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613-Pos Board B399
Activation of Ms Channel by LPC: A Trigger for MscL. Gating in the Absence of Applied Tension
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Mechanosensitive Channel of Large Conductance (MscL) allows bacteria to respond to osmotic stress in the environment. It senses the increase in the lateral pressure in the membrane by sudden hypo-osmotic shock and acts as a safety valve. MscL has one of the largest pores in nature; in its open state it allows the passage of ions and small molecules up to 6.5 kDa. MscL has been used in this study as an externally controlled valve i.e. the opening of the channel is controlled by external stimuli.

Several techniques like patch clamp, EPR spectroscopy has been applied towards elucidating the gating mechanism of MscL. EPR is effective in tracking the initial conformational changes that the protein may undergo during gating. The main challenge in using spectroscopy is that, unlike patch clamp technique, tension cannot be applied directly for opening the channel. L-α-lysophosphatidylcholine, a reported activator of MscL was studied in this work to trigger opening of the channel in a controlled way. In our work we provide evidence that LPC mimics tension in opening the channel. Our findings also clearly show that LPC can be used for phenotypic characterization of MscL mutants, in a much simpler experiment than patch clamp. A clear differentiation in activity between GOF, LOF and WT MscL is observed at 4 µM LPC. In conclusion, we characterized an activator with which the mechanism of channel gating can be studied in a controlled way.

614-Pos Board B400
Piezo1 Gating: Comparison Between Whole Cell Currents and the Patch Philip Gottlieb, Chilman Bae, Frederick Sachs.
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Piezo1 channels gate with mechanical stress in the membrane and gating involves both activation and inactivation. In HEK293 cells transfected with Piezo1 and subjected to pressure stimuli, cell-attached patch recordings showed that the inactivation rate slowed as extracellular divalent ions were reduced. With >1mM Mg2+, activation had no measurable latency and the inactivation rate was rapid but stress dependent, suggesting that Mg2+ may act as an open channel blocker (the effects of Ca2+ are in progress). Without divalents there was no inactivation, but surprisingly, activation now had a pronounced latency (~500 ms). Inactivation may actually represent adaptation of the local stimulus by the cytoskeleton and not overt channel closure. To disrupt the cytoskeleton we treated cells with cytochalasin D before patching and found inactivation was unaffected suggesting cytoskeletal adaption was not the cause. The attempt of the inverse experiment, we increased cytoskeletal stress by swelling the cells osmotically, but that too didn’t affect the inactivation rate.

For analogy close to the in situ situation, we evoked whole cell Piezo1 currents by indenting cells with a glass probe. Like the patch, removing extracellular divalent ions reversibly reduced the inactivation rate. However, in contrast to patch recordings, Cytochalasin D caused a loss of whole cell current and cell swelling increased the evoked currents. These results suggest that the forces that gate Piezo1 in whole cell mode propagate through the cytoskeleton, and that divalent ion block may be responsible for inactivation.

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615-Pos Board B401
A High-Throughput Technique for Screening Novel Antibacterial Agents Targeting Bacterial Mechano-sensitive Channels
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Mechanosensitive channels are activated by sensing membrane tension. MscL is a homopentamer of a subunit with transmembrane inner (TM1) and outer (TM2) helices and TM1s line the ion/water permeable pore. We have analyzed the gating properties of MscL using patch-clamp experiments and simulations such as all-atom (AA) molecular dynamics (MD). However, in AA MD simulations, we need to apply about 10 times bigger membrane tension than experimentally applied to open the pore. In this study, we performed coarse-grained (CG) MD simulations to reproduce the opening process of MscL under appropriate conditions based on the experimental ones and to find the differences of the conformational changes of transmembrane helices of the E-Coli Mechanosensitive Channel MscL. These respond respectively to large and small osmotic pressures, and eliciting ion conductances of ~5nS and ~1nS. Both MscL and MscS exhibit strong homology across all bacteria. In this study we report the use of a combination of spectroscopy to determine if MscL and MscS channels in a family of tethered bilayer membrane systems as a high-throughput technique that can be used to screen for potential lead compounds for the development of novel antibacterial agents that interfere with the function of MS channels. Using either a swept frequency Bode profile or a single frequency impedance measure, plates of 96 electrodes may be screened simultaneously. Typical conditions are a measurement of resistance in the range of 100-1000 kΩ in response to excitation over frequency in the range of 0.1Hz to 1kHz. The robustness of the tethered membrane permits modulation of the MS channel conductance through an alteration in the membrane thickness. This can be achieved through the application of large transmembrane potentials or the dilution of the membrane lipids with surfactants possessing varying hydrophobic chain lengths.