protein MreB is known to rotate in the short axis and is dependent on cell wall assembly. When cells were treated with the MreB depolymerizing antibiotic A22 and cell wall growth inhibiting antibiotics, the oscillatory motion of the DNA loci was not disrupted. This suggests that loci oscillatory motion is not related to the observed rotation of MreB, but is driven by an as of yet undiscovered source.

Platform: Calcium Signaling

202-Plat
Conformational Switching Mechanisms Underlying the Activation of Stromal Interaction Molecule 1 (STIM1)
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Physiological Ca^{2+} signalling in T lymphocytes and various other mammalian cells depends on the STIM-ORAI pathway of store-operated Ca^{2+} entry. STIM1 and STIM2 are Ca^{2+} sensors located in the endoplasmic reticulum (ER) membrane, with ER-luminal domains that monitor cellular Ca^{2+} stores and cytoplasmic domains that gate ORAI channels in the plasma membrane. Physiological stimulation_ through the T cell receptor, the Fcε receptor of mast cells, or various G protein-coupled receptors in other cells_ initiates a sequence of ER Ca^{2+} depletion, dimerization or oligomerization of the STIM luminal domain, and targeting of STIM to ER-plasma membrane junctions. STIM at ER-plasma membrane junctions recruits and directly activates the ORAI channel. Here we demonstrate that dimerization of STIM1 ER-luminal domains triggered by dissociation of Ca^{2+} initiates an extensive conformational change in STIM1 cytoplasmic domains that involves apposition of the predicted coiled-coil 1 (CC1) regions, physical extension of the STIM1 cytoplasmic domain, and increased exposure of the STIM1 polybasic C-terminal tail. Together these conformational changes promote interaction of the STIM1 C-terminal region with the plasma membrane, the first essential step for communication of STIM1 with ORAI calcium channels in the plasma membrane.

203-Plat
Distinct Roles of STIM1 and STIM2 C-Terminal Orai-Coupling Domains
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Store-operated Ca^{2+} entry (SOCe) is essential for Ca^{2+} homeostasis and signaling. SOCe is mediated by STIM proteins which function as ER Ca^{2+} store sensors, coupling with and activating PM Orai Ca^{2+} channels. While STIM1-Orai1 coupling is well characterized, the coupling between STIM2 and Orai1 shows some important functional differences from STIM1. The molecular basis of these differences remains uncharacterized. We examined the STIM2 C-terminal (S2-Ct) region that has considerable homology with the known functional coupling domains of S1-Ct. We examined the comparative functions of STIM1 and STIM2 fragments using a combination of Ca^{2+} imaging, patch-clamp current analysis, and analysis of the pharmacological modifier, 2-APB. Deletion of the ''variable'' C-terminal region (534-833) immediately downstream from the STIM-Orai activating region of STIM2 (SOAR2; 435-533) from either whole STIM2 or S2-Ct, had little effect on the activation of Orai1 channels. Similarly, deletion from S2-Ct of the N-terminal region (325-433) upstream from SOAR2, had little effect on Orai1-activation by S2-Ct. Thus the cytosolic regions outside SOAR2 seem to be less important for mediating STIM2 coupling to and activate Orai1. Interestingly, SOAR2 expression alone is sufficient to mimic some of the different coupling properties that distinguish full length STIM2 from STIM1, including the poor intrinsic coupling to activate Orai1 and the strong enhancement of Orai1 activation induced by 2-APB. To gain further insights on how the two SOAR domains couple and activate Orai1, we constructed a series of SOAR1 and SOAR2 chimeras. Using these chimeras, our results reveal that the Sα1-Sα3 helices in the SOAR molecules are important for defining the distinct Orai1 activating properties of STIM1 and STIM2.

