providing a Ca2+ influx pathway (store operated calcium entry, SOCE) that is essential for proper immune cell function. Careful titration of the protein concentration of STIM1 and Orai1 thus determines the amount of Ca2+ influx and subsequently its downstream effects. Because changes in the degree and quality of glycosylation can affect immune cell development and function, we investigated the impact of altered glycosylation on SOCE. Indeed, inhibition of oligosaccharyltransferases increased SOCE in Jurkat T cells. To delineate the contribution of Orai1 and Stim1, we mutated their potential N-glycosylation sites. However, conventional replacement of the consensus asparagines by glutamines in STIM1 (N/Q) led to a reduction in Orai1 mediated currents. Other amino acid substitutions at the same positions led to variable degrees of current modification with one mutation leading to significantly increased current sizes. Interestingly, this mutation correlated with a change in Orai1 protein concentration and led to a change in the STIM1:Orai1 stoichiometry towards an optimized ratio for Orai1 activation. Noise analyses of Orai1 mediated sodium currents revealed an increase in the number of active channels, with little change in open probability or estimated single channel conductance. The phenotype of the mutant could only be partially mimicked by alteration of wildtype protein ratios between Orai1 and STIM1, suggesting an additional influence of the mutation on the EF-SAM domain stability and function. Our current data suggests that our gain-of-function STIM1 mutant may overcome the negative cooperativity which limits interaction of wildtype STIM1 with Orai1.

3453-Pos Board B314

Gating and Assembling Mechanisms of CRAC Channels

Hongying Zheng[1], Changlong Hu[2], Junjie Hu[3], Lih Kuo[1], Shenyuan L. Zhang[1].
[1]Department of Systems Biology and Translational Medicine, Texas A&M Health Science Center, Temple, TX, USA, [2]Department of Physiology and Biophysics, School of Life Sciences, Fudan University, Shanghai, China, [3]Department of Genetics and Cell Biology, College of Life Sciences and Tianjin Key Laboratory of Protein Sciences, Nankai University, Tianjin, China.

Sustained calcium entry through Calcium Release-Activated Calcium (CRAC) channels is essential for T cell activation and proliferation via sensing the depletion of endoplasmic reticulum (ER) calcium-store by STIM1 subunits and the opening of pore-forming Orai1 subunits. However, the molecular mechanism underlying this process remains elusive. Using gain-of-function human Orai1 point mutants with Aspartate and Proline substitutions on a conserved Glycine residue (98) in the middle of transmembrane segment (TM) 1, we have previously suggested that the putative conformational change at this G98 site (gating-hinge) is a key step toward channel opening. Herein, we found in the mutation studies that the elongated side-chains at this 98-site could constitutively gate the corresponding channels through a conserved ''bent-hinge'' mechanism without affecting permeation pathway. We further demonstrated in Orai1-G98X mutants that the spontaneous opening of Orai1 channels is independent of STIM1 and store content. A truncated Orai1-G98X mutant without both intracellular N- and C- termini exhibited similar store-independent calcium entry as full-length Orai1-G98X mutants, indicating that Orai1 TM region is fully capable of and responsible for pore-forming and channel assembling. Moreover, truncated Orai3 proteins lacking both N- and C- termini remained responsive to 2-aminoethyl diphenylborinate stimulation, but not to store-depletion, indicating the structural requirement for channel assembling and pore formation. These results provide molecular details of the assembling and gating mechanisms of CRAC channels and support the Glycine ''gating-hinge'' hypothesis.

3454-Pos Board B315

Super-Resolution Imaging Reveals a Multi-Array Arrangement of Catsper Channel on the Sperm Tail

Jean-Ju Chung[1], Sang-Hee Shim[2], Xiaowei Zhuang[2], David E. Clapham[1].
[1]Howard Hughes Medical Institute, Children's Hospital Boston, Harvard Medical School, Boston, MA, USA, [2]Howard Hughes Medical Institute, Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA.

Increase in intracellular Ca2+ initiates hyperactive motility of sperm, the high amplitude and often asymmetric movement of the tail. Cationic channel in sperm (CatSper) genes encode a complex of Ca2+ channel in sperm required for hyperactivation and male fertility. However, it is unclear how the CatSper-mediated Ca2+ entry increases flagellar bending and induces hyperactivated motility. Using high-resolution fluorescence microscopy based on high-accuracy localization of photoswitchable fluorophores, or stochastic reconstruction microscopy (STORM), combined with electron microscopy, we examined three-dimensional distribution of CatSper channels and flagellar proteins on the sperm tail. The CatSper channels form a unique three-dimensional multi-array arrangement, which may explain the characteristic patterns of hyperactivated motility.

