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Electrophysiological Characterisation of TMEM16A Currents in Esophageal Squamous Cell Carcinoma Cells
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TMEM16A is a calcium activated chloride channel that has been linked to a number of cancer types including prostate, head and neck and esophageal squamous cell carcinomas (ESCC). The TMEM16A gene has been shown to be amplified and overexpressed in ESCC. To assess the presence of functional TMEM16A channels in human TE-11 cells, an established ESCC cell line, whole-cell current measurements were performed in the presence of different intracellular calcium concentrations using both conventional and planar (QPatch, Sophion) patch clamp technology. Channel characterization was based on the voltage, time and calcium dependence of the currents. Pharmacological validation was also undertaken.
In the presence of 338 nM intracellular free Ca^{2+}, the chloride currents showed time dependent activation and deactivation and a characteristic outward rectifying current-voltage relationship consistent with TMEM16A. These currents were blocked by 100 μM CaCCinh-A01 (95 ± 2 % inhibition, n = 17). The CaCCinh-A01 sensitive chloride current density was increased from 20.5 ±10.7 pA/pF (n = 7) in the absence of intracellular Ca^{2+}, to 171 ± 26 pA/pF (n = 11) in the presence of 1 μM intracellular Ca^{2+}, indicating these currents are calcium dependent as expected for TMEM16A. The currents were also sensitive to other non-specific calcium activated chloride channels blockers e.g. niflumic acid and DIDS. Treatment of TE-11 cells with shRNAs targeting TMEM16A significantly decreased the chloride conductance in TE-11 cells consistent with them being mediated by TMEM16A. Data generated using conventional patch clamp was in good agreement with that from the QPatch, thus validating the application of the QPatch platform for this type of study. The data provides the first electrophysiological characterisation in esophageal squamous cell carcinoma cells of an endogenous Ca^{2+}activated chloride current, confirming the presence of functional TMEM16A in these cancer cells.

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Electrophysiological Properties of TMEM16A Calcium-Activated Chloride Channels
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The functional properties and physiological roles of the calcium-activated chloride channels (CaCCs) encoded by family members of transmembrane proteins of unknown function 16 (TMEM16) have been rigorously studied. Nevertheless, a poorly understood characteristic of CaCCs, namely the channel rundown, significantly interferes electrophysiological measurements of these channels. In our studies, the rundown problem of CaCCs was circumvented by normalizing the Ca^{2+}-induced current to the maximally-activated current within a short period of time in which the rundown was insignificant. Activation of the TMEM16A-encoded CaCC, also known as ANO1, by divalent cations Ca^{2+}, Sr2+, Ba2+, and $Mg2^{+}$ were thus characterized. Ca^{2+}, Sr2+, and $Ba2^{+}$ were found to activate the ANO1 channel with different affinities, but these three divalent cations activate current to the same maximal level. On the other hand, $Mg2^{+}$ was unable to induce current in ANO1, yet, appeared to compete with Ca^{2+} to shift the Ca^{2+} concentration-dependent activation curve. We also studied the permeability of the ANO1 pore for various anions, and discovered that the anion occupancy in the pore was inversely correlated with the apparent affinity of the ANO1 inhibition by niflumic acid (NFA), a known CaCC blocker. In contrast, activating the channel by different divalent cations or the different degree of channel activation did not affect NFA inhibition, suggesting that NFA inhibits the channel by altering the pore function rather than by changing the channel gating. In conclusion, our study characterized functional properties of ANO1 without interference from channel rundown, and revealed properties of the channel that have not yet been documented.