204-Plat
Miro: A Driver of the Kinesin Motor
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Kinesin-1 is the primary anterograde microtubule-based motor for mitochondria. Kinesin-1 binds to a unique cargo-binding adaptor protein, Milton, and Milton attaches to the outer mitochondrial membrane protein Miro, tethering kinesin-1 to mitochondria. The distribution of mitochondria within neurons is particularly important. Defects in this transport may underlie several neurodegenerative diseases such as Parkinson's disease. Recent work has identified Miro as a calcium (Ca) dependent regulator of kinesin-1-mediated mitochondrial motility. Miro contains two GTPase domains that flank two Ca-binding EF-hand domains. In this work, we show that purified Miro protein binds both Ca and guanine nucleotides in vitro, but not adenine nucleotides. We also report that Miro does not undergo large conformational changes upon binding Ca or guanine nucleotides, as measured by small angle x-ray scattering. These findings suggest that the presence of a protein binding partner may be required for Ca- and/or nucleotide-induced conformational changes.

205-Plat
ORAI1 Calcium Signaling Regulates the Release of the Atopic Dermatitis Cytokine TSLP
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The cytokine thymic stromal lymphopoietin (TSLP) is highly expressed in human epithelial keratinocytes in atopic dermatitis, and bronchial epithelial cells in asthma. Numerous studies suggest that TSLP acts as a master switch that triggers both the initiation and maintenance of atopic dermatitis, as well as the progression from atopic dermatitis to asthma. While many of the inflammatory actions of TSLP have been described, little is known about the molecular pathways that trigger TSLP expression by epithelial cells and how this leads to itch. Here we probe the signaling mechanisms that lead to TSLP production in human epithelial cells and identify the ion channel ORAI1 as a novel regulator of TSLP secretion. Using pharmacology and siRNA-mediated knockdown, combined with electrophysiological, biochemical and imaging techniques, we show that a variety of itch-causing compounds trigger ORAI1-dependent calcium signaling in human epithelial cells and that ORAI1 is required for the resulting expression and secretion of TSLP. With a combination of cellular and behavioral experiments, we determine that TSLP directly activates sensory neurons to cause itch-evoked scratching in a TRPA1-dependent manner. Our findings demonstrate that ORAI1 is an essential regulator of the atopic dermatitis cytokine and itch-causing compound, TSLP.

206-Plat
Excision of the Cacnb2 Gene in Mice Results in Augmented SR-Ca Release and Impaired Cardiac Function In Vivo
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In mammalian cardiac myocytes, L-type Ca channels comprise at least the pore-forming $Cav_{1.2}\alpha_1$ and an auxiliary Ca_vß2 subunit. Since Ca_vß2 null mice die at embryonic day 10.5, we used our recently described inducible Ca_vß2-KO mouse line. The patch clamp technique was applied to investigate basic properties of L-type Ca channels as well as its ß-adrenergic responsiveness. We employed video-imaging for global Ca transients and ultrafast 2D confocal imaging for studying Ca sparks. High resolution transthoracic echocardiography allowed us to characterise cardiac function and morphology *in vivo*. Despite the absence of the Ca_vß2-subunit, the L-type Ca current's voltage dependence was unchanged but its amplitude was reduced by 25%. Isoproterenol stimulation resulted in a comparable I_{Ca} increase in myocytes from both, mygliol and tamoxifen injected mice. Unexpectedly, global Ca transients displayed an increased steady-state amplitude and the SR-Ca content was significantly augmented as was fractional Ca release. Analysis of Ca sparks revealed a higher amplitude while their frequency was down by more than 50%. When investigating the heart *in vivo* we found indications of dilated cardiomyopathy concomitant with a decreased fractional shortening and ejection fraction. Echocardiographic studies in Doppler mode indicated massive alterations in the E/A wave ratio. From these data we conclude that in adult mice the impairments resulting from Ca_vß2-KO went well beyond solely altering L-type Ca channel function to largely modulating the SR-Ca release process and causing afflicted cardiac functions *in vivo*.
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207-Plat
Rate-Dependent X-Ros Signaling in Cardiac Ventricular Myocytes
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