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Structural and Biophysical Characterization of Inner Ear Tip Link Variants
Yoshie Narui, Marcos Sotomayor.
Chemistry and Biochemistry, The Ohio State University, Columbus, OH, USA.
At the molecular level, human hearing relies upon the tip-to-tip interaction of two unique non-classical cadherins, protocadherin 15 (Pcdh15) and cadherin 23 (Cdh23). Together, these two proteins form a filament called the tip link that connects neighboring stereocilia of mechanosensitive hair cells. Sound waves cause the stereocilia to deflect applying a force to the tip link and opening a nearby transduction channel. Disruption of the tip link caused by loud sound or chemical treatments eliminates transduction currents and illustrates that tip link integrity is critical for mechanosensing. Recent studies have found that remodeling of the tip link after disruption is a dynamic process, which leads to the formation of atypical complexes that may incorporate alternatively spliced variants or isoforms of Pcdh15. Our current work focuses on understanding these unusual tip links and comparing them with the prototypical tip link. Here, we present the crystal structure of a new complex formed from Cdh23 and isoform 2 of Pcdh15 refined at 3.5 Å resolution. While the molecular structure reveals subtle differences between the two complexes, the binding affinity between Cdh23 and isoform 2 of Pcdh15 is notably different from Cdh23 and isoform 1 of Pcdh15 as observed in analytical size exclusion chromatography. These results clearly demonstrate that alternative heterotypic tip link structures form stable protein-protein interactions *in vitro* and provide no evidence to support the existence of homotypic Pcdh15-Pcdh15 tip links. Additional studies will focus on other Pcdh15 isoforms and determine how changes in the tip link structure alter the mechanical properties of the filament.

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Probing the Mechanosensitivity of Piezo1 Channels
Charles D. Cox[1], Boris Martinac[1,2].
[1]Victor Chang Cardiac Research Institute, Sydney, Australia, [2]St Vincent's Clinical School, University of New South Wales, Australia.
Piezo channels are a new family of cation-selective mechanosensitive ion channels, which have been shown to be integral in numerous physiological processes. Central to these physiological roles is the ability to sense mechanical force. Whether this force is transmitted directly from the lipid bilayer or from tethering to the cytoskeleton and/or ECM is unknown. The 'gold standard' for testing the 'inherent' mechanosensitivity of ion channels has become purification and subsequent reconstitution of mechanosensitive channel proteins into liposomes of known lipid composition. This is experimentally difficult and time consuming and is a process that has to be determined empirically for individual channel proteins. An underused paradigm for the study of channel mechanosensitivity is the production of cytoskeleton deficient membrane 'blebs' [1]. In order to study the inherent mechanosensitivity of Piezo1 channels using this paradigm we used transiently transfected HEK293 cells. Herein we show that; a) membrane blebs can be formed by the addition of both Hypo- and Hyper-osmolar solutions of sodium gluconate in HEK293 cells, b) this treatment induces significant cell death after 2 hours as determined by trypan blue exclusion assays, c) these resulting blebs are deficient in a major cytoskeletal component (actin) and d) Piezo1 channels can be activated in this environment and have a lower pressure threshold of activation. This has important implications with respect to Piezo1 channels being gated according to the force from lipids concept [2]. In addition we also assess the affect of mutating a number of aromatic residues in the most highly conserved region of the Piezo sequence on the mechanosensitivity of the channel.
References:
[1] Zhang, Y., et. al., (2000) J. Physiol.(London) 523, 117-130.
[2] Anishkin, A., et. al., (2014) PNAS 111(22): 7898-7905.
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Sensing Force by Trigeminal Neurons of Acutely Mechanosensitive Birds
Eve R. Schneider, Marco Mastrotto, Willem J. Laursen, Vincent P. Schulz, Jena B. Goodman, Owen H. Funk, Patrick G. Gallagher, Elena O. Gracheva, **Sviatoslav N. Bagriantsev**.
Yale School of Medicine, New Haven, CT, USA.
