translocation step in the case of a transporter or a pore gating step in K2P channels.

### 2151-Pos Board B288

**Can CIC-2 Chloride Channel be Activated by Hyperpolarization Alone in Cells Dialyzed with Non-Permeant Anions?**

José J. De Jesús-Pérez, Alejandra Castro-Chong, Ru-Chi Shieh, Carmen Y. Hernández-Carbollo, José A. De Santiago-Castillo, Jorge Arreola

1. Institute of Physics, Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico
2. Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan
3. ReliaXpert, San Luis Potosi, Mexico

Open and closing of ion channels is driven by conformational changes triggered by either an intrinsic voltage sensor in voltage-gated channels or by ligand binding in ligand-gated channels. However, CIC-2, a two pore Cl channel is activated by hyperpolarisations despite of lacking of an intrinsic voltage sensor. The structural information available to date strongly suggests that in the closed conformation each pore of CIC-2 is occluded by the negatively charged side chain of a glutamate residue. Although the gating mechanism is unknown, there is evidence which indicates that intracellular anions and extracellular protons regulate gating. The present work was aimed to determine the contribution of pore occupancy caused by intracellular anions in gating the mouse CIC-2 channel. In our experiments, CIC-2 was readily activated by hyperpolarisations when permeant anions such Cl−, SCN−, Br− and I− were present on either side of the membrane. However, CIC-2 was not activated in cells dialyzed with acetate (0 Cl−/C0) and exposed to [H+]i at [H+]o = 10−13 M. In contrast, the channels were opened by increasing [Cl−], at [H+]i = 10−10 M a condition unlikely to protonate the glutamate’s side chain. Importantly, voltage gating occurred when F− or glutamate were present in the cytosolic side. Since these two anions are non-permeant we propose that they must enter the pore and interact with the glutamate side chain from the inside in order to induce opening. Thus, we propose that a strong hyperpolarisation drives the intracellular anions into the permeation pathway and the subsequent electrostatic interaction between the anions and the negatively charged side chain opens the pore. Our data support the hypothesis that voltage-dependent gating in mouse CIC-2 requires intracellular anions. Supported by grant 219949 from CONACYT.

### 2152-Pos Board B289

**Molecular Basis of Voltage-Dependent Gating in CIC Transporters**

Jan-Philipp Machtmans, Matthias Grieschat, Christoph Fahlke

1. Institute of Complex Systems, Zelluläre Biophysik (ICS-4), Forschungszentrum Jülich, Jülich, Germany
2. Institut für Neurophysiologie, Medizinische Hochschule Hannover, Hannover, Germany

The CIC family encompasses Cl− channels and coupled Cl−/H+ exchangers. CIC channels and transporters both exhibit voltage-dependent gating, the physiological importance of which is illustrated by multiple disease-causing mutations that specifically result in altered voltage sensing. Despite a large body of available functional and structural data, the molecular mechanisms underlying voltage gating in CICs still remain unclear. Using electrophysiological admittance measurements, we recently decomposed voltage-dependent activation of the human CIC-5 transporter into multiple discrete electrogenic transitions (Grieschat M & Alekov AK (2014) Biophys J 107, L13–L15). A key player of the gating machinery appears to be the so-called gating glutamate (Glueint, Glu148 in EcCIC), which is thought to move upon changes in voltage. To better understand the basis of voltage-dependent gating, we conducted molecular dynamics simulations of the bacterial homologue EcCIC. Using a double-bilayer system, we applied small ionic concentration gradients across the membrane that resulted in charge imbalances Δψint and we calculated the resulting potential differences Vm. Analysis of the dependence of Vm on Δψint for various conformations that differed in Cl− binding site occupancy and the protonation state of Glueint, was used to determine charge displacements of the transporter along the electric field. Multiple processes, including protonation and conformational changes of Glueint, binding of Cl− ions and refocusing of the electric field triggered by changes in water accessibility are associated with charge transfer across the membrane and therefore exhibit intrinsic voltage dependence. We calculated gating charges that underline these conformational transitions of the CIC transporter. Based on these calculations, gating charge recordings and capacitance measurements on CIC-5, we propose a molecular description of CIC voltage sensing which attributes fractional gating charges to these partial reactions of the transport cycle.

### 2153-Pos Board B290

**Multiphasic Profiles: Discontinuous Transitions in Conductance-Voltage Data for Ion Channels**

Per Nissen

Norwegian Univ. of Life Sciences, Ås, Norway

Multiphasic profiles, a series of straight lines separated by discontinuous transitions, were first found (Nissen, Annu. Rev. Plant Physiol. 1974) for the concentration-dependence, plotted in linear transformations of the Michaelis-Menten equation, for ion uptake in plants. Reanalyses of recently published data show that conductance-voltage data for ion channels are also well represented as multiphasic. In contrast, the Boltzmann function and other functions giving curvilinear profiles must often be rejected for statistical reasons (very low probabilities by the Runs test that the uneven distribution of points around the profiles is due to chance). Plots of deviates show that the fits to multiphasic profiles are usually better than the fits to curvilinear profiles, i.e. that the data are more precise than apparent from the published plots. As shown by simulation, the better fits to multiphasic profiles are not due to errors (noise) in the data. Adjacent lines are often parallel. The transitions are then necessarily in the form of noncontiguities (jumps). Non-parallel lines are also frequently separated by jumps. Most often, replicate determinations give different multiphasic patterns (number of phases, voltages at which the transitions occur). The use of the averages will then give a meaningless pattern. Whenever the data are sufficiently detailed and precise, multiphasic profiles are also found for a variety of other processes and systems, biological as well as nonbiological: 1) pH profiles, including profiles for non-enzymatic model systems. 2) Binding as determined by fluorescence anisotropy titration or isothermal titration calorimetry (ITC). 3) Folding and unfolding of proteins. Implications for the opening and closing of single biological channels will be discussed.

### 2154-Pos Board B291

**A Highly Cooperative and Steeply Voltage Gated Channel Triplet**

Shang H. Lin, Benjamin Wu, Marco Colombini

1. University of Maryland, College Park, Greenbelt, MD, USA
2. University of Maryland, College Park, College Park, MD, USA

When reconstituted into planar phospholipid membranes, a 4.5nS (in 1M KCl) membrane channel complex behaves as if it is composed of three channels that are operating in a highly cooperative manner. The channels are steeply voltage-gated (e-fold for 1.8 mV) and well-organized. In the ~70mV range, no voltage gating takes place until the first channel, channel 1, closes at high positive potentials (~70 mV). Only following this closure does channel 2 begin to gate in response changes in voltage. For channel 2, closure takes place at low negative potentials (typically ~25 to ~30 mV). It is only when channel 2 is closed that channel 3 closes routinely but does so at low positive potentials (typically 25-30 mV). Simultaneous reopening of channels 2 and 3 is common, demonstrating especially high cooperativity. These and other remarkable properties support a working model for the gating and cooperativity of these channels. The highlights include anti-parallel orientation of the channels and the voltage sensing which attributes fractional gating charges to these partial reactions of the transport cycle.