WT-MscS and the alanine mutants have been undertaken to experimentally test the predictions from the modelling studies.

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2268-Plat

Novel Mutations in the Extracellular Cap of the Mammalian Mechanosensitive Channel TREK-1

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The Twik related potassium channel 1 (TREK-1) is one of the best studied mechanosensitive mammalian channels. TREK-1 is known to play very important roles in depression, ischemia and vasoregulation. TREK-1 belongs to the family of background or "leak" potassium channels are constitutively open at rest and have a central role in the tuning of neuronal resting membrane potential, duration of action potential and regulation of neurotransmitter release. These K^+ channels are members of the family of K2P channels subunits containing four transmembrane and two pore domains. The functional channel is formed by two subunits and is predicted to have a two-fold symmetry.

Here we show the feasibility of the use of microbial genetics to study the structure-function relationship of this mammalian channel. The advantage of this approach is that we can directly screen for channels with altered phenotypes and correlate this altered function with structural changes. We have successfully expressed a functional TREK-1 channel in bacterial cells, and show that it can partially rescue the slow growth phenotype of an E .coli strain deficient in three mayor potassium transporters. Furthermore, using random mutagenesis and bacterial screens we have isolated five mutants that better remediate the potassium deficiency of this bacterial strain. Because these mutants clustered in a stretch of 20 amino acids in the extracellular cap of the TREK-1, we think that we have found a functional "hot spot" by utilizing the power of bacterial genetics.

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Omega 6 Polyunsaturated Fatty Acid-Containing Phospholipids Enhance Neuronal Cell Mechanics and Touch in C. Elegans

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Mechano-electrical transduction (MeT) channels embedded in neuronal cell membranes are essential for touch and proprioception. Little is understood about the interplay between native MeT channels and membrane phospholipids, in part because few techniques are available for altering plasma membrane composition in vivo. Here, we leverage genetic dissection, chemical complementation, and optogenetics to establish that arachidonic acid (AA), an omega 6 polyunsaturated fatty acid (PUFA), enhances touch sensation and mechanoelectrical transduction activity while incorporated into membrane phospholipids in C. elegans touch receptor neurons (TRNs). We found that arachidonic acid acts cell autonomously, since we show that enzymes needed for its synthesis are expressed in TRNs. We also established that the membrane viscoelastic properties of TRNs lacking omega 6 PUFAs are altered (i.e., membrane bending and viscosity), yielding less flexible membranes than wild type, as determined by atomic force microscopy (AFM) based single-tether extrusion. Our data suggest that the defect in touch sensation likely reflects a loss of mechanotransduction rather than lack of excitability or downstream signaling. These findings establish that polyunsaturated phospholipids are crucial determinants of both the biochemistry and mechanics of mechanoreceptor neurons and reinforce the idea that sensory mechanotransduction in animals relies on a cellular machine composed of both proteins and membrane lipids.

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Touch Activates Mechanosensitive Ion Channels in Merkel Cells In Vitro

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Merkel cell-neurite complexes are gentle touch receptors that mediate slowly adapting type I (SAI) responses. Since their description in 1875, Merkel cells have been proposed to be mechanosensory cells that transduce mechanical stimuli into electrical signals that activate somatosensory neurons. Consistent with this model, conditional knockout mice that lack Merkel cells show the loss of touch-evoked SAI responses. Moreover, in vitro studies on cultured Merkel cells report calcium elevation in Merkel cells in response to swelling or membrane stretch. Previous studies support the contribution of Merkel cells to touch sensation, however, the central question of whether Merkel cells are intrinsically touch sensitive is unanswered. To tackle this problem, we performed live-cell imaging and electrophysiological recordings from mouse Merkel cells. Merkel cells were dissociated from the epidermal skin of transgenic Atoh1/nGFP mice, whose Merkel cells selectively express green fluorescent protein. GFP-positive cells were purified using fluorescence-activated cell sorting and cultured for 1-5 days. Individual Merkel cells were stimulated by families of displacements ($\leqq$0.3-μm steps) with a glass probe driven by a piezoelectric actuator. Touch-evoked responses were monitored with either ratiometric calcium imaging or tight-seal, whole-cell recordings. Merkel cells displayed reversible calcium responses to focal displacements applied to somata. Moreover, electrophysiological recordings demonstrated mechanically activated inward currents at a negative holding potential. Peak inward currents ranged from 107.3-431.3 pA. The 10-90% operating range of these mechanically activated currents was 1.7 ± 0.1 μm (N=10 cells, mean ± SE). Like mechanosensitive currents in hair cells and somatosensory neurons, Merkel-cell currents adapted exponentially to sustained stimuli. Quantitative PCR indicated that Merkel cells expressed both Piezo1 and Piezo2 genes. Together, these data demonstrate that Merkel cells are intrinsically mechanosensitive in the absence of other skin cells or somatosensory neurons.

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Molecular Mechanisms of Deafness Mutations Disrupting Tip-Link Function in Hair-Cell Mechanotransduction

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Hair-cell tip links are fine filaments that directly convey mechanical force to inner ear mechanotransduction channels. These filaments are made of protocadherin-15 (PCDH15) and cadherin-23 (CDH23), two deafness-related proteins that feature long extracellular domains interacting tip-to-tip in a calcium dependent manner. Here we combine X-ray crystallography, molecular dynamics simulations, and binding experiments to explore the molecular mechanisms by which deafness mutations disrupt tip-link function in hair-cell mechanotransduction. We find that these mutations disrupt tip links through impaired interaction between PCDH15 and CDH23 (PCDH15-R113G and PCDH15-I108N), impaired calcium binding (CDH23-D1010G), subtle weakening of structural stability (CDH23-S47P), or impaired folding (PCDH15-D157G). Interestingly, the biochemical effects of each of these deafness mutations correlate with the severity of the reported inner-ear phenotype. Our results shed light on the molecular mechanisms of hair-cell sensory transduction and may help develop tailored treatments for cadherin-mediated deafness.

Platform: Force Spectroscopy

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Disulfide Bonds are Allosteric Regulator of Mechanical Stability

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Disulfide bonds are known to stabilize proteins against perturbations such as temperature or denaturants. Since mechanical force is the most common protein denaturant in vivo, there is increasing interest in the role that disulfide bonds have in the mechanical unfolding of proteins. For instance, disulfide bonds reduce the contour length of stretched proteins by limiting the extensibility up to the covalently linked cysteines. However very little is known about the effect of native disulfide bonds in the strength of structural clamps that determine mechanical stability.

Here, we use single molecule atomic force spectroscopy to study the mechanical effects of disulfide bonding in immunoglobulin domains (Ig) that are constitutively under force.

We observe that the formation of native disulfide bonds in immunoglobulin domains triggers drastic differences in the rupture forces, although the two cognate cysteines do not link together the β-strands of the mechanical clamp motif. In the case of bacterial gram-negative pilin FimH, disulfide increases the most probable unfolding force from 297 pN to 425 pN at 400nm/s. In contrast, the disulfide in the 69th immunoglobulin domain of human titin decreases the unfolding force at a pulling rate of 1200nm/s from 271 pN to 195 pN. We observe that both in titin and in the Fim pilus, the disulfide bonds are remarkably conserved along the entire stretched molecular architecture. Hence, many Igs in elastic segment of titin and in all Fim Igs are predicted to change mechanical stability depending of their oxidation state. Our results