when the temperature was returned to 23°C. In marked contrast, conductance due to ΔF508 CFTR channels exhibited a transient increase within a minute after the temperature challenge, followed by a quasi-exponential decline of about 80-90% of the initial conductance ($t_{1/2}$ = 4 minutes at 37°C). The temperature induced decrease in ΔF508 CFTR conductance was not reversed by returning the temperature to 23°C. The second-site reverent construct, R553M/ ΔF508 CFTR, previously shown to rescue CFTR function in mammalian cells (Teem et al. 1993. *Cell*. 73:335-346), exhibited a thermal response that was indistinguishable from wild type. Preliminary data suggests that this ''thermal instability'' that is readily detectable when ΔF508 CFTR is expressed in *Xenopus* oocytes, reflects an intrinsic structural defect in the channel protein that results in a temperature-sensitive alteration in gating and could potentially trigger the retrieval of surface protein documented in mammalian cells.

1677-Pos

Riding the Conformational Wave to the Open Channel State in the CFTR Chloride Channel

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The pore structure of the CFTR chloride channel is unknown. We showed previously that R352 in TM6 forms a salt bridge with D993 in TM9; charge-destroying mutations at either site destabilized the open state, affecting conductance, selectivity, and pore blockade. Other pairs of interacting residues also contribute to stabilizing the open state. We continued these experiments to determine how steps leading to the dimerization of the NBDs upon binding of nucleotide relate to the steps leading to pore opening, using single-channel recordings of WT-CFTR and channels bearing a cysteine or alanine at 352, 993, or both. In R352C-CFTR, but not R352A-CFTR, modification of the cysteine by positively-charged $MTSET^+$ and $MTSEA^+$ recovered the stability of the open state. In D993C-CFTR, but not D993A-CFTR, negatively-charged $MTSES^-$ recovered the stability of the open state. In contrast, D993C-CFTR modified by $MTSET^+$ retained the instability of the open state. The R352C/D993C-CFTR double mutant exhibited instability of the open state in both the absence and presence of DTT, suggesting that R352C did not form a disulfide with D993C. In WT-CFTR, exposure to AMP-PNP led to greatly prolonged channel openings, as expected. However, this response was not found for R352A-CFTR. Surprisingly, R352C/D993C-CFTR could be latched open by the bifunctional crosslinker, MTS-2-MTS, such that channels could not close upon washout of ATP. MD simulations based on CFTR homology models (see Dawson Lab abstract) predict conformational states in which R352 and D993 approach each other to within van der Waals distances. These results suggest that the binding of ATP at CFTR's NBDs initiates a conformational wave, which leads to a change in pore structure from the closed to the open state, the latter being stabilized by inter-TM interactions including the R352-D993 salt bridge. (Support: NIH-2R56DK056481-07)

1678-Pos

Homology Modeling and Molecular Dynamics Simulation Predict Side-Chain Orientations and Conformational Changes in the Pore of the CFTR Chloride Channel

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We recently presented two homology models of the CFTR chloride channel, one based on homology to the prokaryotic ABC transporter, Sav1866, and a second based on a 5 ns molecular dynamics (MD) simulation of the first (Alexander et al, *Biochemistry* in press, 2009). Predictions for side-chain orientations were in excellent agreement with the results of a cysteine scan of transmembrane segment six (TM6) using both channel-permeant and channel-impermeant, thiol directed probes. Here we present the results of an extended MD simulation, along with the results of a cysteine scan of TM12. Scanning results confirm the model predictions for ''pore-lining'' and ''not pore-lining'' residues in TM12 and support the notion that pore narrowing prevents the reaction of deeper-lying cysteines in TM12 toward larger, thiol-directed probes like $MTSET^+$ and $MTSES^-$ when these compounds enter the channel from the outside. The extended MD simulation predicts movements of pore elements that are in agreement with previously reported results of state-dependent reactivity of a cysteine at position 337 (Norimatsu et al, *Biophysical Journal* 96(3):468a-469a, 2009), and the postulated formation of a salt-bridge between R352 (TM6) and D993 (TM9) (Cui et al, *Biophysical Journal* 91(5):1737-48, 2008, and poster from the McCarty Lab). Supported by NIH, the Cystic Fibrosis Foundation, the American Lung Association, the Wellcome Trust, and the BBSRC.