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Monitoring Substrate-Driven Structural Changes in a CLC Chloride-Proton Antiporter with Double Electron-Electron Resonance Spectroscopy
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ClC-ec1, a prokaryotic Cl-/H^{+} antiporter in the CLC family, has been crystallized under many conditions. Yet, aside from local structural differences at the chloride binding site, only one major conformation is observed. This failure of X-ray crystallography to reveal the unknown conformational states in the transport cycle motivates the use of alternative approaches. Using 19F-NMR, a previous study demonstrated that a tyrosine residue > 20 Å from the chloride-transport pathway undergoes antiport-specific structural changes (Elvington et al. 2009. EMBO J). We combine computational and experimental approaches to further investigate possible protein movements during the antiport cycle. A computational model of ClC-ec1 based on the inverted topology repeat hypothesis (Forrest et al. 2008. PNAS) predicts structural changes that alter the accessibility of pathways to the central Cl- and H^{+} binding sites. To test the model, we introduced nitroxide paramagnetic spin labels to the ClC-ec1 homodimer via site-directed spin labeling and monitored structural changes using double electron-electron resonance (DEER) spectroscopy. Preliminary results are consistent with the inverted-topology repeat model and suggest that the antiport cycle involves structural changes beyond local movement at the chloride-binding site.

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Testing the Limits of Stoichiometric Exchange in a CLC-Type Transporter
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The CLC transporters mediate the stoichiometric exchange of 2 Cl^{-}: 1 H^{+} in prokaryotes and in eukaryotes. The CLC Cl^{-} transport pathway is defined by 3 anionic binding sites, S_{ext}, S_{cen} and S_{int} which can be occupied either by Cl^{-} ions or by a conserved glutamate, Glu_{ex}. Three conformation of the pathway have been observed where (i) the protonated Glu_{ex} is out of the pathway and the 3 sites are occupied by Cl^{-}, (ii) Glu_{ex} occupies the S_{ext} while Cl^{-} ions are bound to S_{cen} and S_{int}, and (iii) Glu_{ex} in S_{cen} sandwiched by two Cl^{-} ions in S_{ext} and S_{int}. While the role of S_{cen} and S_{ext} as well as that of Glu_{ex} as the external gate is well documented, the rate-limiting step regulating the exchange of ions between S_{int} and S_{cen} remain poorly understood. Here we investigate (i) how robust is the coupling stoichiometry of the CLCs and (ii) the role of Sint in the transport cycle.
To this end we reconstituted CLC-ec1 in proteoliposomes and used Cl^{-} and H^{+} flux assays to measure transport. To test the robustness of the stoichiometry of coupled transport we are measuring simultaneous Cl^{-} and H^{+} fluxes under extreme Cl^{-} gradients and at voltages as high as 180 mV. To identify the role of S_{int} in transport we measured H^{+} transport driven by voltage at different symmetrical Cl^{-} concentrations. As expected H^{+} transport increases when [Cl^{-}] is raised from 0.02 to ~0.3 M. Interestingly, we found that H^{+} transport decreases for [Cl^{-}] >0.3 M and is drastically inhibited at 1 M [Cl^{-}]. This result suggests that at high [Cl^{-}] Glu_{ex} becomes ''sandwiched'' between Cl^{-} ions in S_{ext} and S_{cen}, indicating that the internal site does play a role in the transport cycle.

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Conformational Changes Required for Chloride Ion Permeation in the CLC-ec1 Exchanger
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In the classical view, channels and transporters are different in their transport rates and mechanisms. Interestingly, the CLC protein family contains both chloride ion channels and H^{+}/Cl- exchangers. The CLC-ec1 exchanger, for which high-resolution structures and functional data are available, provides a framework to understand the mechanical features that distinguish between coupled and uncoupled transport.
Although mutational studies have identified key residues regulating chloride transport and coupling to protons, the detailed permeation mechanism remain unresolved. We performed molecular dynamics simulations to better understand the structural features that regulate chloride permeation. The simulations show that the distance between residues S107 and Y445, forming the internal gate, fluctuates extensively. These fluctuations are correlated to the conformation of helix O, which can be straight or kinked as seen in the X-ray structure. Potential of mean force calculations show that, in its straight conformation, helix O limits the fluctuation of the internal gate and thus impede ion permeation. On the contrary, the kinked conformation of helix O frees Y445 thereby favoring the opening of the internal gate and the translocation of a Cl- ion between the central and internal binding sites. Breaking salt-bridges by the protonation of glutamic acid near the intracellular mouth of the pore favors fluctuations and opening of the gate.