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# A "mix-and-match" approach to designing Ca2+ microdomains at membrane-contact sites

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**Abbreviations:** Membrane contact sites (MCS), endoplasmic reticulum (ER), sarcoplasmic reticulum (SR), Ca2+ concentration ([Ca2+]), nicotinic acid adenine dinucleotide phosphate (NAADP), inositol trisphosphate (Ins(1,4,5)P3), inositol trisphosphate receptor (Ins(1,4,5)P3R), sarco/endoplasmic reticulum Ca2+ ATPase (SERCA), ryanodine receptor (RyR)

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**$Ca^{2+}$ microdomains are critical for regulating cellular activity and often form at membrane contact sites. Such sites between lysosomes and the ER potentially provide a platform for signaling by the $Ca^{2+}$ mobilizing messenger NAADP. However, at present we know little of how $Ca^{2+}$ release events are coordinated at these experimentally intractable junctions. We therefore developed a computational model of lysosome-ER microdomains, which suggested that small leaks of $Ca^{2+}$ from the lysosome couple to $Ca^{2+}$-sensitive Ins(1,4,5)$P_3$ receptors on the ER to generate global, microdomain-dependent $Ca^{2+}$ signals. Here we discuss how the "mix-and-match" arrangement of different $Ca^{2+}$ signaling proteins on the "source" and "target" membranes might generate functionally heterogeneous $Ca^{2+}$ microdomains.**

Membrane contact sites (MCS) are specialized structures, whereby cellular membranes are closely apposed (10–50 nm).[1] Such an arrangement facilitates information flow between different cellular compartments.[1] $Ca^{2+}$ is a universal signaling currency that often makes use of MCS to direct cellular activity.[2] The restricted space within MCS allows formation of local $Ca^{2+}$ microdomains where the $Ca^{2+}$ concentration ($[Ca^{2+}]$) is thought to reach high, micromolar levels (~10 μM) levels.[2] These microdomains facilitate activation of downstream $Ca^{2+}$-dependent processes. A high $[Ca^{2+}]$ within the dyadic junction of cardiac cells, for example, couples excitation of the sarcolemma to cellular contraction, driven by $Ca^{2+}$ release from the sarcoplasmic reticulum (SR).[3] Similarly, high $[Ca^{2+}]$ microdomains at ER-mitochondria MCS drive excitation-metabolism coupling.[4] Recently, MCS between lysosomes and the endoplasmic reticulum (ER) have been identified.[5] These sites provide an anatomical basis for functional coupling of lysosomal $Ca^{2+}$ stores with the ER through the $Ca^{2+}$ mobilizing messenger, NAADP.[6] NAADP is thought to act via two-pore channels to evoke $Ca^{2+}$ signals from acidic organelles that are then amplified by the ER to drive complex $Ca^{2+}$ behavior.[7,8] However, the function of lysosome-ER MCS and putative $Ca^{2+}$ microdomains contained therein is currently unknown.

In our recent paper,[9] we developed a model of $Ca^{2+}$ dynamics within these putative lysosome-ER microdomains and the continuous bulk cytosol to simulate cellular $Ca^{2+}$ responses. We modeled leaks of $Ca^{2+}$ from the "source" lysosome membrane, such as those produced in response to NAADP. These leaks activated $Ca^{2+}$-sensitive inositol trisphosphate receptors (Ins(1,4,5)$P_3$Rs) on the "target" ER membrane and generated global $Ca^{2+}$ responses that were microdomain-dependent. Interestingly, we also found that varying either the distribution or density of the lysosomal $Ca^{2+}$ leak altered the mode of coupling. For example, selectively increasing the magnitude of the lysosomal $Ca^{2+}$ leak into the microdomain, akin to channel clustering, broadened the concentration range over which NAADP produced responses. A uniform increase in $Ca^{2+}$ leak density into both the microdomain and