1679-Pos

Identification of Possible Binding Sites for the CFTR Pore Blocker, GlyH-101

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The last decade has seen the discovery by means of high throughput screening of a wide range of small-molecule modulators of the CFTR chloride channel. These compounds act by altering anion conduction, channel gating and/or trafficking of the CFTR protein. However, binding sites for these molecules on CFTR or other cellular constituents have yet to be identified. GlyH-101 is a CFTR modulator that blocks the channel by entering from the extracellular side and binding to a site within the pore. In an effort to identify possible GlyH-101 binding sites within the pore of the CFTR channel, we applied the small-molecule docking program, ''Glide'' (Schrodinger, Inc.), to a series of molecular models of CFTR, derived by means of molecular dynamics simulation from a homology model based on the prokaryotic ABC transporter, Sav1866 (Dawson and Locher, Nature 443: 180-185, 2006; Alexander et al., Biochemistry in press, 2009). One of the potential GlyH-101 binding sites identified by Glide lies in close proximity to two residues in the sixth transmembrane segment (TM6), F337 and T338, where substituted cysteines are ''protected'' by GlyH-101 from reaction with thiol-directed probes (Norimatsu et al., Biophys. Journal 96: 468a-469a, 2009). These results suggest an approach to identifying the binding site(s) for GlyH-101 and other small molecules within the CFTR protein. Supported by NIH, Cystic Fibrosis Foundation, American Lung Association, the Wellcome Trust, and the BBSRC.

Mechanosensitive Channels

1680-Pos

Mechanosensitivity of a Voltage-Gated Ion Channel, $Na_v1.5$

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Voltage-gated ion channels are often found in tissues where electrical and mechanical stimuli coexist. The mechanosensitive, voltage-gated sodium channel $Na_v1.5$ (encoded by SCN5A) is expressed in two such electromechanical organs, the heart and the gastrointestinal tract. Mutations in SCN5A are frequently pathogenic and may affect mechanoelectrical coupling. The aim of this study was to assess mechanical sensitivity of $Na_v1.5$ at the molecular level. SCN5A was expressed in HEK cells and studied using a pipette pulled and fire polished to ensure that a small number (2-50) of channels were reliably present in cell-attached micropatches. This allowed resolution of both single channel events and averaged behavior. Both positive and negative pressures (up to 50mmHg) produced visible patch distention, an increase in patch current at all voltages and large hyperpolarizing shifts in steady-state voltage-sensitivity of activation and inactivation. From voltage dependence of activation at rest ($V_{1/2}$ = −30mV at 0mmHg), pressure resulted in graded shifts of $V_{1/2}$ for activation and inactivation of −0.71mV/mmHg and −0.72mV/mmHg, respectively. Channel kinetics were predictably affected by the voltage shifts, but channel opening and fast inactivation were otherwise unaffected by pressure. Single channel traces showed that unitary conductance was unaffected, rather peak currents appeared to increase due to an increase in the number of active channels in the patch. These effects were minimally reversible for as long as 30 minutes after a single stretch stimulus. Patch excision resulted in an immediate shift of activation $V_{1/2}$=-75mV and loss of stretch sensitivity. Application of the inhibitor of actin polymerization, cytochalasin D, diminished sensitivity to stretch (−0.42mV/mmHg). Our work demonstrates that mechanical stress at physiologically relevant levels affects voltage sensing of $Na_v1.5$ channels, without affecting the pore, channel gate and fast inactivation. Supported by NIH DK52766.

