Supplementary information

ClC-3 is an intracellular chloride/proton exchanger with large voltage-dependent nonlinear capacitance

Raul E. Guzman[1,2], Matthias Grieschat[1], Christoph Fahlke[1,2], and Alexi K. Alekov[1]

[1]Institut für Neurophysiologie, Medizinische Hochschule Hannover, D-30625 Hannover, Germany

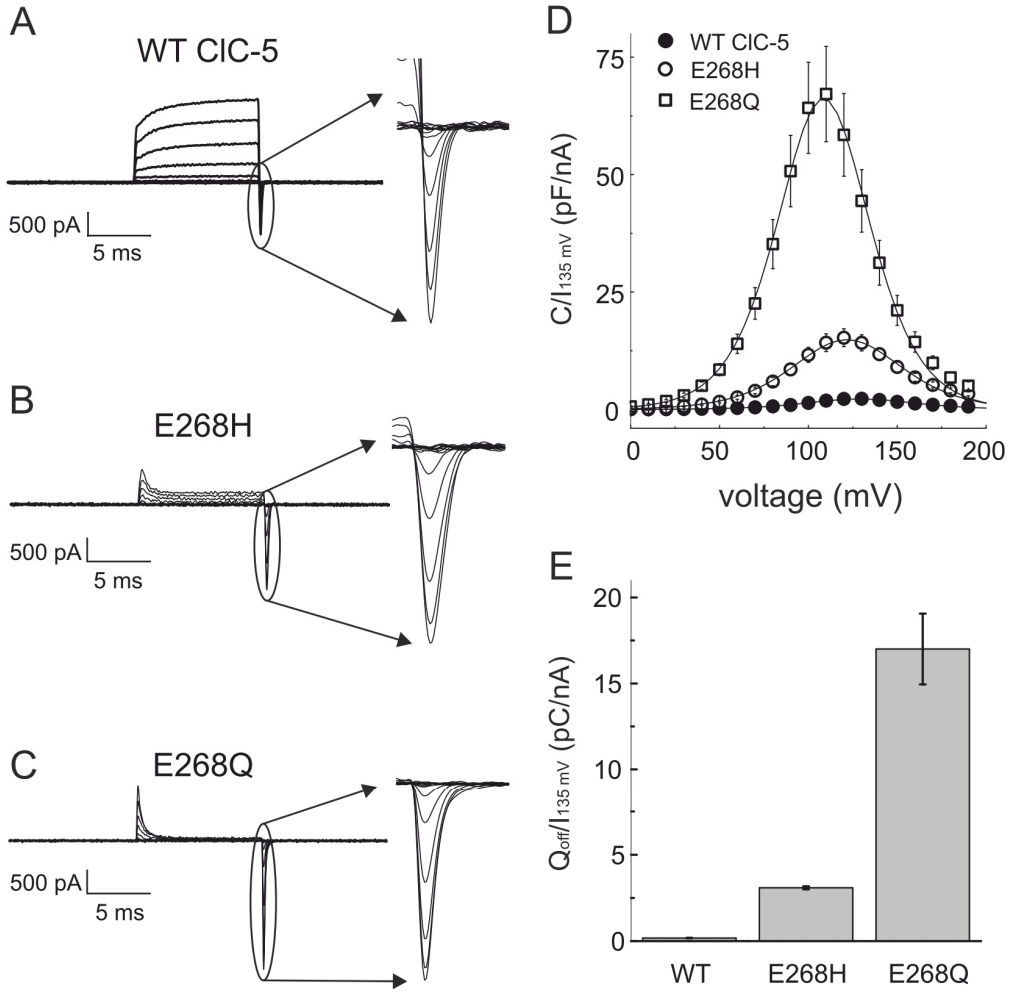
[2]Institute of Complex Systems, Zelluläre Biophysik, Forschungszentrum Jülich, 52425 Jülich, Germany

Correspondence to: A. K. Alekov Email: alekov.alexi@MH-Hannover.de, alexi.alekov@gmail.com, postal address: Medizinische Hochschule Hannover, OE4230, Carl-Neuberg-Str.1, D-30625 Hannover, Germany, Fax: ++49 511 532 9391, or

