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Review

Organization and function of membrane contact sites ☆

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ABSTRACT

Membrane-bound organelles are a wonderful evolutionary acquisition of the eukaryotic cell, allowing the segregation of sometimes incompatible biochemical reactions into specific compartments with tailored microenvironments. On the flip side, these isolating membranes that crowd the interior of the cell, constitute a hindrance to the diffusion of metabolites and information to all corners of the cell. To ensure coordination of cellular activities, cells use a network of contact sites between the membranes of different organelles. These membrane contact sites (MCSs) are domains where two membranes come to close proximity, typically less than 30 nm. Such contacts create microdomains that favor exchange between two organelles. MCSs are established and maintained in durable or transient states by tethering structures, which keep the two membranes in proximity, but fusion between the membranes does not take place. Since the endoplasmic reticulum (ER) is the most extensive cellular membrane network, it is thus not surprising to find the ER involved in most MCSs within the cell. The ER contacts diverse compartments such as mitochondria, lysosomes, lipid droplets, the Golgi apparatus, endosomes and the plasma membrane. In this review, we will focus on the common organizing principles underlying the many MCSs found between the ER and virtually all compartments of the cell, and on how the ER establishes a network of MCSs for the trafficking of vital metabolites and information. This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

1. Functions of MCSs

MCSs display a wide variety of functions including, but not limited to regulation of immune response, apoptosis, organelle dynamics and trafficking. At the molecular level, the function of MCSs most often involves calcium (Ca^{2+}) and/or lipid exchange between two compartments. Trafficking of both Ca^{2+} and lipids must be governed by strict rules. In the case of Ca^{2+}, tight coordination is required because it is a very potent signaling molecule that acts locally, and which can be toxic when deregulated (reviewed in [1]), and in the case of lipids because their variety contributes to determining the identity and physicochemical properties of all membrane-bound compartments (reviewed in [2]). Ca^{2+} and lipids also have in common diffusion-limited properties. For Ca^{2+}, diffusion is limited by the abundance of Ca^{2+}-binding activities in all compartments of the cell, while diffusion of lipids is limited by their insolubility in most of the aqueous cellular volume. MCSs allow localized and targeted exchange of both Ca^{2+} and lipids to coordinate homeostasis across the many compartments of the cell. Since the ER is both the main site of lipid synthesis and the main cellular Ca^{2+} store, it is not surprising that MCSs involving the ER provide an important function in both of these exchange reactions.

1.1. Calcium signaling

The ER [Ca^{2+}] is in the millimolar range, while that of the cytosol is nanomolar (reviewed in [1]). This difference is established by Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} ATPases (SERCA), which pump Ca^{2+} into the ER lumen, and by Plasma Membrane Ca^{2+} ATPases (PMCA), which pump it out of the cell (Fig. 1B). This gradient is used in signaling events whereby the opening of ER Ca^{2+} channels (the Ryanodine Receptors, RyR and the Inositol-1,4,5-triphosphate Receptor, IP_3R) causes a transient surge in cytosolic [Ca^{2+}], triggering a number of downstream events by activating Ca^{2+}-binding proteins. Ca^{2+} signaling governs processes as diverse as memory, vision, fertilization, muscle contraction, proliferation, cell migration, immune response and transcription. Ca^{2+} does not only signal to the cytosol, but also to other compartments. Intracellular Ca^{2+} diffusion is slowed by the presence of many Ca^{2+}-binding activities. This slow diffusion maintains steep local [Ca^{2+}] gradients and non-equilibrium conditions, which are best illustrated by such spectacular phenomena as Ca^{2+} oscillations and Ca^{2+} waves [1]. On the small scale, slow Ca^{2+} diffusion promotes the formation of cytosolic Ca^{2+} microdomains, where the [Ca^{2+}] differs from that of the bulk cytosol. Microdomains of this kind are often created at MCSs, which coordinate vast movements of the Ca^{2+} pool across different compartments.

