protoplast formation increases the probability of mechanosensitive activity in a patch. Furthermore, a new mechanosensitive channel conductance (~1nS) is revealed in ~40% of patches. Tests so far indicate that 0.5M KCl incubations do not cause the same effect. Further analysis of these novel activities will enhance our knowledge of mechanosensitive channels in bacteria.

1528-Pos Board B438

Combining Scanning Probe and Confocal Microscopy to Investigate the Biophysical Properties of the Primary Cilium

Stephen J. Kocher, David N. Sheppard, Terry J. McMaster.

Primary cilia are non-motile specialised sensory organelles that protrude from the surface of epithelial cells. In the kidney, they are 200 nm in diameter and extend approximately 10 μm in length into the lumen of the nephron. Ciliary dysfunction is linked to autosomal dominant polycystic kidney disease, the most common of the inherited cystic diseases. The manner in which the cilium bends in response to fluid flow along the nephron and the mechanisms leading to the intracellular calcium release are widely debated. To gain insight into the mechanism of ciliary bending, we investigated the biophysical properties of the primary cilium. Here, a TCS SP5 Confocal Microscope (Leica, Wetzlar, Germany) was used to identify cells with a primary cilium and then position a cantilever in a Nanowizard II Atomic Force Microscope (JPK, Berlin, Germany) in the near vicinity of the cilium. We controllably probed individual primary cilia with the AFM cantilever tip to obtain a vertical deflection map in one plane. By probing primary cilia at different heights from the surface of the cell, we produced AFM deflection volume maps. This detailed probing of the mechanical response of the cilium is a mechanical analogue of the z-stack optical imaging employed in confocal microscopy. Thus, using our technique, we have quantified the rigidity of the primary cilium.

Funded by BBSRC, EPSRC and JPK Instruments (Berlin, Germany)

1529-Pos Board B439

Bacteria Regulate Turgor Pressure in Order to Grow

Teuta Pilizota, Joshua W. Shaevitz.

The extreme concentrations of chemicals in a bacterium's cytoplasm generate an osmotic pressure that inflates the cell. It is thought that *E. coli* use a number of interconnected systems to adapt to changes in external pressure, allowing them to maintain turgor and live in environments that range more than two-hundred-fold in external osmolality. To date, how this adaptation is achieved and why pressure is required for survival have been poorly addressed. Previous measurements of osmoregulation in bacteria have been unable to directly probe a single cell's adaptation, focusing instead on the activity of various transporters, or changes in population growth rates. I will show that different mechanisms used by bacteria can be explored using fluorescence imaging to monitor changes in cell shape during adaptation on a single cell level with a time resolution on the order of seconds. This type of measurement allows the different adaptation pathways to be studied individually as well as in defined groups. Furthermore, I will demonstrate that using a number of interconnected systems; bacteria actively regulate their turgor pressure to a preset value, despite changes in their local environment. This precise value of the turgor pressure is required for cell growth and I will make direct connections between pressure adaptation and growth rate in challenging environments.

1530-Pos Board B440

Molecular Organization of the Distal Cilium, a Potential Mechanotransduction Site, in Campaniform Receptor of Drosophila Melanogaster

Xin Liang, Johnson Madrid, Henri Saleh, Jonathon Howard.

The observation of a very short latency between stimulus and cell response in mechanosensation led to the gating-spring concept. The central idea is that the gating of mechanosensitive channels is directly controlled by a compliant structure, the gating spring. This model suggests a transduction apparatus based on the physical organization of several molecular components. We are studying the identities and organization of these molecular components by using campaniform receptor in fly as a model system.

