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Coupling of Anion Selectivity and Proton Transport in CLC-ec1
Tao Jiang. Email: tjiang@illinois.edu.
University of Illinois at Urbana-Champaign, Urbana, IL, USA.
CLC transporters catalyze transmembrane exchange of chloride ions and protons necessary for pH regulation of key physiological processes. Based on abundant structural information obtained for a bacterial CLC (CLC-ec1), the chloride pathway is defined by three anionic binding sites that span the length of the membrane. Recent results have suggested that protons might be transported by hopping through water chains between the extracellular and cytoplasmic gates in CLC-ec1. A fundamental question concerning the CIC Cl-/H+ antiporters is the mechanism of proton transport coupled to anion binding. Cl- binding facilitates functional H+ transport, while NO3- or SCN- appear to reduce or completely abolish H+ coupling to anion movement. To investigate the coupling mechanism between anion binding to the central binding site (Scn) and water-mediated proton transport, we first applied free energy perturbation calculations to study the Scn anion selectivity. The binding free energy of NO3- and SCN- indicates that binding of these ions is unfavorable compared to Cl- binding. To compare the stability of water wires in the presence of different Scn binding anions, we employed molecular dynamics simulations starting from a snapshot including a preformed water wire structure, with Scn occupied by either Cl-, NO3-, or SCN-, respectively. The average lifetime of the water wire is greatly reduced upon the binding of NO3-, while the SCN-binding breaks the wire immediately by inserting between the water molecules. Based on these results, we suggest that Scn anion selectivity affects the coupled proton transport in two ways: first, Scn selectivity determines whether the anion can bind stably; and second, whether the bind anions can support the transient water wire which is required for conduction of the protons.

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Conformational Changes Outside the Ion Pathway are required for Transport in a CLC-Type Cl-/H+ Exchanger
Daniel Basilio, Kristin Noack, Alessandra Picollo, Alessio Accardi.
Weill Cornell Medical College, New York, NY, USA.
The CLC proteins catalyze transport of chloride ions (Cl-) through cellular membranes in muscle, kidney, bone, and neurons. While some CLCs are ion channels others are H+-coupled secondary active transporters mediating the stoichiometric exchange of 2 Cl- for 1 H+. The exchange mechanism of the CLCs is unclear. All proposed models postulate that the only conformational changes taking place during transport are the movements of a conserved glutamate’s side chain in and out of the Cl- permeation pathway. This hypothesis is supported by structural and functional work. However, others have suggested that regions distal to the Cl- pathway might also be involved in transport. To test whether transport entails only local or also global rearrangements we constrained the movement of helices J, O, P and Q, which do not line the Cl- or H+ pathways in CLC-ec1, a CLC prokaryotic homologue. If exchange involves the relative movement of these helices then these constraints should reduce the transport rate. In a cys-less background we introduced pairs of cys-linked doubly ungated mutant. Results show increases 2-fold, while that of Cl- transport is steeply voltage-dependent, increasing much more dramatically over the same range. We aim to explore this finding using the CLCβ homologue, CLC-eca, by monitoring anion fluxes in reconstituted liposomes.

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An Optical Assay of the Transport Activity of CLC-7
Ilaria Zanardi, Silvia De Stefano, Giovanni Zifarelli, Michael Pusch.
IBF CNR, Genova, Italy.
Osteoporosis, characterized by excessive osteoclast mediated bone resorption, affects millions of people. CLC-7 is a chloride-proton exchanger member of the CLC protein family localized in lysosomes and in the ruffled border of osteoclasts. Loss of function of CLC-7 leads to osteopetrosis, neurodegeneration and lysosomal storage disease. The osteopetrotic phenotype is explained by the fact that the ion transport activity of CLC-7 is essential for the osteoclast mediated bone resorption. Thus, blocking CLC-7 can be expected to provide an effective treatment of osteoporosis. Here, we describe a purely optical assay of CLC-7 function employing the E2GFP-dsRed CI-/H+ exchanger, which we call Fluc (previously named Fluc-Exchanger, which we call Fluc (previously named Fluc). Fluc is phyogenetically unrelated to any protein of known function. It has four transmembrane helical spans, and assembles as an oligomer. In some organisms, Fluc is a homo-oligomer, and in others, the functional Flch channel, which is a mixed tetramer of subunits encoded by consecutive Fluc genes in an operon that share low sequence identity. Knockout of genes that encode Fluc proteins renders organisms much more sensitive to environmental F-. We present functional data for several purified Fluc homologues reconstituted in liposomes and in planar lipid bilayers, including single channel recordings. Macroscopic F+ currents show high selectivity for Cl- over H+, single channels show high constitutive open probability, occasional substates, and a main-state conductance of the order of 5 pS. The oligomeric structure of Fluc is assessed by static light scattering in detergent micelles and a functional “Poisson-dilution” assay in transport-active liposome membranes.

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Dissection of Proton Inhibition Mechanism in the H+ /Cl- Exchanger, CLC-7
Daniel Silverman, Joseph A. Mindell.
National Institute of Neurological Disorders and Stroke, Bethesda, MD, USA.
CLC-7 is a voltage-activated H+ /Cl- exchanger that serves a critical yet somewhat uncertain role in lysosomal membranes of diverse mammalian cell types. Despite trafficking to acidic vesicles in the cell, proton inhibition is a functional characteristic of mammalian CIC H+/Cl- exchangers in general and of CLC-7 in particular. We sought to dissect the mechanism of proton inhibition.