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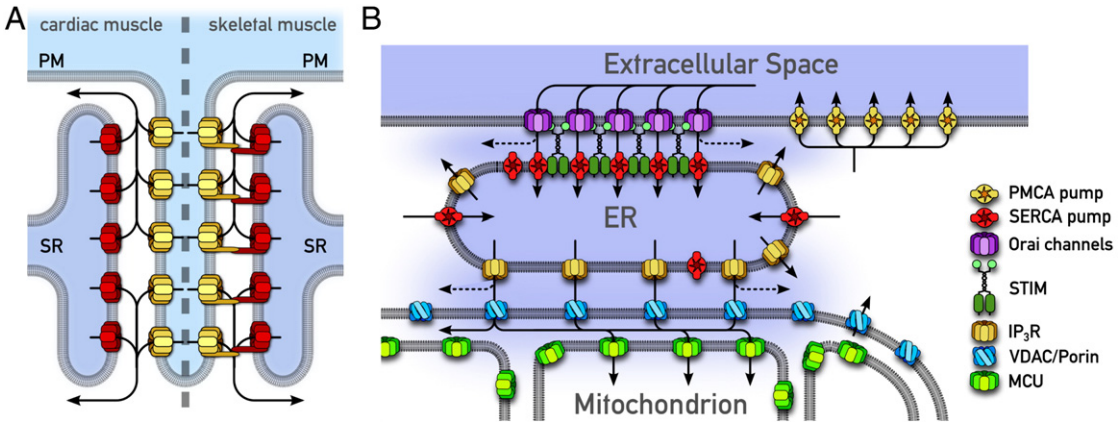

Fig 1. MCSs and Ca^{2+} homeostasis. A) Organization of the junctional membrane complexes in striated cardiac and skeletal muscles. DHPR (yellow) is found in T-tubules, which invaginate from the cell surface into the cell interior. DHPR gates the opening of RyR (red) in cardiac cells by increasing the local [Ca^{2+}] (Ca^{2+}-gated Ca^{2+} release), in skeletal cells by physically contacting them across the cytosolic space between the SR and the PM (allosteric coupling). B) Organization of Ca^{2+} microdomains at MCSs. The blue color represents the local [Ca^{2+}]. A steep [Ca^{2+}] gradient is established by constitutive Ca^{2+} pumping performed by SERCA (red) towards the ER lumen, and PMCA (yellow) towards cell exterior. ER–PM MCSs allow direct ER Ca^{2+} refilling *via* STIM/Orai-dependent (purple/green) SOCE with minimal Ca^{2+} escape towards the bulk cytosol. ER–mitochondria MCSs allow direct Ca^{2+} exchange from the ER channel IP_3R (tan) to the mitochondrial uptake machinery VDAC (blue) and MCU (green).

1.1.1. Excitation–contraction in muscle cells

A classical contact site example is the spatial arrangement of the muscle ER (called Sarcoplasmic Reticulum, SR) and the Plasma-Membrane (PM), and its role in generating the signal that results in muscle contraction [3]. Muscle contraction is triggered by a massive influx of Ca^{2+} into the cytosol, which activates myosin movement along actin filaments in the sarcomeres (Fig. 1A). This Ca^{2+} influx is the result of the synergistic activation of two mechanisms: 1) the opening of voltage-gated PM Ca^{2+} channels, such as the dihydropyridine receptor (DHPR), which respond to a change in PM potential originating at the neuromuscular synapse, and 2) the synchronous opening of the main SR Ca^{2+} channel RyR (reviewed in [4]). The synchronization of both events is ensured by their physical coupling at MCSs between the SR and the PM. The myocyte PM is invaginated in between sarcomeres. These invaginations create transverse tubules (T-tubules), which project into the center of the cell. These T-tubules are closely bracketed by SR tubules, forming characteristic junctional membrane complexes (JMC) [5], referred to as dyads, where one T-tubule associates with one tubule of SR, or triads, where one T-tubule is bracketed by two tubules of SR (Fig. 1A). RyRs are found on the SR tubules while DHPRs are on the opposing T-tubules. It is this proximity between RyR and DHPR that allows coupled gating of both channels. Interestingly, although the spatial proximity between both types of Ca^{2+} channels is conserved, the way DHPR "talks" to RyR depends on the muscle type. In cardiac muscle, myocardial RyR (RyR2) senses the Ca^{2+} emanating from DHPR. Ensuing RyR opening is therefore part of a positive feedback loop [6]. In non-pulsatile skeletal muscles, however, DHPR is thought to directly open skeletal muscle RyR (RyR1) through a physical connection between both channels [7,8]. According to this model, a voltage-dependent change in DHPR conformation is transmitted to RyR1 *via* the C-terminus of DHPR cytoplasmic β_{1a} subunit. This subunit of DHPR bridges the cytosolic gap in between T-tubule and SR to directly bind RyR1 and gate its opening [9]. In both cases, close apposition of SR and PM at MCSs allows the coordination of cellular events, which permits rapid and precise muscular contraction.

