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channel activity is mainly due to an increased open-time. The results shown here suggest that NBD1 and NBD2 may employee different chemical mechanisms in binding ATP and that NBD1 can be a potential molecular target for developing CFTR potentiators for CF-related mutants. The effects of different nucleotides (for example, GTP and UTP) on NBD1 and NBD2 will be studied to gain a better understanding of the chemical mechanism underlying nucleotide-NBD interaction.

1672-Pos
The Inhibition Mechanisms of the Regulatory Domain of Cyctic Fibrosis Transmembrane Conductance Regulator
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The cyctic fibrosis transmembrane conductance regulator (CFTR) is a member of the human C subfamily of ATP-binding cassette (ABC) transporters but functions as a chloride channel. Activity of CFTR is tightly controlled not only by ATP binding-induced NBD1-NBD2 dimerization but also by phosphorylation of the unique regulatory (R) domain by protein kinase A (PKA). The R domain has multiple phosphorylation sites for which only Ser737 and Ser768 are inhibitory. The underlying mechanisms are unclear because neither the structure of the R domain nor its interactions with other parts of CFTR have been fully illuminated. Here I applied the crystal structure of bacterial transporter Sav1866 and sulfhydryl-specific crosslinking strategy to determine which part of CFTR interacts with the R domain regulating channel activity. The results show that diamide-induced disulfide bond crosslinking of S768C to H950C, K951C, H954C or S955C from cytoplasmic loop 3 (CL3) inhibited the channel activity and inhibition was reversed by DTT. Similarly, disulfide crosslinking of S737C to H954C, S955C or Q958C also suppressed the channel activity. Furthermore, mutation of these residues to alanines weakened the curcumin-induced relative PKA-dependence which was completely removed by deletion of the R domain. Finally, activation of a double mutant H950R/S768R CFTR did not need any PKA while either H950R or S768R construct needed it. These results suggest that S768 and S737 may form putative H-bonds with hydrophilic residues of CL3 and thus inhibit the channel activity in the unphosphorylated state. In the phosphorylated state, a putative ferrous bridge involving H950, H954, C832, D836, H775 and H667 at the CL3-R interface may inhibit the channel activity. All these observations are consistent with the recent electron cryomicroscopy-based structural model on which the R domain is closed to cytoplasmic loops regulating channel gating.

1673-Pos
CFTR Cytosolic Loop Mutations Allosterically Promote ATP Independent Channel Gating
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CFTR channel gating normally depends on ATP binding and NBD dimerization. Optimal CFTR channel activation further requires phosphorylation of the R domain. How ATP binding at the NBDs and phosphorylation of the R domain regulate CFTR channel gating are not fully understood. In the present study, we demonstrate that mutations in the CFTR Cytosolic Loops (CL) markedly promote channel opening in the absence of ATP and NBD2, presumably by an allosteric mechanism. In excised inside-out patches, we observed that single or double mutations of K978 and K190 in CL 3 and 1 induced large ATP independent currents (5-70% of current before removing ATP). These mutant channels deactivated with a slow time constant (49.11 ± 4.58 sec) when ATP was removed by Hexokinase/glucose and subsequent bath perfusion. A K978 point mutation greatly increased the ATP sensitivity of channel activation by decreasing the EC_{50} (by 8-fold) for ATP activation, which is consistent with the slow deactivation following ATP removal. K978 mutations markedly enhanced G551D channel activity, a disease mutant that fails to respond to ATP, and Δ1198-CFTR, a mutant that lacks NBD2, indicating that the K978 mutations affect channel gating downstream of NBD dimerization. Interestedly, R domain phosphorylation further stimulated K978/G551D and K978/Δ1198 combined mutants, indicating that the R domain regulates channel activity independently of NBD dimerization. Similarly, K978 mutations also increased the activation rate at low dose (3 U/ml) of PKA, indicating that K978 mutations also enhance the PKA sensitivity of channel activation. Our results support an allosteric gating mechanism in which loops 1 and 3 functionally link ATP binding and NBD dimerization to CFTR channel opening.

