tension were carried out. Restraints were slowly introduced to induce gating. The simulations produced a set of open channel structures that were analysed using a range of structural features such as pore radius and helix tilt.

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1518-Pos Board B428

Cardiolipin Effects on the Gating Behaviour and Reconstitution of MscL and MscS

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The *m*echanosensitive *c*hannels of *l*arge (MscL) and *s*mall (MscS) conductance act as osmosensors in bacterial cells against hypo-osmotic shock. MscL has been extensively studied by reconstitution into liposomes[2,3], however MscS has proved more difficult to reconstitute, requiring high protein-lipid ratios[4,5]. We recently published an improved reconstitution method for both MscL and MscS in soy azolectin[6], a mixture that contains lipids, sugars and sterols. We have expanded these results and show here the effect of both individual and mixtures of lipids on the reconstitution and channel gating behaviour of co-reconstituted MscL and MscS. Introduction of the highly charged lipid cardiolipin causes rapid gating of MscS (Fig 1A) in comparison to soy azolectin (Fig 1B), indicating that lipid charge may play a significant role on channel gating dynamics.

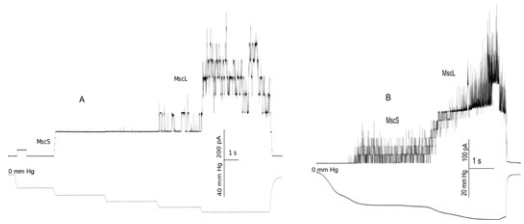

Fig 1 (A) MscS/MscL co- reconstitution in soy azolectin. (B) MscS/MscL co- reconstitution in mixture of phosphatidyl ethanolamine/ phosphatidyl choline/cardiolipin at a wt/wt ratio of 7:2:1, recordings at a pipette voltage of +30 mV.

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1519-Pos Board B429

Prediction and Verification of Critical Tension Sensing Residues in the E. coli Mechanosensitive Channel of Small Conductance (EC-MSCS)

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We have previously used a combination of random mutagenesis and molecular dynamics simulations to identify and understand critical residues for tension sensation in MscL (mechanosensitive channel of large conductance) [Maurer et al JBC 2003, 278, 21076-21082; Elmore et. al Biophysical J., 2003, 85, 1512-1524]. In MscL, we were able to demonstrate a strong correlation between lipid-protein interaction energy, determined using a molecular dynamics simulation of the closed state structure, and mutant channel function. Upon mutation of amino acid residues in transmembrane domains that exhibited significant lipid interaction over the course of the simulation displayed were more likely than other transmembrane residues to display either a loss of function or a gain of function phenotype in bacterial assays. Here, we have employed a similar computational analysis to identify potentially critical lipid-binding residues for tension sensation in MscS. Molecular dynamics simulations of a closed state model of MscS were carried out in the presence of an explicit lipid membrane. Energetic analysis of lipid-protein interactions in the first transmembrane domain (TM1) and the second transmembrane domain (TM2) indicated ten residues (TM1: L35, I39, L42, I43, R46, N50, R54; and TM2: R74, L78, I82) with increased lipid interactions. Using site directed mutagenesis these residues were mutated to alanine and their ability to rescue MscL/MscS/MscK null E. coli from osmotic downshock was determined. Generally, mutating lipid interacting residues to alanine resulted in MscS channels that exhibited decreased ability to rescue bacteria from osmotic downshock, following a similar trend to our previous studies of MscL.

1520-Pos Board B430

Membrane Tension and Cytoplasmic Crowding Pressure: The Multimodal Mechanism of the Mechanosensitive Channel Mscs

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MscS is the major osmolyte efflux valve that regulates turgor in *E. coli* during osmotic downshock by opening a 16 Å pore in response to membrane tension. Here we present data strongly suggesting that this channel not only gates by tension in the membrane but also senses the degree of cytoplasm hydration. Crystal structures of the homoheptameric MscS complex reveal the transmembrane domain connected to the large hollow cytoplasmic domain (cage) through the narrow gate region formed by pore-lining TM3 helices. Computational exploration of the conformational space of MscS and a set of experimental constraints allowed us to reconstruct the resting state and the closed-to-open transition involving straightening of TM3s. The reconstructed TM2-TM3 interhelical contacts, necessary for force transmission from the membrane to the gate, were stabilized by the D62-R131 salt bridges connecting the TM and 'cage' domains. Simulations have demonstrated that the cage domain is not rigid; it changes its shape with gating and compacts under hypo-osmotic or crowding stress. Simulated compaction of the cage destabilizes the D62-R131 salt bridges. In patch-clamp experiments, crowding agents (PEGs, dextrans, ficoll) added to the cytoplasmic side lead to fast inactivation, and the same phenotype was produced by D62N/R mutations disrupting the slat bridges. Thus, initially considered as a size-limiting pre-filter, the cage appears to act as a large-osmolyte pressure sensor regulating the connection of the gate with the peripheral tension-receiving helices. Because lateral tension acting on the transmembrane domain and osmotic /crowding stress perceived by the cage all converge on the gate, the channel is structurally designed to reconcile the opening stimulus (membrane tension) with the inhibitory action of cytoplasmic crowding pressure which provides feedback on the hydration state of the cytoplasm and terminates small solute release.

1521-Pos Board B431

Functional Analysis of Mutations in the TRESK K2P Potassium Channel Associated with 'migraine with Aura'

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An inherited mutation in the KCNK18 gene has been shown to be associated with 'migraine with aura'. This is a common, debilitating, recurrent headache disorder associated with transient and reversible focal neurological symptoms. KNCK18 encodes the TWIK-related spinal cord potassium channel (TRESK), a member of the K2P family of potassium channels. The F139WfsX24 mutation, segregates perfectly with typical migraine with aura in a large pedigree and functional characterization of this mutation demonstrates that it causes a complete loss of TRESK function and that the mutant subunit suppresses wild-type channel function through a dominant-negative effect, thus explaining the dominant penetrance of this allele (Lafreniere et al, doi:10.1038/nm.2216). This identifies a role for TRESK in the pathogenesis of typical migraine with aura and further supports the role of this channel as a potential therapeutic target. In this study we have examined the electrophysiological properties of other mutations identified in the human KCNK18 gene and find that several of these variants also produce a dramatic dominant-negative phenotype.

1522-Pos Board B432

Identification of Gating Mutations in the Trek-1 k2p Potassium Channel by Functional Complementation in K+ uptake Deficient Yeast

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TREK-1 is a member of the K2P family of potassium channels. These channels appear to have a unique gating mechanism compared to other types of K+ channel and this may be a reflection of their overall asymmetric structure. In order to address which domains of the channel may be important for channel gating we took advantage of an unbiased random mutagenesis approach which selects for activatory mutations by complementation in a K+ auxotrophic strain of yeast (SGY1528). Wild-type TREK-1 did not complement the growth of this strain on low [K+] media. However, screening a randomly mutated TREK-1 library yielded a number of mutations which robustly complemented growth. One of the mutants has been identified previously through its effects on pH-gating (Glu-321). However many novel mutations were also identified. Intriguingly, the majority of these mutations were located in the TMs and/or close to the selectivity filter of TREK-1. Electrophysiological analysis of these