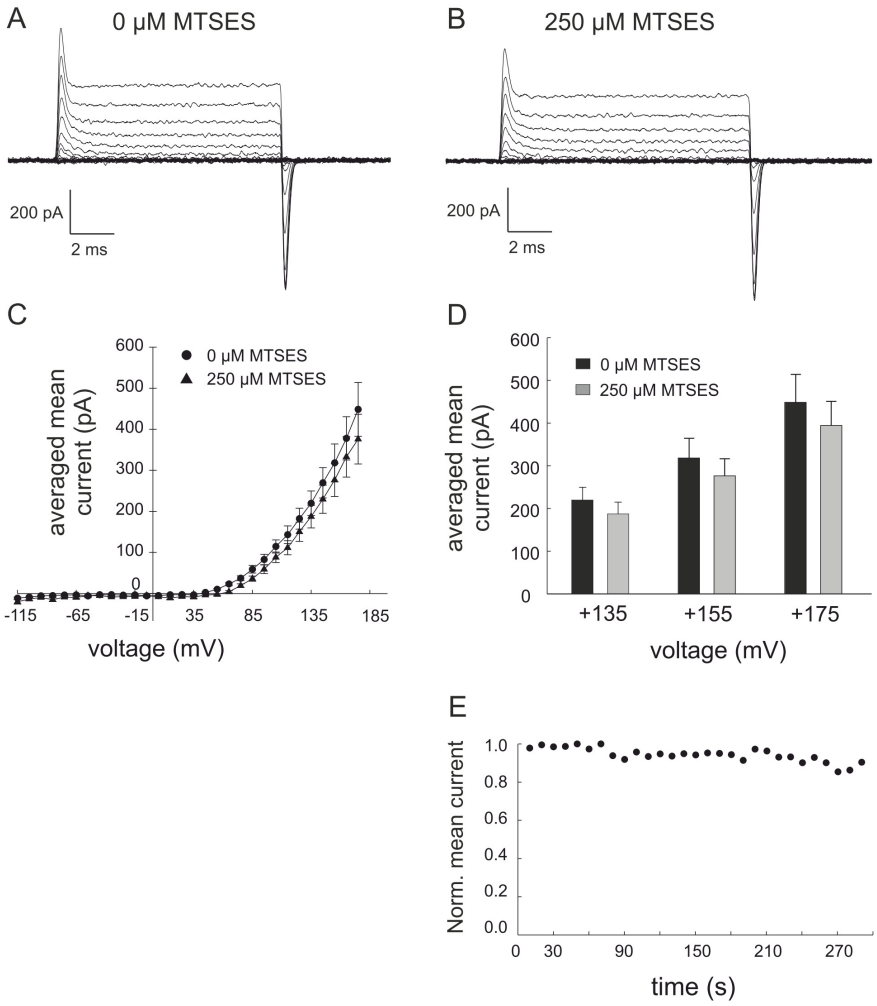
Ch. Fahlke, E-mail: c.fahlke@fz-juelich.de, postal address: Institute of Complex Systems, Zelluläre Biophysik, Forschungszentrum Jülich, D- 52425 Jülich, Germany

Running title: Functional specialization of ClC transporters

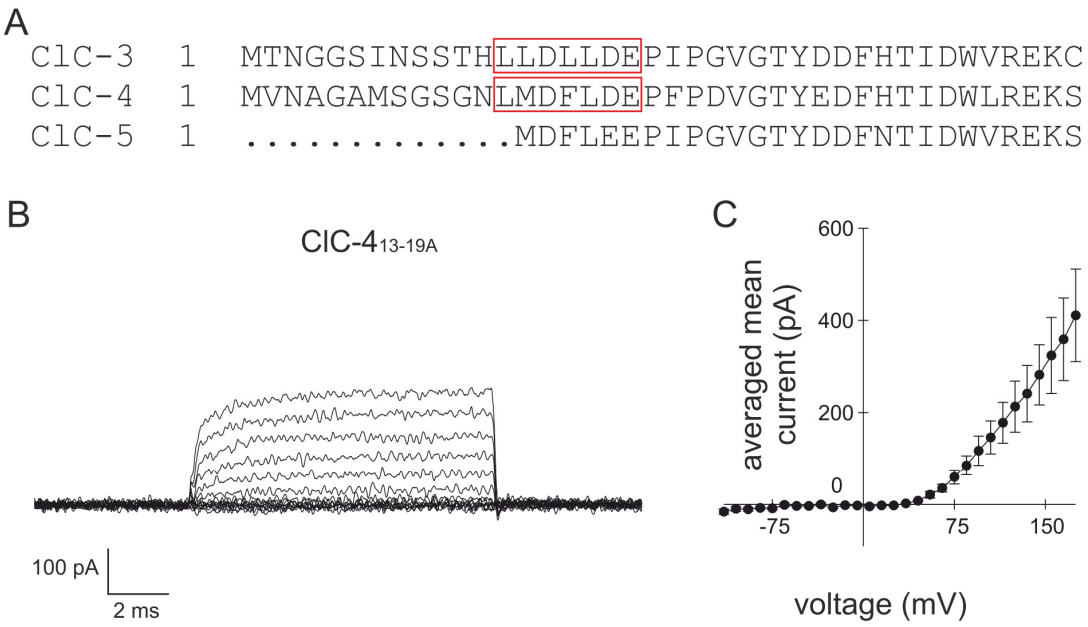
The supplementary information contains 4 figures (Suppl. Fig. S1-S4) and additional supplementary methods.