3455-Pos Board B316

Contribution of the Epithelial Sodium Channel to Chondrocyte Regulatory Volume Increase

Rebecca Lewis, Lucy Gentles, Richard Barrett-Jolley.
University of Liverpool, Liverpool, United Kingdom.

Chondrocytes are the cells of articular cartilage, responsible for the production and maintenance of the extracellular milieu. They exist in a constantly changing osmotic environment and to survive such osmotic changes they must be able to quickly and effectively regulate their volume. Cells use regulatory volume increase (RVI) to oppose osmotic shrinkage. It has previously been shown that the epithelial sodium channel (ENaC) is important to this process in rat hepatocytes[1]. Here we investigate the possible contribution of ENaC to RVI mechanisms in canine chondrocytes.

Chondrocytes were isolated from cartilage according to standard methods[2]. Amiloride sensitive single-channel activity reversed at a membrane potential of -1 ± 5mV ($n = 5$), mean conductance was 9 ± 0.4pS ($n = 5$) and kinetics were slow. The calculated E_{Na} under these conditions was -6mV, which coupled to the very small conductance would be consistent with this channel being an ENaC. Channel open probability in control conditions was 0.3 ± 0.06 and decreased by $97 \pm 2\%$ after application of the ENaC inhibitor, amiloride (10μM; $n = 3$).

Upon exposure to hypertonic solution, cell volume decreased significantly by $35 \pm 3\%$ ($n = 5$; $p<0.001$). Within 20 minutes of reaching their smallest size, cells under control conditions had returned to $92 \pm 4\%$ of their original volume, not significantly different to starting volume ($p=0.07$). When 100nM benzamil, a specific ENaC inhibitor, was added to the hypertonic solution, cells shrank by $41 \pm 3\%$ ($n = 5$) and were unable to return to their original volume.

These data suggest that ENaC contributes to RVI in canine chondrocytes.

1. F. Wehner, C. Bohmer, H. Heinzinger et al., *Cellular Physiology and Biochemistry* **10** (5-6), 335 (2000).
2. R. Lewis, K. Asplin, G. Bruce et al., *J Cell Physiol* **226** (8) (2011).

3456-Pos Board B317

Electrostatics in the NMDA Receptor Transduction Pathway Alter Gating

Rashek Kazi, Iehab Talukder, Lonnie P. Wollmuth.
Stony Brook University, Stony Brook, NY, USA.

Most excitatory synaptic transmission in the mammalian central nervous system is mediated by the neurotransmitter glutamate. Fast glutamatergic signaling is critical to cell-to-cell transmission, normal brain development, and learning and memory. NMDA receptors are a glutamate receptor subtype with high calcium permeability and slow gating kinetics. Although gating - the process of ligand binding/unbinding resulting in pore opening/closing - is a critical component of NMDA receptor function, our understanding of it remains incomplete. We used the GluA2 crystal structure to generate a rudimentary GluN1/GluN2A homology model. From this, we found that the NMDA receptor linkers, which connect the extracellular ligand binding domain (LBD) to the pore-forming transmembrane domain (TMD), contain a network of proximally lying charged residues. We hypothesized that the proximity of these charged residues might influence gating. To test this, we substituted proximal residue pairs with cysteines (to induce cross-linking). We found, for example, that currents in GluN1/GluN2A (E638C, K785C) receptors potentiated 150-fold when exposed to the reducing agent DTT. This suggests that these residues are proximal and have state-dependent positioning. To test that these residues interact electrostatically, we created charge reversal mutants and quantified single-channel activity. We found profound gating perturbations as each mutant showed significant differences in gating properties (open channel probability, mean closed time, and mean open time). We are currently in the process of performing double-mutant cycle analysis on double-charge reversal mutants to quantify the degree of charge-charge interaction. Thus, by making superficial, albeit critical, observations from our model, we are able to provide a potential physiological role for electrostatic interactions in NMDA receptor gating.

3457-Pos Board B318

Yellow Optogenetics with *Volvox* Channelrhodopsin Variants

Franziska Schneider[1], Matthias Prigge[1], Satoshi P. Tsunoda[1], Ofer Yizhar[2], Karl Deisseroth[2], Peter Hegemann[1].
[1]Humboldt University, Berlin, Germany, [2]Stanford University, Stanford, CA, USA.

Channelrhodopsins (ChR) are light-gated ion channels and rhodopsins with internal ion conducting pores. In 2001 ChR1 and ChR2 cDNAs were identified in