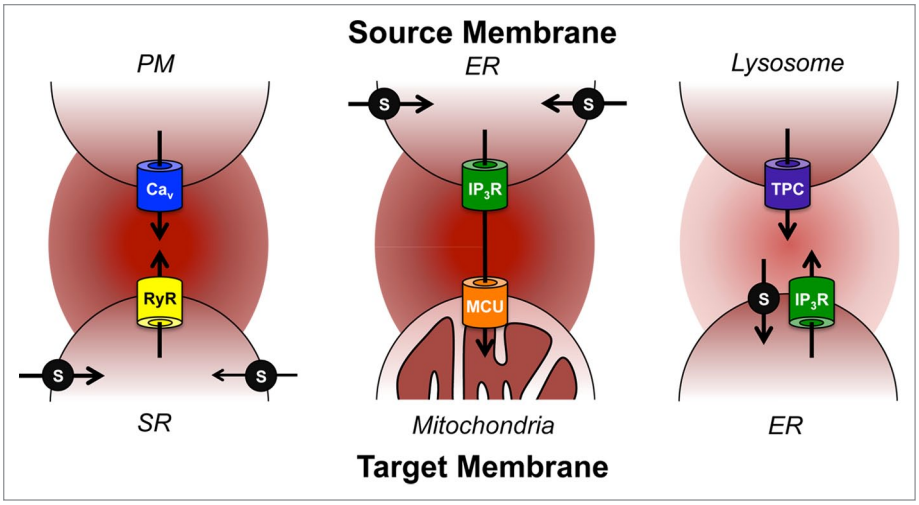


**Figure 1.** Heterogeneity of $Ca^{2+}$ microdomains at membrane contact sites. MCS between the source (top) and target (bottom) membranes allow functional $Ca^{2+}$ microdomains to form between them. $Ca^{2+}$ influx through voltage gated $Ca^{2+}$ channels ($Ca_v$) in the PM-SR MCS of the dyadic cleft (left) forms a high [$Ca^{2+}$] microdomain (dark circle) to initiate $Ca^{2+}$ release from low-affinity ryanodine receptors (RyR). $Ca^{2+}$ release through Ins(1,4,5)$P_3$Rs ($IP_3R$) in ER-mitochondria MCS (center) also forms a high [$Ca^{2+}$] microdomain to facilitate mitochondrial $Ca^{2+}$ uptake by the low-affinity mitochondrial uniporter (MCU). $Ca^{2+}$ release through two-pore channels (TPC) in lysosome-ER MCS (right) forms a low [$Ca^{2+}$] microdomain (light circle) due to the presence of SERCA (S) but which is nevertheless able to activate high-affinity Ins(1,4,5)$P_3$Rs.

non-microdomain compartments, mimicking channel overexpression, also had a broadening effect. However, in these simulations, microdomains acted to modulate the frequency of global $Ca^{2+}$ oscillations rather than initiate them. Lysosome-ER $Ca^{2+}$ microdomains can either drive or shape complex $Ca^{2+}$ signals, depending on their composition.[9]

Unlike other microdomains,[4,10] the lysosome-ER microdomains simulated within our model did *not* achieve high [$Ca^{2+}$]. Although kinetically distinct, $Ca^{2+}$ signals in the microdomain were only marginally greater in amplitude than within the bulk cytosol.[9] However, this result is not as counterintuitive as it may first seem. As $Ca^{2+}$-release from Ins(1,4,5)$P_3$Rs on the target membrane can be activated by relatively low [$Ca^{2+}$],[11] small leaks of $Ca^{2+}$ from lysosomes can raise microdomain [$Ca^{2+}$] sufficiently to initiate $Ca^{2+}$-induced $Ca^{2+}$-release via the Ins(1,4,5)$P_3$R. This arrangement differs from other $Ca^{2+}$ microdomains. For example, ryanodine receptors (RyRs) on the target membrane in the cardiac dyadic cleft have a lower affinity for $Ca^{2+}$-induced activation than Ins(1,4,5)$P_3$Rs (~0.8–1 μM, compared with ~0.1–0.3 μM).[12] They therefore require higher [$Ca^{2+}$] for activation to generate a high [$Ca^{2+}$] microdomain. Moreover, the mitochondrial $Ca^{2+}$ uptake machinery on the target membrane in ER-mitochondria microdomains has an even lower affinity for $Ca^{2+}$ (estimated between 10–100 μM).[13,14] Thus, microdomain [$Ca^{2+}$] may "match" $Ca^{2+}$ affinity of target proteins.

The unexpectedly low microdomain [$Ca^{2+}$] in our model was due to the presence of sarco/endoplasmic reticulum $Ca^{2+}$ ATPase (SERCA) pumps within the microdomains, which efficiently take up the released $Ca^{2+}$.[3] SERCA pumps were required to balance a basal $Ca^{2+}$ leak through the Ins(1,4,5)$P_3$R at a resting level of Ins(1,4,5)$P_3$. This arrangement differs from other studies utilizing RyR models[10] because, unlike Ins(1,4,5)$P_3$Rs, RyRs can be gated directly by $Ca^{2+}$. As such, these models contain no basal RyR leak and therefore do not require balancing $Ca^{2+}$ uptake mechanisms within the microdomain. This allows for the accumulation of high [$Ca^{2+}$] upon activation of the RyRs.

Our hypothesis, that peak [$Ca^{2+}$] within microdomains can be determined by the level of microdomain SERCA, may also extend to ER-mitochondria microdomains. Here, the ER acts as the source membrane whereby Ins(1,4,5)$P_3$Rs create high microdomain [$Ca^{2+}$] to facilitate low affinity mitochondrial $Ca^{2+}$ uptake. It is therefore of note that Ins(1,4,5)$P_3$Rs are enriched within ER-mitochondria MCS, while SERCA is not.[15] The potential absence of SERCA within these microdomains might permit spontaneous activity within these junctions, potentially accounting for the proposed maintenance of mitochondrial bioenergetics by basally active Ins(1,4,5)$P_3$Rs.[16] $Ca^{2+}$ signaling proteins may be "mixed" together to generate functionally heterogeneous microdomains.

Computational models of $Ca^{2+}$ dynamics often have a modular design, whereby individual models for each $Ca^{2+}$ transport process are assembled together to generate an appropriate, relevant system. This rule is consistent at different levels of computational complexity.[9,10,17] We suggest that viewing "real" microdomains in this modular fashion can aid our understanding of their architecture in live cells. As discussed, the variable expression of individual $Ca^{2+}$ channels and pumps within MCS, on either the source or target membrane, can profoundly alter the properties of microdomains. This "mix-and-match" approach may account for the functionally diverse behaviors that microdomains coordinate. (**Fig. 1**)

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.


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