nature* 325, 717–720. doi: 10.1038/325717a0
- Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T (1998). Close contacts with the endoplasmic Reticulum as determinants of mitochondrial Ca²⁺ responses. *Science* 280, 1763–1766. doi: 10.1126/science.280.5370.1763
- Rodney GG, Williams BY, Strasburg GM, Beckingham K, Hamilton SL (2000). Regulation of RYR1 activity by Ca²⁺

- and calmodulin. *Biochemistry* 39, 7807–7812. doi: 10.1021/bi0005660
- Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, et al. (2005). STIM1, An essential and conserved component of store-operated Ca²⁺ channel function. *J Cell Biol* 169, 435–445. doi: 10.1083/jcb.200502019
- Rossi D, Pierantozzi E, Amadsun DO, Buonocore S, Rubino EM, Sorrentino V (2022). The sarcoplasmic Reticulum of skeletal muscle cells: A labyrinth of membrane contact sites. *Biomolecules* 12, 488. doi: 10.3390/biom12040488
- Rossi D, Scarcella AM, Liguori E, Lorenzini S, Pierantozzi E, Kutchukian C, Jacquemond V, Messa M, De Camilli P, Sorrentino V (2019). Molecular determinants of homo- and heteromeric interactions of Junctophilin-1 at triads in adult skeletal muscle fibers. *Proc Natl Acad Sci U S A* 116, 15716–15724. doi: 10.1073/pnas.1820980116
- Ruas M, Rietdorf K, Arredouani A, Davis LC, Lloyd-Evans E, Koegel H, Funnell TM, Morgan AJ, Ward JA, Watanabe K, et al. (2010). Purified TPC isoforms form NAADP receptors with distinct roles for Ca²⁺ signaling and endolysosomal trafficking. *Curr Biol* 20, 703–709. doi: 10.1016/j.cub.2010.02.049
- Saeki T, Suzuki Y, Yamamura H, Takeshima H, Imaizumi Y (2019). A junctophilin-caveolin interaction enables efficient coupling between ryanodine receptors and BK(Ca) channels in the Ca(2+) microdomain of vascular smooth muscle. *J Biol Chem* 294, 13093–13105. doi: 10.1074/jbc.RA119.008342
- Sahu G, Wazen RM, Colarusso P, Chen SRW, Zamponi GW, Turner RW (2019). Junctophilin proteins tether a Cav1-RyR2-KCa3.1 tripartite complex to regulate neuronal excitability. *Cell Rep* 28, 2427–2442.e2426. doi: 10.1016/j.celrep.2019.07.075
- Schneider MF, Chandler WK (1973). Voltage dependent charge movement of skeletal muscle: A possible step in excitation-contraction coupling. *Nature* 242, 244–246. doi: 10.1038/242244a0
- Scorrano L, De Matteis MA, Emr S, Giordano F, Hajnoczky G, Kornmann B, Lackner LL, Levine TP, Pellegrini L, Reinisch K, et al. (2019). Coming together to define membrane contact sites. *Nat Commun* 10, 1287. doi: 10.1038/s41467-019-09253-3
- Shen D, Wang X, Li X, Zhang X, Yao Z, Dibble S, Dong XP, Yu T, Lieberman AP, Showalter HD, Xu H (2012). Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release. *Nat Commun* 3, 731. doi: 10.1038/ncomms1735
- Somlyo AV, Somlyo AP (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. *J Pharmacol Exp Ther* 159, 129–145.
