we used yeast as a reconstitution system to identify the minimal components sufficient for in vivo unipporter activity. First, we considered Dictyostelium discoidium and showed that it has a highly simplified unipporter machinery: the expression of DmMCU, a single transmembrane component alone is sufficient to reconstitute mitochondrial calcium unipporter activity. Second, to establish human unipporter 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 unipporter to study the evolution and function of this channel.

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 distribution of the two models 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.

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 Ca2+). When GECI-Orai1 and the CRAC-activating domain (CAD) of STIM1 were expressed in HEK 293 cells, we visualized the activity of single Orai1 channels by fusing 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 Ca2+ and by direct detection of Ca2+ 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 distinguishable from drosophila Orai. In addition, we report a new, 4.25 Å resolution X-ray structure of drosophila Orai reveals an extended 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.