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Properties of Human Pieoz1 Bearing Two Mutations Identified in Xerocytosis

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Piezo1 is a cation selective channel isolated from eukaryotes which shows voltage dependent inactivation. Xerocytosis is a condition that causes hemolytic anemia of red blood cells associated with either of two individual mutations in the gene encoding Piezo1. In this work we have incorporated both mutations together into the Piezo1 gene and characterized the properties of the new channel. The protein has a substitution of arginine for methionine at position 2225 (M2225R) and a lysine for arginine at position 2455 (R2455K). The double mutant showed no voltage dependent inactivation, a property also seen for the single mutant R2455K. We measured the pressure dependence of the channel in cell attached mode and found it to be shifted significantly leftward (Boltzmann). The ability to activate Piezo1 at low pressure has allowed us to determine more accurately the kinetics of Piezo1 channel gating. The conductance of the double mutant is ~ 30 pS. Whole cell currents were significantly larger, indicating that the double-mutant channel is more sensitive to cell mechanical stimulation. We have co-transfected Piezo1 and MscL and sequentially activated both channels with increasing pressure, allowing us to compare area changes within a single patch. The double mutated channel appears to have changes similar to those found for MscL. We also show that the channel is blocked by the addition of GsMTx4, a peptide inhibitor for mechanical channels.

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Arachidonic Acid is Essential for Normal Touch Sensation

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Touch, proprioception, and blood pressure regulation rely on mechanoreceptor neurons and mechano-electrical transduction (MeT) channels to convert mechanical cues into electrical signals. The protein partners that form native MeT channels are known only for a small group of mechanoreceptor neurons, including the touch receptor neurons responsible for gentle body touch in C. elegans. Such MeT channels are formed from at least four membrane proteins: two amiloride-sensitive sodium channel proteins (MEC-4 and MEC-10) and two putative lipid binding protein subunits (MEC-2 and MEC-6). It remains unknown how touch activates MeT channels, but interactions between the channel and the lipid membrane are likely to be essential. To learn more about interactions between MeT channels and specific lipids, we utilized C. elegans strains deficient in the synthesis of long-chain, poly-unsaturated fatty acids (PUFAs) and identified arachidonic acid (AA) as the sole PUFA required for full touch sensitivity. Touch receptor neurons retain normal morphology in PUFA-deficient mutants and normal touch sensitivity can be restored by exogenous PUFAs. We will present evidence garnered from behavioral and optogenetic studies suggesting that long-chain PUFAs are needed to activate native MeT channels, but dispensable for subsequent events linking sensory signaling in touch receptor neurons to behavior. Our data suggest that AA and its non-metabolizable analog ETYA directly modulate the activity of the MeT channel.

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Measuring Transmembrane Helix Separation on the MscS Mechanosensitive Channel using Pulsed Electron-Electron Double Resonance (PELDOR) Spectroscopy

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The heptameric mechanosensitive channel of small conductance (MscS) provides an essential function in Escherichia coli as it prevents cell lysis by opening in response to increased bilayer tension, during extreme osmotic shock. The gating of MscS has been extensively studied by three approaches: X-ray crystallography [1], cwEPR [2] spectroscopy and EMD simulations [3], but each of them has defined different closed and open structures of the channel. The proposed models report very different arrangements of the transmembrane helices. To resolve the discrepancy, we have attached spin labels to cysteine mutants on key structural elements within all three MscS transmembrane helices, specifically chosen to discriminate between the competing models. These distances from multiple mutants provide an independent assessment of the validity of the competing models. In addition, they offer the advantage of measurements on MscS in solution under physiological conditions. The resulting pulsed electron-electron double resonance (PELDOR) spectra allow the accurate measurement of transmembrane helix separation, offering a direct comparison for the different models. The distance distributions for the open crystal structure of MscS match the experimental data [4]. We also report two new crystal structures of spin labeled MscS which are in complete agreement with the PELDOR data and demonstrate the accuracy of PELDOR for complex ion channels. PELDOR is a powerful experimental tool which can be used for interrogating the conformation of transmembrane regions of multimeric membrane proteins.