1681-Pos

Integrin-Dependent and -Independent Potentiation of L-type Calcium Current (Cav1.2) by Cell Stretch

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Stretch-induced (myogenic) contraction of vascular smooth muscle (VSM) requires calcium influx through L-type calcium channels (Cav1.2). Integrins play a role in this process because alpha5 beta1 and alphaVbeta3 integrin blocking antibodies prevent myogenic constriction. Recent studies in our lab indicate that Cav1.2 current is potentiated by alpha5beta1 integrin activation and requires phosphorylation by PKA and c-Src of Cav1.2 C-terminal residues. To test

whether Cav1.2 channels are mechanosensitive and the possible role of integrins in this process, patch clamp methods were used to investigate the properties of either native or heterologously expressed Cav1.2 cannels by stretch of single cells plated onto a flexible substrate. Thin silicone membranes were coated with either fibronectin (FN) or poly-l-lysine (PLL) to assess integrin-dependent and -independent responses, respectively, and stretched using two blunt micropipettes driven in equal and opposite directions by piezoelectric translators. Graded stretch to 130% of resting cell length induced graded increases in Cav1.2 current (up to 63%) in HEK 293 cells expressing the neuronal channel isoform (Cav1.2c). The increase in current was ~2-fold greater for cells adhering to FN than for cells on PLL. On FN, 130% longitudinal stretch of primary VSM cells induced ~50% increases in Cav1.2 current. However, the magnitude of stretch-activated Cav1.2 current was the same on FN or PLL for cells expressing a Cav1.2 construct containing two C-terminal mutations (Y2122F/S1901A) to prevent phosphorylation by PKA and c-Src, or for cells expressing a Cav1.2 construct with the C-terminus truncated. Our results suggest that the Cav1.2 channel can be potentiated by membrane stretch, with one component due to intrinsic mechanosensitivity of the channel and a second component due to signaling through an integrin-dependent process.

1682-Pos

Dissecting the Molecular Mechanism of How Force Activates Yeast TRP Channel TRPY1

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Though clear for the prokaryotic mechanosensitive (MS) MscL and MscS, how mechanical force activates eukaryotic MS channels remains poorly understood. Several members of transient receptor potential (TRP) channel family are apparently involved in animal mechanosensations and are therefore promising MS-channel candidates. The yeast homolog, TRPY1, expressed in the yeast vacuolar membrane, can clearly be gated by hyper-osmotic shock *in vivo* and by directly stretching excised vacuolar membrane patches under patch clamp. Here, we investigated the structure-function relationship of TRPY1 by mutagenesis, aiming at dissecting how force is sensed by TRPY1 and how force opens the channel gate. TRP channels share general organization with well-studied voltage-gated potassium channels, being tetramers with each subunit consisting of six transmembrane helices (S1-S6) and N- and C-terminal cytoplamic domains. We found that the C-terminal cytoplasmic domains of TRPY1 harbor Ca^{2+} binding motifs, which confer TRPY1 Ca^{2+} activation. We demonstrated that the Ca^{2+} activation and the force activation are synergistic and the two gating mechanisms act in parallel. We proposed that force is perceived by the transmembrane domains. Our further in-depth analyses showed that strategic insertions of long peptide linkers before S4-S5 linker and after S6 can surprisingly yield functional channels with largely intact mechanosensitivity, highlighting the crucial roles of the pore module in TRPY1 mechanosensitivity. Together with our detailed scanning mutagenesis, we will discuss possible molecular mechanisms on how force activates TRPY1.

1683-Pos

Inactivation of the Bacterial Mechanosensitive Channel MscL Involves Flexible Transmembrane Helices and a 'Dry' Gate

