that the C-terminus is involved in channel gating. The mutation E2117D slowed the inactivation rate 8-fold. We made heteromeric channels in HEK cells by co-transfecting with high and low conductance mutants. This created active channels with two new unitary conductances indicating that pore formation occurs at the subunits' interface. The number of new conducting states supports the trimer structure as revealed by Cryo EM. This is the first demonstration of functional heteromers of Piezo channels. Heteromeric formation is likely to be important for understanding the physiological activity of these channels.

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Pore Determinants of Mechanosensitive Piezo Channels

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Piezo proteins have been proposed as the long-sought-after mechanosensitive (MS) cation channels in mammals that play critical roles in various mechanotransduction processes, such as touch sensation and vascular development. However, their ion-conducting pore and ion permeation mechanisms have remained undefined. Here we identify domains and specific residues in Piezo1 that control the essential pore properties, including unitary conductance, ion selectivity and pore blockage, pinpointing the ion-conducting pathway. By uncovering the *bona fide* ion-conducting pore, these findings not only provide definitive proof that Piezo proteins are genuine pore-forming subunits of MS cation channels, but also shed light on elucidating the ion permeation and gating mechanisms of this prototypic class of mammalian MS cation channels.

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Characterization and Physiological Role of a Bacterial-Like Mechanosensitive Channel in Trypanosoma Cruzi Osmoregulation

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In order to complete its life cycle, Trypanosoma cruzi-the protozoan parasite that causes Chagas disease- faces various environmental changes as it propagates from an insect vector to a mammalian host. Previous studies have shown that T. cruzi has a robust osmoregulatory response, however the osmosensors involved in the detection and compensation pathways have not been identified. Mechanosensitive channels, which are activated by a stretch of the plasma membrane, have been associated with sensing of environmental changes in other organisms, but the function of these channels in T. cruzi is still unknown. In silico analysis of T. cruzi genome reveals the presence of mechanosensitive channels similar to the ones described in bacteria.

We hypothesize that a bacterial-like mechanosensitive channel, TcMcS, is involved in osmoregulatory processes in T. cruzi.

Overexpressing mutants using a tetracycline inducible system were developed to investigate the role of TcMcS in T. cruzi osmoregulation. Knockout mutants mediated by CRISPR/Cas9 were generated to test the essentiality of the protein. The localization and expression pattern of TcMcS varied in the three main life stages of T. cruzi. TcMcS seems to be localized in the contractile vacuole of epimastigote and trypomastigote forms, and in the plasma membrane of intracellular amastigote forms.

Under hyposmotic stress, cells overexpressing TcMcS swell significantly less than wild- type parasites. Under EGTA treatment, this advantage was eliminated, suggesting that calcium plays a role in the osmoregulatory response. Known mechanosensitive channel blockers, including gadolinium and streptomycin, were found to elicit significant differences in the parasite's ability to detect and compensate for the osmotic stress. Overall, our results support the idea that TcMcS is involved in sensing and compensation of osmotic stress in T. cruzi.

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Systematic Discovery of the 'Force-From-Lipid' Principles

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The functioning of bacterial mechanosensitive channels is governed by the lipid environment in which they are embedded. Several physical parameters such as lipid charge, saturation and topography, work in concert during transmission of tension from the bilayer onto the ion channel, thus making it a difficult task to dissect the contribution of each parameter to the channel's mechanosensitivity.

The elasticity modulus, level of saturation, fatty acid length and the electrical charge of the lipids ultimately modify the lateral pressure profile of the bilayer at the hydrophobic interface with the ion channels. Even if we are currently unable to measure the lateral pressure profile directly, these changes can be indirectly measured in patch-clamp experiments by using MscS and MscL as probes.

Our previous experimental results have shown how the lipid Cardiolipin differentially affects MscS and MscL in artificial bilayers, and our current research shows how lipid saturation plays an essential role in mechanosensitivity and gating frequency of these channels.

By focusing on the change of the channel-activity parameters such as activation threshold, frequency of gating, open probability and hysteresis, we aim to generate a model of the 'force-from-lipid' principles through the systematic patch-clamping of liposomes of controlled composition.

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Expression and Biophysical Characterization of Bacterial Mechano-Sensitive Ion Channel of Large Conductance into Mammalian Cells

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Mechanobiology is an emerging field of science focused on the role of physical parameters in determining cell morphology and physiology. Mechanosensistive ion channels represent one of the more important cellular elements sensing and transducing mechanical forces in chemical signaling activating biological pathways.

The mechanosensitive channel of large conductance (MscL) was the first mechano-sensitive ion channel to be isolated, cloned and sequenced, and therefore is the more characterized. In Escherichia Coli, MscL acts as an emergency release valve when the tension in the membrane lipid bilayer is getting near to the lytic limit. In the past, the MscL has been used as a comparative model for newly discovered mechano-sensitive channels, and recently, it has been recognized as a tool for potential application in nanotechnology. For example, the MscL have been successfully expressed in mammalian cells to achieve controlled delivery of bioactive molecules through application of mechanical forces.

In the present work, we report the expression of the MscL channel into mammalian cells, in order to develop an experimental model of cells highly sensitive to mechanical forces. In order to fully characterize the biophysical features of this transmembrane protein, we developed an optical tweezers setup integrated with a patch-clamp recording system to apply calibrated forces on single cells and simultaneously record the electrophysiological response of the genetically modified cells. We strongly believe that heterologous cellular expression of the MscL may become a useful tool to achieve targeted modulation of cellular activity, as well as to understand the role of mechanical properties of the materials currently used for the development of cellurized scaffolds.

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The Role of the C-Terminal Domain on the Gating Properties of Corynebacterium Glutamicum Mechanosensitive Channel MscCG

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The gram-positive soil bacterium Corynebacterium glutamicum exports a large amount of glutamate through the mechanosensitive channel MscCG. This process is utilized for the world industrial glutamate production. MscCG belongs to a subfamily of bacterial MscS-like channels, which function in osmoregulation, however this channel shows structural and functional differences compared to MscS. To understand the role of the carboxyl terminal domain of MscCG, the chimeric channel MscS-(C-MscCG), which is a fusion protein between the carboxyl terminal domain of MscCG and the MscS channel, was examined by the patch clamp technique. We found that the chimeric channel exhibited MS channel activity in E. coli spheroplasts characterized by a lower activation threshold and slow channel closing compared to MscS. The chimeric channel MscS-(C-MscCG) was also successfully reconstituted into azolectin liposomes and exhibited gating hysteresis in a voltage-dependent manner, especially at high pipette voltages. Moreover, the channel remained open after releasing pipette pressure at