- Somlyo AP, Somlyo AV (2003). Ca²⁺ sensitivity of smooth muscle and nonmuscle myosin II: Modulated by G proteins, kinases, and myosin phosphatase. *Physiol Rev* 83, 1325–1358. doi: 10.1152/physrev.00023.2003
- Specia DJ, Ogata G, Mandikian D, Bishop HI, Wiler SW, Eum K, Wenzel HJ, Doisy ET, Matt L, Campi KL, et al. (2014). Deletion of the Kv2.1 delayed rectifier potassium channel leads to neuronal and behavioral hyperexcitability. *Genes Brain Behav* 13, 394–408. doi: 10.1111/gbb.12120
- Spinozzi S, Liu C, Chen Z, Feng W, Zhang L, Ouyang K, Evans SM, Chen J (2020). Nexilin is necessary for maintaining the transverse-axial tubular system in adult cardiomyocytes. *Circ Heart Fail* 13, e006935. doi: 10.1161/circheartfailure.120.006935
- Sun XH, Protasi F, Takahashi M, Takeshima H, Ferguson DG, Franzini-Armstrong C (1995). Molecular architecture of membranes involved in excitation-contraction coupling of cardiac muscle. *J Cell Biol* 129, 659–671. doi: 10.1083/jcb.129.3.659
- Suski JM, Lebedzinska M, Wojtala A, Duszynski J, Giorgi C, Pinton P, Wieckowski MR (2014). Isolation of plasma membrane-associated membranes from rat liver. *Nat Protoc* 9, 312–322. doi: 10.1038/nprot.2014.016
- Sweeney HL, Houdusse A (2010). Structural and functional insights into the Myosin motor mechanism. *Annu Rev Biophys* 39, 539–557. doi: 10.1146/annurev.biophys.050708.133751
- Szabadkai Gr, Bianchi K, Várnai Pt, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla Ts, Rizzuto R (2006). Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca²⁺ channels. *J Cell Biol* 175, 901–911. doi: 10.1083/jcb.200608073
- Takeda Y, Nystoriak MA, Nieves-Cintrón M, Santana LF, Navedo MF (2011). Relationship between Ca²⁺ sparklets and sarcoplasmic reticulum Ca²⁺ load and release in rat cerebral arterial smooth muscle. *Am J Physiol-Heart Circ Physiol* 301, H2285–H2294. doi: 10.1152/ajpheart.00488.2011
- Thakore P, Pritchard HAT, Griffin CS, Yamasaki E, Drumm BT, Lane C, Sanders KM, Feng Earley Y, Earley S (2020). TRPML1 channels initiate Ca(2+) sparks in vascular smooth muscle cells. *Sci Signal* 13. doi: 10.1126/scisignal.aba1015
- Thiffault I, Specia DJ, Austin DC, Cobb MM, Eum KS, Safina NP, Grote L, Farrow EG, Miller N, Soden S, et al. (2015). A novel epileptic encephalopathy mutation in KCNB1 disrupts Kv2.1 ion selectivity, expression, and localization. *J Gen Physiol* 146, 399–410. doi: 10.1085/jgp.201511444
- Thillaiappan NB, Chavda AP, Tovey SC, Prole DL, Taylor CW (2017). Ca(2+) signals initiate at immobile IP3 receptors adjacent to ER-plasma membrane junctions. *Nat Commun* 8, 1505. doi: 10.1038/s41467-017-01644-8
- Tiscione SA, Vivas O, Ginsburg KS, Bers DM, Ory DS, Santana LF, Dixon RE, Dickson EJ (2019). Disease-associated mutations in Niemann-Pick type C1 alter ER calcium signaling and neuronal plasticity. *J Cell Biol* 218, 4141–4156. doi: 10.1083/jcb.201903018
- Toglia P, Cheung KH, Mak DO, Ullah G (2016). Impaired mitochondrial function due to familial Alzheimer's disease-causing presenilins mutants via Ca(2+) disruptions. *Cell Calcium* 59, 240–250. doi: 10.1016/j.ceca.2016.02.013
- Trebak M (2012). STIM/Orai signalling complexes in vascular smooth muscle. *J Physiol* 590, 4201–4208. doi: 10.1113/jphysiol.2012.233353
- Valm AM, Cohen S, Legant WR, Melunis J, Hershberg U, Wait E, Cohen AR, Davidson MW, Betzig E, Lippincott-Schwartz J (2017). Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature* 546, 162–167. doi: 10.1038/nature22369
- van Oort RJ, Garbino A, Wang W, Dixit SS, Landstrom AP, Gaur N, De Almeida AC, Skapura DG, Rudy Y, Burns AR, et al. (2011). Disrupted junctional membrane complexes and hyperactive ryanodine receptors after acute junctophilin knock-down in mice. *Circulation* 123, 979–988. doi: 10.1161/circulationaha.110.006437
- Verma M, Wills Z, Chu CT (2018). Excitatory dendritic mitochondrial calcium toxicity: Implications for Parkinson's and other

- neurodegenerative diseases. *Front Neurosci* 12. doi: 10.3389/fnins.2018.00523
