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we used yeast as a reconstitution system to identify the minimal components sufficient for in vivo uniporter activity. First, we considered Dictyostelium discoideum and showed that it has a highly simplified uniporter machinery: the expression of DdMCU, a single transmembrane component alone is sufficient to reconstitute mitochondrial calcium uniporter activity. Second, to establish human uniporter activity, the coexpression of MCU and - the animal specific protein - EMRE is necessary, whereas expression of MCU alone is insufficient. Our work established yeast as a powerful in vivo reconstitution system for the uniporter to study the evolution and function of this channel.

895-Plat
Fluctuations in Calcium Concentration Alter the Temporal Dynamics of Calcium-Dependent Signaling Cascades
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Calcium signaling is often localized in spatially restricted ‘‘microdomains,’’ which may involve only 1-100 calcium ions. Fluctuations in the local calcium concentration can arise from calcium influx and association/dissociation with calcium buffers [Weinberg and Smith. Biophys J 106(12): 2693 (2014)]. However it is unclear to what extent these fluctuations alter calcium-dependent signaling cascades. We construct a Markov model of a calcium-dependent signaling cascade and compare the first hitting time distribution for a Markov model that accounts for calcium fluctuations, a phase-type distribution that can be calculated from the infinitesimal generator matrix, with the corresponding model that neglects these fluctuations. In general, when calcium fluctuations are much faster than the characteristic time for the signaling cascade, the distributions for the two processes are similar. However, when the time scale of calcium fluctuations is on the same order as the signaling cascade or slower, the mean and variance of the hitting time is increased, in particular when the number of calcium ions is small, a consequence of a long-tailed hitting time distribution. These ‘‘rare events’’ comprising the long tail can be significant and have a physiological impact. We further study calcium fluctuations in two settings: calcium-dependent synaptic vesicle release [Bollmann et al. Science 289, 953 (2000)] and a calcium-release site model composed of calcium-activated calcium channels [DeRemigio and Smith. Cell Calcium 38: 73 (2005)]. In these models, we demonstrate the conditions for which calcium fluctuations alter the distribution, mean, and variance of the timing for synaptic vesicle release and calcium-release site activation, respectively. Under physiological conditions, the mean hitting time can be increased orders of magnitude when calcium fluctuations are accounted for, demonstrating a significant influence on intracellular signaling.

896-Plat
Visualizing Calcium Influx through Single Orai1 Channels
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Orai is the pore-forming subunit of two-component channels that mediate store-operated calcium entry (SOCE). When activated by the ER resident calcium sensor STIM, Orai channels possess high selectivity for calcium but an extremely small conductance (~10 fS in 2 mM Ca^{2+} from noise analysis) that has precluded direct recording of single channel currents. We have developed an approach to visualize the activity of single Orai1 channels by fusing human Orai1 to a fluorescent, genetically-encoded calcium indicator (GECI). Whole-cell recording in HEK 293 cells co-transfected with STIM1 showed that GECI-Orai1 is fully functional as a CRAC channel, with normal activation rates, current amplitudes, ion selectivity and rectification, and Ca^{2+}-dependent inactivation. When GECI-Orai1 and the CRAC-activating domain (CAD) of STIM1 were co-expressed at low levels and imaged using a TIRF microscope, cells exhibited sporadic fluorescence transients the size of diffraction-limited spots and the brightness of a few activated GECI proteins. Transients typically rose to a maximum within 100-200 milliseconds and fell into two classes according to duration: briefer ‘‘flickers’’ lasting only a few hundred milliseconds, and longer ‘‘pulses’’ lasting ~1 to several seconds. The frequency of both classes increased as CAD expression levels were increased. GECI-Orai1 transients exhibited physiological characteristics expected for single Orai1 channels in the plasma membrane, including rapid response to changes in extracellular calcium, enhancement by valinomycin-induced hyperpolarization, and block by trivalent cations. When single molecule GECI-Orai1 traces were aligned by the rise in green fluorescence, a corresponding rise in mCherry-CAD fluorescence, indicative of binding, was observed. These data indicate that GECI-Orai1 transients correspond to the opening of single Ora1 channels gated by CAD. These first recordings of single channel Orai1 currents reveal unexpected dynamics, and when paired with CAD association, support multiple single channel states.

