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a complete removal of ATP, raising the possibility that gating motion in the TMDs of modified L102C channels is independent of that in their NBDs. Finally, MTSET modification of L102C is state-dependent, meaning that L102C-cysless-CFTR channels do not respond to the treatment of MTSET in the absence of ATP. These findings suggest that either position 102 moves during gating or gating motion in other regions of CFTR alters its reactivity towards MTSET. Overall, our preliminary data reveal several interesting yet enigmatic aspects of TM1 that call for further studies.

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Three Charged Amino Acids in the Outer Vestibule of CFTR Stabilize the Open Pore Architecture

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Cystic fibrosis transmembrane conductance regulator (CFTR) carries 6 extracellular loops (ECL1-6). ECL4 bears N-linked oligosaccharide chains while the functions of other ECLs remain unknown. Few charged amino acids of ECL1 have been identified as sites of CF disease mutation, including R117C/G/P/H/L, D110H/Y/N/E, and E116K/Q. It was reported that D110H-, E116K-, and R117C/L/P-CFTR possibly impair channel stability but not R117H. We asked whether these amino acids are directly involved in ion conduction and permeation of CFTR or contribute to stabilizing the outer vestibule architecture. We used cRNA injected oocytes combined with electrophysiological technique to probe the possible function of these amino acids. We found that: (1) Mutants R117A-, D110R-, and E116R-CFTR exhibited multiple open states with significantly shortened burst duration compared with WT-CFTR, while charge-retaining mutants R117K-, D110E-, and E116D-CFTR showed mainly the full open state which rescued the open burst duration similar to WT-CFTR; (2) R117A-, D110R-, and E116R-CFTR unlike WT-CFTR failed to be locked into the open state by AMP-PNP; (3) The function of R117C-, D110C-, and E116C-CFTR were not modified by extracellular $MTSES^-$ or $MTSET^+$; (4) R117C-, D110C-, and E116C-CFTR were weakly blocked by GlyH-101 compared to WT-CFTR, while GlyH-101 strongly blocked T338A- and R352A-CFTR and completely lost its effect on R334C- and R334A-CFTR. R334, T338, and R352 are amino acids in TM6 which is the most important TM that determines ion permeation in CFTR. The data so far suggest that: (1) R117, D110, and E116 are not involved in ion conduction and permeation of CFTR directly; (2) The three charged amino acids contribute to stabilizing the CFTR channel pore; (3) The three charged amino acids probably interact with their partners to help maintain CFTR's outer vestibule architecture. (NIH-R01-DK056481).

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Effects of Genistein and Curcumin on Non ATP-Hydrolytic CFTR Mutants

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The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel plays an important role in salt and water transport across epithelia and defective function due to mutations in the CFTR gene cause cystic fibrosis (CF). Numerous small molecules have been shown to increase the activity of CFTR mutants presumably by binding to the CFTR protein. Among the many CFTR potentiators, genistein is perhaps the most extensively studied. Recently a component of the spice turmeric, curcumin was reported to strongly activate wild type and mutated CFTR including F508del and G551D mutations. Recently we found that genistein and curcumin have a synergistic effect in the potentiation of G551D-CFTR (Yu, Miki et al. J Cystic Fibrosis 10: 243 - 252, 2011). However, the mechanism through which these compounds increase the CFTR activity is still unclear.

To study the mechanisms of genistein and curcumin, we investigated the effects of genistein and curcumin on the non ATP-hydrolytic CFTR mutants, K1250A- and E1371S- CFTR, expressed in CHO cells using whole-cell clamp technique. The reflect to the single channel currents. Because of their open probability close to 1, the whole-cell currents are thought to reflect the amplitude of their single channel currents.

Curcumin did not significantly affect the whole-cell currents obtained from CHO cells expressing K1250A- or E1371 S-CFTR whereas genistein induced a voltage-dependent block on them. Interestingly a combined application of genistein and curcumin induced a voltage-independent reversible reduction in K1250A- or E1371A-CFTR whole-cell currents. This current reduction seemed to be mainly induced by the genistein and curcumin accessing to CFTR proteins from the external side.