Mechanosensation is a fundamental way animals interact with the environment, but it remains the least well understood at cellular and molecular level. Somatosensory ganglia of the standard laboratory species house a highly diverse population of neurons, where low-threshold mechanoreceptors - the neurons that innervate light touch receptors in the skin - represent only a small fraction. This heterogeneity significantly impedes progress in understanding functional roles of somatosensory neurons in light touch perception. Here, we explored functional specialization of somatosensory ganglia from animals which have taken the sense of touch to the extreme - tactile foraging ducks. These animals have acutely mechanosensitive bill innervated by trigeminal (TG) neurons, and as such provide an opportunity to study general principles of mechanotransduction from an unconventional standpoint. We found that, in contrast to species without tactile specialization, the majority (85%) of duck TG neurons are large-diameter myelinated mechanoreceptors expressing the mechano-gated ion channel Piezo2. Electrophysiological analyses showed that mechanosensitivity of duck TG neurons has been optimized in three ways. Compared to mouse cells, duck neurons exhibit (i) lowered threshold of mechano-activation, (ii) elevated signal amplification gain, and (iii) prolonged kinetics of inactivation, all of which increase the amount of depolarizing charge entering the cell upon mechanical stimulation. Thus, duck TG neurons have augmented intrinsic ability to convert mechanical force into excitatory ionic current, which explains the acute mechanosensory properties of the duck bill. Our studies emphasize a key role of the intrinsic mechanosensory ability of somatosensory neurons in touch physiology, reveal an evolutionary strategy utilized by vertebrates to hone tactile perception, and suggest a novel model system to study the sense of touch at the cellular and molecular level.
Schneider ER, Gracheva EO, Bagriantsev SN et al, PNAS 2014 (e-pub Sept 22).

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Is Cryptochrome a Primary Sensor of Extremely Low Frequency Magnetic Fields in Childhood Leukemia?
Patricia L. Bounds[1,2], Niels Kuster[1,3].
[1]Foundation for Research on Information Technologies in Society (IT'IS), CH-8004 Zurich, Switzerland, [2]Department of Chemistry and Applied Biosciences, Swiss Federal Institute of Technology, CH-8093 Zurich, Switzerland, [3]Deptartment of Information Technology and Electrical Engineering, Swiss Federal Institute of Technology, CH-8092 Zurich, Switzerland.
Extremely low frequency magnetic fields (ELF MF) are classified as possibly carcinogenic to humans, but the biophysical mechanisms of a causal relationship remain unclear. A cryptochrome-based radical pair mechanism (RPM) has been invoked as the primary MF sensor in animal magnetoreception to explain effects from MF strengths in the nT range. Model studies of the RPM in aprotic solution require cryogenic temperatures and MFs in the μT range to elicit marginal responses, implying that physiological responses evoked by radical pairs in biological milieu are unlikely. We explore ideas about how signals from transient radical pairs in cryptochrome might be transduced and amplified in mutagenic responses. The cryptochrome-based RPM involves blue light activation of the flavin adenine dinucleotide (FAD) cofactor followed by electron transfer (ET) from a conserved triad of tryptophan residues. However, intramolecular ET involving additional conserved aromatic residues in cryptochrome likely extends beyond the canonical triad. Further, ascorbate, which is present at millimolar concentrations in leukocytes, is likely to transfer an electron to the ultimate amino acid radical formed during one-electron reduction of FAD. The ascorbyl radical has been proposed as a potential radical pair partner in the RPM.
The flavin semiquinone radical may be reoxidized to the resting state via ET to O_2 to form $O_2^{\bullet-}$. Increased levels of the ascorbyl radical and $O_2^{\bullet-}$ would contribute to oxidative stress pathways in the cell. Oxidative stress responses in cancer are thought to be mediated in part by the mitogen activated protein kinase (MAPK) signaling pathway. We have presented evidence for alteration of MAPK activation in response to ELF MF.
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A Structure-Function Approach to Understanding the Dual Functions of the Plant Mechanosensitive Ion Channel MSL10
Grigory Maksaev, Kira Veley, Elizabeth Haswell.
Biology Dept, Washington University in Saint Louis, Saint Louis, MO, USA.
MscS-Like (MSL) 10 is a member of the MscS superfamily of mechanosensitive ion channels and one of 10 MSL proteins in the model flowering plant Arabidopsis thaliana. Unlike Escherichia coli MscS, MSL10 contains 6 transmembrane helices, and only its C-terminal TM helix shows homology with EcMscS's pore-lining domain. MSL10 has been shown to provide a mechanosensitive activity in plant cells and Xenopus oocytes. However, its structural organization and function in plants are just beginning to be elucidated. We have shown that MSL10 is involved in one or more signal pathways that do not require its ion conducting ability. Instead, MSL10's intracellular N-terminus was shown to have a regulatory function in the induction of programmed