897-Plat
Functional Reconstitution and Structural Flexibility of the CRAC Channel Orai
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Calcium (Ca^{2+}) release-activated Ca^{2+} (CRAC) channels mediate Ca^{2+} influx across the plasma membrane in response to Ca^{2+} store depletion in the endoplasmic reticulum (ER). CRAC channel function requires two components: the plasma membrane Ca^{2+} channel Orai and its regulator located in the ER membrane, the Ca^{2+} store sensor STIM. Using liposomes reconstituted with a purified fusion protein of human Orai1 and cytosolic fragments of human STIM1 (hO1-SS), we show that Orai1 and STIM1 are sufficient to form active CRAC channels in vitro. Reconstituted hO1-SS recapitulates CRAC channel properties as shown by detection of sodium (Na^{+}) flux in the absence of Ca^{2+} and by direct detection of Ca^{2+} flux. 2-APB, a known CRAC channel inhibitor, blocks both fluxes. Our findings confirm that human STIM1 gates the pore of Orai1 and demonstrates that the two proteins are sufficient to form functional channels in the absence of other cellular factors. Previously, we published the crystal structure of drosophila Orai in a closed state. Here we present low-resolution X-ray diffraction data of human Orai1, which indicate an overall structure that is indistinguishable from drosophila Orai. In addition, a new 4.25 Å resolution X-ray structure of drosophila Orai reveals an extended conformation of the fourth transmembrane helix (M4) at the periphery of the channel that extends into the cytosol and is strikingly different from the arrangement of these helices in the previous structure. The comparison of structures reveals conformational flexibility that starts from the M4 helices and continues into the cytosolic M4 extension helices. In all of the structures, the pore adopts the same conformation and remains closed. The conformational flexibility observed for the M4 and M4 extension helices may have a role in the binding of STIM and in signal transduction from the ER.

898-Plat
Thermodynamic and Structural Analysis of Calmodulin Interaction with the Skeletal Muscle Ryanodine Receptor
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In skeletal muscle, the calcium sensor calmodulin (CaM) plays a key role in excitation-contraction coupling, the process of translating neuronal stimuli into mechanical contraction of muscle, by regulating the opening and closing of the calcium channel ryanodine receptor (RyR1). By interacting with this channel differently at high and low calcium, CaM acts as a feedback regulator of calcium levels during muscle contraction: at low calcium, CaM weakly activates RyR1, while calcium-CaM inhibits it. We are investigating the interaction between CaM and its established binding site on RyR1 (CaMBD, residues 3614-3640) using a multi-faceted approach combining biophysical (fluorescence spectroscopy) and structural (solution NMR) methodologies. Förster resonance energy transfer (FRET) experiments in an auto-fluorescent biosensor construct (YFP-CaMBD-CFP) enabled us to determine Gibbs free energies of binding in the absence and the presence of calcium. Using this system, we systematically explored the thermodynamics of molecular recognition between the two biomolecules in high and low calcium environments, as well as the roles played by individual RyR1 residues and each CaM lobe at the interface. To gain additional insights into the interplay between the processes of calcium- and target-binding to CaM, we analyzed the interaction between wild-type or mutated RyR1 CaMBD sequences and CaM mutants in which the calcium-binding sites in one domain had been rendered non-functional. Overall, these experiments show that CaM C-domain binding to molecular determinants in the N-terminus of RyR1 CAMBD dominates the interaction, both in the presence and the absence of calcium. However, the interaction is three orders of magnitude stronger at high calcium levels. To obtain residue-specific information of the binding interface, we are currently undertaking NMR studies of isotopically labeled CaM (wild-type and calcium-binding mutants) in the absence and presence of wild-type and mutated RyR1 sequences.

899-Plat
Stretch-Induced Changes in Atrial Ca Signaling
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Atrial myocytes undergo stretch during diastole as do ventricular myocytes. We have identified a novel mechanism that links cellular stretch in ventricular myocytes to the tuning of Ca^{2+} release from the sarcoplasmic reticulum (SR)(Prosser et al. Science 2011;333:1440-5). This mechanism, ‘‘X-ROS