bleb patches appears to be larger than proposed previously (Honore et al., 2006, PNAS **103**:6859).

1310-Pos Board B154

Rapid And Efficient Co-reconstitution Of Bacterial Mechanosensitive Ion Channels Of Small And Large Conductance Into Liposomes

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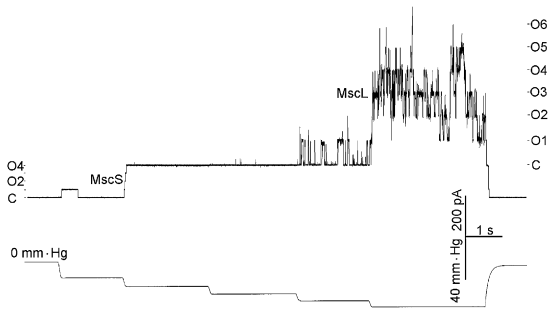
Bacterial mechanosensitive (MS) channels protect bacterial cells from osmotic shock, acting as emergency relief valves (1,2). *E. coli* has three such channels, the MS channel of large conductance (MscL), the MS channel of small conductance (MscS) and the MS channel of mini conductance (MscM). Both MscL and MscS have been extensively studied using the patch-clamp technique in giant spheroplasts (3,4). However, only MscL incorporates efficiently in liposomes (2,5). Here we report the first example of co-reconstitution of both MscS and MscL into azolectin liposomes. We also report reconstitution efficiencies of both proteins into liposomes of different lipid composition and using different incorporation methods.



1. Martinac, B., J. Cell Sci., 117, 2449-2460 (2004).
2. Perozo, E., Nat. Rev. Mol. Biol., 7, 109-119 (2006).
3. Martinac, B., Buechner, M., Delcour, A.H., Adler, J., Kung, C. PNAS, 84, 2297-2301 (1987).
4. Booth, I.R., Edwards, M.D., Black, S., Schumann, U., Miller, S. Nat. Rev. Microbiol., 5, 431-440 (2007).
5. Häse, C.C., Le Dain, A.C., Martinac, B., J Biol. Chem, 270, 18329-18334 (1995).

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1311-Pos Board B155

Modulating The Conductance And Ionic Preference Of MscL, A Biological Nanovalve

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The bacterial mechanosensitive channel of large conductance, MscL, has many properties that are ideal for use as a nanosensor. Previous studies have shown that the pore size is huge (>30Å), it can be translated *in vitro* or synthetically synthesized, and it can spontaneously assemble into a functional complex. In addition, the modality of the channel can be changed; studies have shown that the sensor can be engineered to be sensitive to light, pH, and post-translational chemical modification as well as modulated by different heavy metals and redox. Hence, many studies have suggested its potential in nano-technological applications such as nano-scaled sensors in microchips and drug delivery systems. On the other hand, its large conductance may actually be limiting in some microchip applications, and even though some molecules pass through the MscL nanovalve, modifying its ionic preference could have advantages for vesicular release of charged compounds. Here we demonstrate that MscL can be molecular engineered to have altered conductance or ionic preference to better serve specific purposes. We found that constricting the cytoplasmic loops between the pore and a C-terminal cytoplasmic helical bundle of the channel by bridging cysteines, or coordinating heavy metals with histidines, can decrease the channel conductance as much as 50%; in both instances, the change is reversible. In addition, we found that the ionic preference of the channel can be modified by altering residues near the pore; changing the ionic preference of MscL towards anionic alters the permeability of spermidine, a polycationic organic compound. In summary, our results demonstrate that the conductance and ionic preference of the MscL nanovalve can be modified, and thus designed for specific applications. Keywords: MscL; channel conductance; ionic preference; Nanotechnology.

1312-Pos Board B156

Adaptive Behavior of Bacterial Mechanosensitive Channels in Excised Patches is Coupled to Membrane Mechanics

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MscS, a tension-driven osmolyte release valve residing in the inner membrane of E. coli, exhibits complex adaptive behavior, whereas MscL, its functional counterpart, was considered non-adaptive. When a membrane patch is held under a constant non-saturating pressure gradient, MscS exhibits desensitization (mode-shifting) manifested as a reversible closure followed by complete inactivation. Attempts to utilize MscL as a non-adaptive 'reference' channel revealed that a prolonged exposure of patches to sub-threshold tensions right-shifts activation curves for both MscS and MscL with similar magnitudes and time courses. MscS channels were also found to retain a 'memory' of prior desensitization, returning to the mode-shifted state after being fully opened by a saturating pressure pulse. When recorded in the whole-spheroplast mode under positive pipette pressure, MscS shows no desensitization whereas some inactivation still occurs. We thus link desensitization observed specifically in excised patches with mechanical relaxation of the inner leaflet not attached to the glass pipette, which may create a distribution of tension less favorable for opening. To further characterize and separate the processes of desensitization and inactivation in MscS we applied multi-pulse pressure protocols to excised patches. The results indicated that membrane tension slows reversible closure (desensitization) but speeds up inactivation and strongly impedes the process of recovery from inactivation. These dependencies indicate that the MscS channel contracts in the plane of the membrane when it reversibly desensitizes, but further expands when it inactivates. This calls for models with a more compact gate formed by the TM3 helices in both closed and inactivated states. In contrast, the peripheral transmembrane helices (TM1-TM2) can assume different conformations to confer a larger in-plane area for the inactivated state.

1313-Pos Board B157

Gating Of Bacterial Cyclic Nucleotide Gated (bCNG) Channels In Response To Membrane Tension

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We have identified, cloned, and characterized a new family of bacterial cyclic nucleotide gated (bCNG) ion channels. While we have demonstrated that these channels gate in response to cyclic adenosine monophosphate (cAMP), all bCNG channels exhibit significant homology to the pore lining helix (TM3) of the mechanosensitive channel of small conductance (MscS). This homology suggests that these channels might gate in response to mechanical stress in addition to ligand binding. To test this hypothesis, we have explored the ability of bCNG channels to rescue MscL/MscS/MscK null *E. coli* from osmotic downshock. While some homologues, such as bCNG from *Synechococcus sp. PCC 6803,* show rescue similar to that observed for wild-type *E. coli* MscS, other homologues, such as bCNG from *M. loti,* do not exhibit osmotic rescue. In the bCNG channel family, the numbers of transmembrane domains varies from two to six, with high homology between pore lining helices. Our current data implies that bCNG channels with greater homology to MscS are more likely to be mechanosensitive.

1314-Pos Board B158

Caveolae Act As Membrane Reserves Which May Limit $I_{Cl,swell}$ Activation During Cardiac Myocyte Swelling

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The channel responsible for swelling-activated chloride current ($I_{Cl,swell}$) is a mechanosensor which responds to changes in membrane tension during cell swelling, and regulates cell volume. It has been proposed that the $I_{Cl,swell}$ channel (or elements that regulate this) are dependent on caveolae, and we have previously shown that disrupting caveolae increases the rate of hyposmotic cardiac myocyte swelling. Here we test the hypothesis that the role of caveolae as a membrane reserve limits activation of $I_{Cl,swell}$. Rat ventricular myocytes were treated with methyl- β-cyclodextrin (MBCD) to disrupt caveolae and exposed to 0.02T solution (until cell lysis) or 0.64T solution for 10-15 min (swelling). Maximum cell volume achieved prior to lysis was calculated from a video image. Swollen cells (0.64T) were fixed for electron microscopy, and the negative inotropic response to swelling used as an index of $I_{Cl,swell}$