Activation of Ms Channel by LPC
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were reduced. It senses the increase in the lateral pressure induced by a 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 con-

trolled by external stimuli.

Several techniques like patch clamp, EPR spectroscopy has been applied to-

wards 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-lyso phosphati-
dyline, 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 mutations, in a much simpler experiment than patch clamp. A clear differentiation in ac-
tivity between GOF, LOF and WT E. MscL is observed at 4 μM LPC. In conclu-

sion, we characterized an activator with which the mechanism of channel
gating can be studied in a controlled way.

Piezo1 Gating: Comparison Between Whole Cell Currents and the Patch

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Piezo1 channels gate with mechanical stress in the membrane and gating in-

volves 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 to

the inverse experiment, we increased cytoskeletal stress by swelling the cells os-
motically, 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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A High-Throughput Technique for Screening Novel Antibacterial Agents

Targeting Bacterial Mechanosensitive Channels

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Mechanosensitive ion channels are essential for maintaining cellular homeosta-
sis. It has been proposed that they function as osmotically activated emergency valves, guarding against membrane rupture by opening pores to release excess-
tive turgor pressure. Interfering with their function suggests many novel mecha-

nisms to complement conventional antimicrobials to kill or inhibit growth of

bacterial pathogens. Typical of this class of mechanosensitive channel are the large conductance homomultimeric bacterial channel, MscL (MW~17kDa) and the small conductance homohexameric bacterial channel, MscS (~37kDa). These respond respectively to large and small osmotic pressures, and elicit ion conductances of ~5nS and ~1nS. Both MscL and MscS exhibit strong ho-
mology across all bacteria. In this study we report the use of a capacitance

despectroscopy of pure 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 re-

sistance in the range of 1kΩ to 100MΩ 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 mem-

brane thickness. This can be achieved through the application of large trans-

membrane potentials or the dilution of the membrane lipids with surfactants

possessing varying hydrophobic chain lengths.

Mechanotransduction in A549 Alveolar Cells via Cell Stretc-Induced

ATP Release

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Mechanotransduction at the cellular/tissue level often involves release of signal-

ing molecules. Among them, purines appear to be the most primitive and wide-

spread chemical messengers in the animal and plant kingdoms. Their release is

highly mechano-sensitive, but the release pathways and regulatory mechanisms

are not well understood. Here, we investigated the effect of unidirectional stretch on ATP release from human lung A549 alveolar cells grown on a flexible sub-

strate. We used real-time luciferin-luciferase bioluminescence imaging combined with IR imaging to simultaneously monitor cellular ATP release and extend of cell stretch. Single 1-s stretch of 15-30% induced transient ATP re-

lease that ceased in 2-3 min and was restricted to a limited number of cells. The number of responding cells increased dose-dependently with the extent of stretch but did not involve cell damage. Calibaration of the ATP response showed that local ATP concentration in the close proximity (~150 μm) to stretch-

activated cells may exceed 1 μM or even 10 μM. These concentrations are suf-

ficient for autocrine/paracrine stimulation of cell surface purinergic receptors on

the neighboring cells. ATP responses were insensitive to putative ATP channel

blockers carbeneoxolone or NPBP (100 μM), inhibitors of pannexin or annion channels respectively, but were abolished by N-ethylmaleimide. Fluoro fluores-

cence measurement of stretch-induced intracellular Ca2+ responses revealed that limited number of cells displayed rapid responses, which peaked in <1-s and ceased in 1-3 min. This is similar to stretch-induced ATP responses and sug-

gests functional connection between the two signals. Experiments show that cell

stretch induces ATP release via cell-regulated process, likely exocytosis. Mechano-sensitive ATP release, via autocrine/paracrine effects, initiates puri-

nergic signaling cascade in other cells and may function as a general intercellular

mechanotransduction paradigm in the lung and other tissues.

ATP Release

Coarse-Grained Molecular Dynamics Simulation Study Focusing on the

Conformational Changes of Transmembrane Helices of the E-Coli Mecha-
nosensitive Channel MscL

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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 simu-

lations, we need to apply about 10 times bigger membrane tension than exper-

imentally applied to open the pore. In this study, we performed coarse-grained

(CG) MD simulations to reproduce the opening process of MscL under appropi-

ate conditions based on the experimental ones and to find the differences of

the conformational changes of transmembrane helices between wild type (WT)

and mutant models (F78N and G222N). We constructed CG MscL model based on the equilibrated all-atom closed model. After constructing, MscL was en-

bedded in the lipid bilayer and water beads were added. Then we performed CG MD simulations for the opening of MscL under 60 bar, about three times bigger membrane tension for 1 μs. Upon membrane stretch, all types of MscL could open its pore during the simulations. In order to analyze the cor-

relation of the conformational changes between TM1 and TM2 helices, we

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