- Vierra NC, Kirmiz M, van der List D, Santana LF, Trimmer JS (2019). Kv2.1 mediates spatial and functional coupling of L-type calcium channels and ryanodine receptors in mammalian neurons. *Elife* 8. doi: 10.7554/eLife.49953
- Vierra NC, O'Dwyer SC, Matsumoto C, Santana LF, Trimmer JS (2021). Regulation of neuronal excitation-transcription coupling by Kv2.1-induced clustering of somatic L-type Ca²⁺ channels at ER-PM junctions. *Proc Natl Acad Sci U S A* 118, ARTN e2110094118. doi: 10.1073/pnas.2110094118
- Vierra NC, Ribeiro-Silva L, Kirmiz M, van der List D, Bhandari P, Mack OA, Carroll J, Le Monnier E, Aicher SA, Shigemoto R, Trimmer JS (2023). Neuronal ER-plasma membrane junctions couple excitation to Ca(2+)-activated PKA signaling. *Nat Commun* 14, 5231. doi: 10.1038/s41467-023-40930-6
- Vig M, Peinelt C, Beck A, Koomoa DL, Rabah D, Koblan-Huberson M, Kraft S, Turner H, Fleig A, Penner R, Kinet JP (2006). CRACM1 Is a plasma membrane protein essential for store-operated Ca²⁺ entry. *Science* 312, 1220–1223. doi: 10.1126/science.1127883
- Voeltz GK, Sawyer EM, Hajnóczky G, Prinz WA (2024). Making the connection: How membrane contact sites have changed our view of organelle biology. *Cell* 187, 257–270. doi: 10.1016/j.cell.2023.11.040
- Wang SQ, Song LS, Lakatta EG, Cheng H (2001). Ca²⁺ signalling between single L-type Ca²⁺ channels and ryanodine receptors in heart cells. *Nature* 410, 592–596. doi: 10.1038/35069083
- Wang S, Tukachinsky H, Romano FB, Rapoport TA (2016). Cooperation of the ER-shaping proteins atlastin, lunapark, and reticulons to generate a tubular membrane network. *eLife* 5, e18605. doi: 10.7554/eLife.18605
- Wang X, Zhang X, Dong XP, Samie M, Li X, Cheng X, Goschka A, Shen D, Zhou Y, Harlow J, et al. (2012). TPC Proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes. *Cell* 151, 372–383. doi: 10.1016/j.cell.2012.08.036
- Wegierski T, Kuznicki J (2018). Neuronal calcium signaling via store-operated channels in health and disease. *Cell Calcium* 74, 102–111. doi: 10.1016/j.ceca.2018.07.001
- Wei C, Wang X, Zheng M, Cheng H (2012). Calcium gradients underlying cell migration. *Curr Opin Cell Biol* 24, 254–261. doi: 10.1016/j.ceb.2011.12.002
- Westhoff M, Del Villar SG, Voelker TL, Thai PN, Spooner HC, Costa AD, Sirish P, Chiamvimonvat N, Dickson EJ, Dixon RE (2024). BIN1 Knockdown rescues systolic dysfunction in aging male mouse hearts. *Nat Commun* 15, 3528. doi: 10.1038/s41467-024-47847-8
- Wilhelm LP, Wendling C, Védie B, Kobayashi T, Chenard MP, Tomasetto C (2017). STARD3 Mediates endoplasmic reticulum-to-endosome cholesterol transport at membrane contact sites. *EMBO J* 36, 1412–1433. doi: 10.15252/embj.201695917
- Wong YC, Ysselstein D, Krainc D (2018). Mitochondria-lysosome contacts regulate mitochondrial fission via RAB7 GTP hydrolysis. *Nature* 554, 382–386. doi: 10.1038/nature25486
- Wu Y, Whiteus C, Xu CS, Hayworth KJ, Weinberg RJ, Hess HF, De Camilli P (2017). Contacts between the endoplasmic reticulum and other membranes in neurons. *Proc Natl Acad Sci U S A* 114, E4859–E4867. doi: 10.1073/pnas.1701078114
- Wu J, Yan Z, Li Z, Yan C, Lu S, Dong M, Yan N (2015). Structure of the voltage-gated calcium channel Cav1.1 complex. *Science* 350, aad2395. doi: 10.1126/science.aad2395
- Yazbeck P, Tauseef M, Kruse K, Amin MR, Sheikh R, Feske S, Komarova Y, Mehta D (2017). STIM1 phosphorylation at Y361 recruits orai1 to STIM1 puncta and induces Ca(2+) entry. *Sci Rep* 7, 42758. doi: 10.1038/srep42758
- Zaichick SV, McGrath KM, Caraveo G (2017). The role of Ca(2+) signaling in Parkinson's disease. *Dis Models Mech* 10, 519–535. doi: 10.1242/dmm.028738
- Zhang X, Li X, Xu H (2012). Phosphoinositide isoforms determine compartment-specific ion channel activity. *Proc Natl Acad Sci U S A* 109, 11384–11389. doi: 10.1073/pnas.1202194109
- Zhao K, Ridgway ND. Oxysterol-binding protein-related protein 1L regulates cholesterol egress from the endo-lysosomal system. *Cell Rep* 19, 1807–1818. doi: 10.1016/j.celrep.2017.05.028
- Zweifach A, Lewis RS (1993). Mitogen-regulated Ca²⁺ current of T lymphocytes is activated by depletion of intracellular Ca²⁺ stores. *Proc Natl Acad Sci U S A* 90, 6295–6299. doi: 10.1073/pnas.90.13.6295