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Evolutionary and Mechanistic Insights into ABC Exporters

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A prevalent model holds that ABC transporter function involves an ATP-driven conformational cycle in which ATP hydrolysis dissociates a tight dimer of nucleotide binding domains (NBDs), so propelling the transmembrane domain (TMD) conformation from outward to inward facing. Detailed characterization of these TMD events lags behind those in the NBDs because the TMDs are structurally diverse and more refractory to structural analysis. Recently we developed a bioinformatic approach that predicts evolutionarily conserved interactions between pairs of sequence positions. Like other approaches, ours gauges how, at each position, amino acid variation across aligned homologous sequences correlates with that at any other position. Distinctively, our approach exploits structural input to optimize performance through a side-chain contact prediction test. We applied this approach separately to the ABCB and ABCC subfamilies (represented by Pgp and CFTR) that are clearly, albeit distantly, homologous for all domains. Their TMD dimers contain 2x6 transmembrane helices in distinct bundles: two 'wings' at the extracellular side and two pairs of intracellular 'loop' (ICL) extensions. Comparing inward to outward facing structures suggests that rigid body motions of these bundles underlie transport mechanism. In our bioinformatic analysis, the precise pattern of predicted position pairs differed between subfamilies, alluding to their deep evolutionary segregation. But some general patterns were shared: pairs separated by one helical turn, and those between helices of the same bundle, were frequently predicted and may provide stability and rigidity to bundles throughout the transport cycle. On the other hand, a few predicted pairs between bundles exhibited strikingly different spatial separation in opposing conformations, such as Q179-V260 (ICL1-ICL2), separated by 5.7 Angstroms in CFTR modeled in the outward conformation but 16.1 Angstroms in the inward conformation. Thus, this approach provides detailed evolutionary and mechanistic insights into large classes of ABC exporters. [DK51767].

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Voltage-Dependent Gating of ClC-2 Chloride Channel

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The gating of ClC-2 Cl^- channel is facilitated by elevated $[H^+]_e$ and $[Cl^-]_i$ by interacting with the protopore gate whilst $[Cl^-]_e$ had not effect. In contrast, the gating of ClC-0 Cl^- channel is facilitated by protonation of the protopore gate by intracellular $[H^+]_i$ in a manner that is dependent on the extracellular $[Cl^-]_e$. To gain insights into the V_m dependent mechanism of ClC-2 expressed in HEK cells, we determine the V_m-dependence of open probability ($P_A(V_m)$) at different $[H^+]_i$, $[H^+]_e$ and $[Cl^-]_i$ using the patch clamp technique. Changing $[H^+]_i$ by 5 orders of magnitude whilst $[Cl^-]_i/[Cl^-]_e$=140/140 or 10/140 mM did not altered the onset kinetics but channel closing became faster at acidic pH_i and $P_A(V_m)$ curves were shifted towards more negative V_m. These results suggest that $[H^+]_i$ did not facilitated gating. In contrast, a same change in $[H^+]_o$ with $[Cl^-]_i/[Cl^-]_e$=140/140 mM enhanced P_A in a bi-phasic manner and shifted $P_A(V_m)$ curves to positive V_m. Importantly, P_A was >0 with $[H^+]_o=10^{-10}$ M and channel closed more slowly when $[H^+]_o$ or $[Cl^-]_i$ increased. This implied that ClC-2 can be gated without protonation and that external H^+ and/or internal Cl^- stabilized the open state. A kinetic analysis of Cl^- currents and $P_A(V_m)$ curves at different $[H^+]_o$ and $[Cl^-]_i$ using a gating scheme coupled to Cl^- permeation indicated that protonation of the protopore gate has negligible V_m- and Cl^--dependence and that the rate constant for closed-open transition of un-protonated channels were facilitated by elevated $[Cl^-]_i$ in a V_m-dependent manner. We propose that the majority of the V_m-dependence is due to a V_m-dependent occupancy of ClC-2 pore by the permeant Cl^- and that the open conformation is stabilized by a V_m-independent protonation and the Cl^- occupancy. Supported by CONACyT.

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Alkaline pH Block of CLC-K Kidney Chloride Channels Mediated by a Pore Lysine Residue

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The human chloride channels ClC-Ka/Kb, as their murine orthologues ClC-K1/K2, are expressed in kidney and inner ear epithelia where they are involved in NaCl reabsorption and endolymph production, respectively. Mutations in ClC-Kb and barttin, an essential CLC-K channel beta subunit, lead to Bartter syndrome. Recently we identified the external residue H497 responsible for block of ClC-Ka at acid pH (Gradogna et al. 2010. J Gen Physiol 136:311). Now we