#37: Soar1 dimer binding to the c-terminus of Orai1 induces clustering

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Store-operated calcium entry (SOCE) is a ubiquitous signaling mechanism in eukaryotic cells crucial for mediating longer term Ca2+ signals and restoring endoplasmic/sarcoplasmic reticulum Ca2+ after ligand induced depletion. The key operators in SOCE are the Ca2+ selective PM Orai1-3 channels and the ER/SR resident, single pass transmembrane calcium sensors STIM1 and STIM2. STIM1 is activated when ER/SR luminal Ca2+ is depleted, inducing it to unfold and bind to Orai1 channels in the PM. Active Orai1 channels create discrete microdomains of high Ca2+ within ER-PM junctions that contain roughly 100-fold greater Ca2+ concentrations than resting cytoplasmic levels. Simulations of Stim-Orai Ca2+ microdomains by Samanta K. et al. reveal that physically coupled Orai1 channels generate more saturated Ca2+ domains at the apposing ER membrane and laterally extend the concentrated domain farther from the channel cluster along the PM surface compared to randomly spaced Orai1 channels within an ER-PM junction. Cryo-EM by Perni S. et al. reveal Orai1 channels within an ER-PM junction are spaced (9-13 nm), roughly the distance between c-terminal binding regions of concatemerized peptides of the Stim-Orai Activating Region (SOAR) of Stim1. Clustering of Orai channels is critical for generating Ca2+ saturated microdomains, however the mechanism of clustering is vague. We have discovered that Orai1 clustering is dependent on the presence of at least two function c-terminal binding domains on Soar concatemer dimers. In HEK Orai1-His cells expressing wildtype Soar-Soar (S-S) concatemers, we observe an increase in puncta formation at the plasma membrane. When one subunit within a Soar concatemer is mutated to F394H (SH-S or S-SH), a residue critical for high-affinity c-terminal binding to Orai1, there is a dramatic decrease in puncta. We also observe that linking a large fluorescent protein, CFP, to the c-terminus of Orai1 can sterically hinder clustering. HEK Orai1-CFP stable cells do not form puncta, regardless of the presence of two functional c-terminal binding domains on Soar concatemers. There is also a functional difference in ICRAC magnitude in HEK Orai1-His cells that is dependent on the presence of two functional Soar subunits. Intriguingly, this dependence is absent in sterically hindered cells. Our data demonstrates that clustering of Orai1 channels is dependent on the presence of two functional Soar subunits, and supports a unimolecular coupling mechanism between Orai and Stim that promotes full channel activity and the generation of Ca2+ saturated microdomains at ER-PM junctions. The Stim2 splice variant Stim2.1 has an additional 8 amino acid insertion within the c-terminal binding domain of the Soar2 region, and concatemerized S-S2.1 peptides behave similar to SH-S or S-SH. This suggests that eukaryotes may have evolved a mechanism mediated through regulation of Stim2 splicing that can affect the clustering dynamics of Orai ER-PM junctions in cells.