1674-Pos
Accessibility of Cysteines Within the NBD Interface in a CFTR Channel
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Opening and closing of a CFTR channel is accompanied by ATP-driven formation and hydrolysis-triggered disruption of a head-to-tail NBD1-NBD2 heterodimer where composite interfacial sites, between the Walker motifs of one NBD and the LSGGQ-like (ABC signature) sequence of the other, each enclose an ATP molecule. Only the ''NBD2'' composite site (containing NBD2 Walker motifs) is catalytically competent. The ATP-bound tight NBD1-NBD2 heterodimer is linked to the open-channel state, but the disposition of the NBDs in the closed-channel state of CFTR, in the absence of ATP or after its hydrolysis, remains unknown. To address this, we assess accessibility to various size MTS reagents of single interfacial target cysteines introduced into the ABC signature sequence of the competent site (at NDB1 position S549), or of the dead site (at NBD2 position S1347), or at mid-interface positions S605 of NBD1 or A1374 of NBD2, in full-length cys-depleted CFTR-C(832-1458)S,expressed in *Xenopus* oocytes and examined in excised patches. Cysteines at all four positions were readily accessible to MTSET in closed channels in the absence of ATP. For channels opening and closing in 3 mM ATP, the reaction rate depended on MTSET concentration and was $\sim 10^5\ M^{-1}s^{-1}$ at 5 μM at position 549, and was similarly rapid at corresponding position 1347. In closed channels without ATP, cysteines at 549, 605, 1347, and 1374 were all also readily accessible to MTS reagents of increasing size, e.g., MTS-biotin, MTS-THAE (trihexylammonium-ethyl) and MTS-verapamil, up to ~11Åx16Åx10Å, suggesting substantial separation between NBD1 and NBD2, throughout the NBD interface, in closed CFTR channels. (Supported by NIH DK51767).

1675-Pos
Strict Coupling Between CFTR's Catalytic Cycle and Gating of its Channel Pore Revealed by Distributions of Open Burst Durations
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CFTR, the ABC protein defective in cystic fibrosis, functions as an anion channel. Once phosphorylated by protein kinase A, a CFTR channel is opened and closed by events at its two cytosolic nucleotide binding domains (NBDs). Formation of a head-to-tail NBD1/NBD2 heterodimer, by ATP binding in two interfacial composite sites between conserved Walker A and B motifs of one NBD and the ABC-specific signature sequence of the other, has been proposed to trigger channel opening. ATP hydrolysis at the only catalytically competent interfacial site is suggested to then destabilize the NBD dimer and prompt channel closure. But this gating mechanism, and how tightly CFTR channel opening and closing are coupled to its catalytic cycle, remain controversial. Here we determine the distributions of open burst durations of individual CFTR channels, and use maximum likelihood to evaluate fits to equilibrium and non-equilibrium mechanisms and estimate the rate constants that govern channel closure. We examine partially- and fully-phosphorylated, wild-type CFTR channels, and two mutant CFTR channels each bearing a deleterious mutation in one or other composite ATP binding site. We show that the wild-type CFTR channel gating cycle is essentially irreversible and tightly coupled to the ATPase cycle, and that this coupling is completely destroyed by the NBD2 Walker-B mutation D1370N but only partially disrupted by the NBD1 Walker-A mutation K464A. [NIH R01-DK051767, NIH FIC R03-TW007829, Wellcome Trust 081298/Z/06/Z]

1676-Pos
ΔF508 CFTR Expressed In *Xenopus* Oocytes Exhibits Unique Thermal Sensitivity
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The deletion of a phenylalanine at position 508 is the most common, disease-related mutation in the CFTR protein. ΔF508 CFTR channels are assembled in mammalian cells, but exhibit two deficiencies thought to underlie the disease phenotype: impaired trafficking and defective gating. The CFTR trafficking defect is partially mitigated at low temperature so that *Xenopus* oocytes are ideal for studying the physical properties of ΔF508 channels under physiological conditions. In order to investigate possible effects of the Phe deletion on the intrinsic stability of CFTR channel function in the plasma membrane, we monitored channel activity at room temperature (~23°C) and during a brief (10-12 minute) period of elevated temperature (28°C to 37°C). In oocytes expressing wild type CFTR, a temperature challenge resulted in a reversible increase in the conductance; a result of a simultaneous increase in single-channel conductance and open probability. Both parameters, however, returned to baseline values

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 ($V_{1/2} = -30$mV 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