1.1.2. The store-operated Ca^{2+} entry

1.1.2.1. Function. Ca^{2+} signaling is not restricted to excitable muscle cells. Non-excitable cells also use Ca^{2+} signaling in important signaling cascades, and they also use MCSs to coordinate these cascades across the many compartments of the cell.

The ER lumen is the site of numerous fundamental calcium-dependent processes, such as protein folding and quality control [10]. After a discharge caused by opening of IP_3R – the main Ca^{2+} channel in the ER of non-excitable cells – it is essential that the luminal [Ca^{2+}] returns rapidly to its normal level. This is ensured by an important cellular mechanism, which triggers the entry of Ca^{2+} ions into the cytosol from the extracellular space in conditions where the ER Ca^{2+} store is depleted.

This Store-Operated Ca^{2+} Entry (SOCE) is not only important for ER function, but also for the proper execution of Ca^{2+}-dependent signaling cascades. By constantly refilling the ER, SOCE allows to sustain elevated cytosolic [Ca^{2+}] during extended periods of times. For instance, SOCE is required for many immune functions, such as T-lymphocyte activation and proliferation (reviewed in [11]).

1.1.2.2. SOCE mechanism. How does a Ca^{2+} channel situated in the PM know that the [Ca^{2+}] in the ER lumen is low? This question has kept researchers busy for decades, until RNAi screens revealed an astoundingly simple device for membrane–membrane communication, made of only two central components: 1) the sensor Stromal Interaction Molecule (STIM) [12,13] and 2) the effector PM Ca^{2+} channel Orai [14–18]. STIM is a type I transmembrane ER protein. It senses luminal [Ca^{2+}] *via* its luminal N-terminus, which bears Ca^{2+}-binding EF-hand motifs [12]. Ca^{2+} depletion in the lumen causes the unfolding of the EF-hands and allows the formation of STIM oligomers [19–21]. Oligomerization is accompanied by a conformational change in the C-terminal cytosolic moiety [22], exposing a polybasic tract of aminoacids near the C-terminus. The polybasic tract can bind to acidic phosphoinositides found in the PM [19,22–24]. As a result, the localization of STIM, which is rather homogenous in the ER in Ca^{2+} replete condition [12,19,25], becomes restricted to a few structures ("puncta") upon ER Ca^{2+} depletion. Examination using TIRF microscopy (see Box 3) indicates that these puncta are MCSs between the ER and the PM [12,25–30]. At these ER–PM MCSs, STIM can recruit the Ca^{2+}-specific channel Orai and gate it by direct physical interaction [31–35]. This system is so simple that it can be recapitulated *in vitro*. Recombinant STIM cytosolic fragments bind to and gate Orai channels produced in yeast [36]. As yeast does not have any of the SOCE components, it is very likely that this gating is direct.

1.1.2.3. SOCE and MCSs: how?. What recruits STIM to the MCSs? A likely model is that in resting conditions, STIM diffuses passively in the plane of the ER membrane, and that upon stimulation, the exposure