The distal cilium of campaniform receptor is in close proximity to the cuticle and directly connects to the extracellular matrix. We found that NOMPC, a candidate of the mechanotransduction channel, exclusively localizes to this region by immunofluorescence with an antibody against N-terminal of NOMPC. We also found that the electrical response of campaniform receptors is absent in *nompC* mutant. These observations suggest that the distal cilium is the transduction site in the campaniform receptor. Transmission EM of this region reveals a particular microtubule-based structure and membrane-microtubule connection (MMC), which we have analyzed quantitatively using image analysis software. This method was applied to several mutants. First, ultrastructural defects in both microtubule-based structure and MMC in this region were found in a *nompC* mutant, suggesting an important role of NOMPC in the structural organization of this region. Second, the microtubule-based structure in DCX-EMAP mutant is disrupted. Although the localization of NOMPC seems normal, the electrical response of campaniform receptor in DCX-EMAP mutant is altered in both amplitude and time course, suggesting that microtubule structure is involved in shaping the electrical response. In summary, our data provide further information about the general architecture of the transduction site in campaniform receptor and the possible roles of several molecular components in its organization.

1531-Pos Board B441

Role of Muscle Specific Caveolin-3 in Mechanosensitive Channel Regulation

Thomas M. Suchyna, HaiXia Huang.

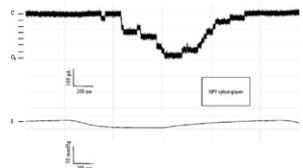
Caveolin-3 (Cav-3) is a small muscle specific scaffolding protein that acts to complex signaling proteins within cholesterol rich raft domains through a small cytoplasmic binding domain. Cav-3 has the unique function of inserting only into the inner membrane leaflet causing expansion of the inner surface that induces curvature and membrane involutions called caveolae. In muscle the dystroglycan complex is localized to the caveolae and is the cortical cytoskeleton attachment point for dystrophin and other proteins which regulate sarcolemma tension. These attributes make caveolae ideal mechanosensing structures and likely locations for mechanosensitive channels (MSCs). To understand the role of Cav-3 in MSC regualtion we depleted membrane cholesterol with methyl β-cyclodextran which caused disruption and internalization of the caveolae protein and increased MSC activity in patch pipette recordings. Overexpression of a Cav-3-GFP fusion protein in well differentiated myotubes also leads to increased MSC activity. Reduction of Cav-3 with miRNA produced no change in MSC activity, though baseline MSC activity is already low in differentiated myotubes. To rapidly and specifically inhibit Cav-3 interactions, we fused the 20 amino acid Cav-3 scaffolding domain the 16 amino acid antennapedia membrane translocation signaling domain (Anten-CSD). Treatment of myotubes with 20 μM Anten-CSD caused internal Ca^{2+} to increase, large blebs to formed on the sarcolemma and Cav-3-GFP aggregation on the surface. There was an increase in MSC activity within 20 minutes after Anten-CBD treatment. We are currently investigating a link between Cav-3 and TRP channels. These studies suggest that Cav-3 may be involved in both localization of MSCs to the sarcolemma and regulating channel activity in myotubes through its ability to complex MSCs with the cortical cytoskeleton.

1532-Pos Board B442

Patch-Clamp Characterization of the MscS-Like Mechanosensitive Channel from *Silicobacter Pomeroyi*

Evgeny Petrov, Dinesh Palanivelu, Paul R. Rohde, Maryrose Constantine, Dan Minor, Boris Martinac.

Based on structural similarity, the Sp7 gene product of a sulphur-compounds decomposing Gram-negative marine bacterium *Silicibacter pomeroyi* belongs to the family of MscS-type mechanosensitive channels. In order to clarify in detail its physiological significance in response to membrane tension we have studied the properties of the Sp7 channel protein using patch-clamp technique. After heterologous expression in MJF465 *E. coli* strain (triple KO E. coli strain: *mscL*$^-$, *mscK*$^-$ and *mscS*$^-$knock-out) devoid of mechanosensitive channels MscL, MscS and MscK, patch-clamp analysis of giant *E. coli* spheroplasts Sp7 showed the typical pressure dependent gating behaviour of a stretch-activated channel (Figure) with a current/voltage dependence indicating a rectifying behaviour similar to MscS from *E. coli*. Except that, Sp7 is characterized by functional differences with respect to conductance and ion selectivity and desensitisation behaviour as compared to MscS.



Supported by the ARC grant DP0769983 and NIH grant U54GM074929.