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MscL, a mechanosensitive channel of large conductance, is an emergency release valve residing in the cytoplasmic membrane of E. coli. Under osmotic shock, when membrane tension approaches the lytic limit of ~10 mN/m, MscL opens a 3 nm pore relieving osmotic stress. The conformational transition in the pentameric complex was previously envisioned as a tilting iris-like motion of tightly coupled pairs of the centrally located TM1 and peripheral TM2 helices. Wetting the hydrophobic constriction formed by the rings of L19 and V23 was identified as is the rate-limiting step over a barrier of more than 50 kT. While adaptation of MscL was reported, the channel was generally considered non-inactivating. Special pressure protocols involving prolonged conditioning steps and short saturating test pulses revealed that after a 30 s exposure to half-saturating pressure (p0.5) in spheroplast patches, about 20% of MscL population reversibly inactivates. The channels return to the resting state within 1 s upon pressure release. Introduction of a flexible double glycine motif (A91G/I92G) in TM2 dramatically increased the rate of inactivation resulting in a 90% silent channel population after a 10 s step to p0.5. Single-channel traces revealed a split of concerted 70 pA opening transitions into a staircase of irregular ~7 pA substates in the double glycine mutant. The additional hydrophilic substitution in the constriction (V23T/A91G/I92G) pre-hydrates the pore, reduces p0.5 by ~55% and, while generating multiple substates, completely abolishes inactivation. In extrapolated-motion simulations TM2 kinks at A91G/I92G and its C-terminal end separates from TM1. We propose that in WT MscL TM1s and TM2s are tightly coupled, whereas in the double glycine mutant the unsupported TM1s may reform the tight hydrophobic seal independent of the positions of TM2s thus creating a tension-insensitive non-conductive state.

1684-Pos

Caveolin and Cholesterol Control of Mechanosensitive Channels in Muscle

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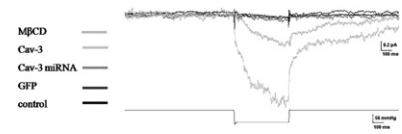
In Duchene muscular dystrophy the loss of the cytoskeletal element dystrophin modifies the localization of sarcolemma components and the stress distribution to those components. There is a change in the distribution of caveolae and dysregulation of mechanosensitive ion channels (MSCs) both of which may contribute to the elevated Ca2+ levels present in dystrophic myotubes. Caveolae are curved cholesterol rich membrane structures associated with dystrophin that contain many signaling molecules. We have shown that overexpression of Caveolin-3 in mouse myotubes increases the incidence of MSCs in patches and average MSC current. However, it has no effect on their kinetics of activation. Cholesterol depletion by MβCD produces an even larger increase in MSC incidence and average patch current, and also significantly decreases the relaxation rate of the membrane as observed by membrane capacitance changes. We have used miRNA against TRP channels (TRPC1, TRPC4, TRPC6, TRPV2) that have been reported to mechanosensitive in various systems to try and determine the identity of the channel revealed by cholesterol depletion. Average patch current and immunofluorescence staining has been used to determine the level of knockdown of the individual TRP channel subunits.



1685-Pos

Clustering and Functional Interaction of MscL Channels

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Mechanosensitive channels allow bacteria to respond to osmotic stress by opening a nanometer size pore in the cellular membrane. While the underlying mechanism has been studied intensively on the basis of individual channels, the work described here sheds light on the behavior of an ensemble of mechanosensitive channels of large conductance (MscL) in the membrane. Evaluating the spatial distribution of MscL channels in the bilayer using patch clamp, fluorescence, neutron scattering and reflection techniques, as well as atomic force microscopy and mathematical modeling, MscL was found to form clusters under a wide range of conditions. Within the cluster, MscL is closely packed, but still active and mechanosensitive. The channel activity, however, is modulated by the presence of neighbouring proteins, indicating functional protein-protein interactions. Collectively, the results demonstrate a potential functional role for self-assembly of MscL in the membrane.

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1686-Pos

A Kinetic Characterization of the Human Erythrocyte Mechano-Activated K^+ Channel Inactivation Process

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Human erythrocyte (hRBC) shows a live span of 120 days, as these cells lack nucleus and organelles a question arises: which is the subjacent molecular process to this tightly controlled programme cell death (the biological clock)? It has been proposed that the increased Ca^{2+} concentration characteristic of the senescent cells is due to a mechanical stress at the microcirculation level. Using the Patch Clamp Technique, we had characterized a mechano-activated K^+