[1] Wang et al., Science 321, 1179 (2008).
[2] Vásquez et al., Science 321, 1210 (2008).
[3] Akitake et al., NSMB 14, 1141 (2007).
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Gate Coupling to the Mechanical Stimulus Depends on Electrostatic Interactions between the Transmembrane and Cytoplasmic Domains

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The mechanosensitive channel MscS residing in the inner E. coli membrane opens directly by tension of 6-10 mN/m. Under sub-saturating conditions (5-8 mN/m), MscS enters a tension-insensitive inactivated state, but quickly recovers to the resting state when tension is released. Extrapolated motion (EMP) and conventional MD simulations have predicted two major stable states for MscS: the crystal-like inactivated state with kinked TM3s and splayed (uncoupled) peripheral TM1-TM2 helices, and the resting state with kink-free TM3s, in which TM1-TM2 pairs align along TM3s and form a buried contact with the gate. The positions of the TM1-TM2 pairs are stabilized by predicted alternating salt bridges interlinking the TM1-TM2 loops (D62) with the equatorial region of the cytoplasmic cage domain. Here we show that in the inactivated state the D62 partner is R128, whereas recovery to the resting state leads to switching to R131. Delayed recovery in the mutants lacking salt bridges (D62R/N, R128D/R131D) strongly supports the necessity of salt bridge formation for this process. Disulfide formation rates indicated that the D62C-R128C pair forms preferentially in the inactivated state whereas the D62C-R131C disulfide forms constitutively in the resting state. Chemical cross-linking analysis suggests these salt bridges are not preserved in the open state. The models show that the switching of the salt bridge partners involves mutual rearrangement of the distal TM3b segments and rotation of the cytoplasmic beta domains about the TM3-beta linker containing conserved P129. The P129A or G substitutions do not affect the rate of inactivation; however, the former increases the rate of recovery. The data illustrate how the cytoplasmic segments influence the overall conformation of the MscS complex, defining the coupling of the channel gate to the mechanical stimulus arriving from the lipid bilayer.

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Organellar Mechanosensitive Channels Regulate the Hypo-Osmotic Response in Fission Yeast

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Living cells challenged by hypo-osmotic shock regulate cell volume to avoid degradation of ion homeostasis and cell rupture. A key molecule of sensing machineries essential for survival upon hypo-osmotic shock is the mechanosensitive channel gated by membrane tension. The bacterial mechanosensitive channel MscS functions directly for this purpose by releasing cytoplasmic solutes out of the cell, and MscS homologs are found in cell-walled eukaryotic organisms, such as plant, algae, and fungi. While MscS is required for osmoregulation in bacteria, it is unknown whether eukaryotic MscS homologs are involved in the osmotic response. Rather, eukaryotic MscS homologs analyzed so far, such as *Arabidopsis thaliana* MscS-like proteins (MSL2 and MSL3) and a *Chlamydomonas reinhardtii* MscS homolog (MSC1), are associated with chloroplast organization. Here we show that the fission yeast MscS homologs, designated Msy1 and Msy2, participate in the hypo-osmotic shock response by a mechanism different from that operated by the bacterial MscS. Upon hypo-osmotic shock, *msy2*$^-$ and *msy1*$^-$ *msy2*$^-$ cells display greater cell swelling than wild-type cells and underwent cell death. Cytoplasmic Ca^{2+} measurements with aequorin and yellow cameleon revealed that cell swelling preceded an intracellular Ca^{2+} increase, which was greater in *msy1*$^-$ and *msy1*$^-$ *msy2*$^-$ cells than in wild-type cells, suggesting that the death of the mutant was due to an excessive Ca^{2+} increase. Fluorescent microscopy showed that Msy1 and Msy2 are localized mainly in the endoplasmic reticulum. These observations suggest that organellar Msy1 and Msy2 regulate intracellular Ca^{2+} and cell volume for survival upon hypo-osmotic shock.