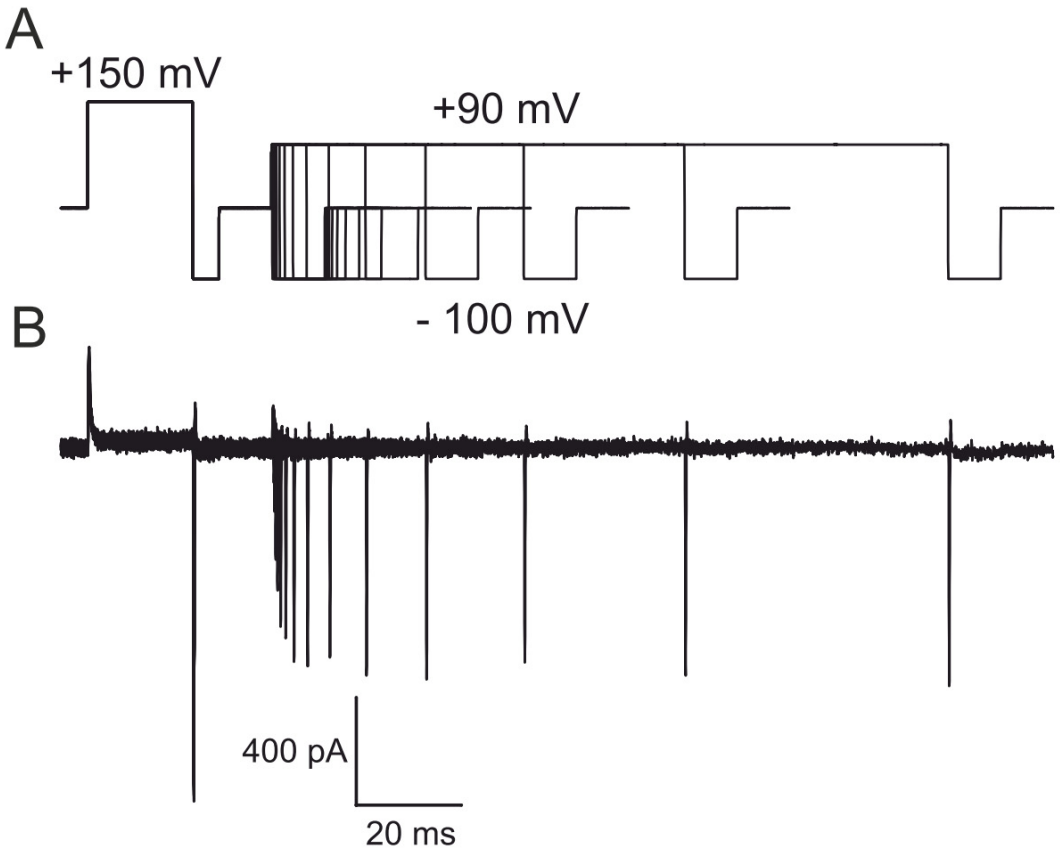
Supplementary Figure S1. Gating charge movements and nonlinear capacitances in ClC-5. **A-C)** Representative whole-cell current recordings elicited by voltage steps from -115 mV to +175 mV for (A) ClC-5 WT, (B) ClC-5 E268H and (C) ClC-5 E268Q. Insets show enlarged the ClC-5 off-gating currents. **D)** Voltage dependent nonlinear capacitances, normalized to the corresponding steady-state current at +135 mV. Solid lines represent fits to the first derivative of a standard Boltzmann function (n = 6 - 7). Fit parameters are enlisted in Table 1. **E)** Off-gating charge (Q_{off}) at +135 mV normalized to the ion transport (I_{135}) at the same voltage (n = 6 - 7).



Supplementary Figure S2. ClC-$3_{13\text{-}19A}$ currents are not blocked by the application of 250 µM MTES. A, B) Representative currents recorded in the absence and after a 5-min incubation in 250 µM MTSES. **C)** Current-voltage relation of ClC-$3_{13\text{-}19A}$ recorded in the absence and in the presence (n=9) of 250 µM MTSES. **D)** Bar graph representation of the effects of MTSES on the mean current amplitude at three different voltages. **E)** Time course of the ClC-$3_{13\text{-}19A}$ current amplitude at +165 mV normalized to the amplitude at the time at which 250 µM MTSES was applied The slight rundown is observed also in the absence of MTSES.



Supplementary Figure S3. Alanine replacement of the dileucine acidic cluster at the N-terminus dos not change the functional properties of ClC-4. A) Sequence alignment of the N-termini of ClC-3, ClC-4 and ClC-5. Red boxes represent the dileucine motif (*1*) mutated in the experiments. **B)** Whole-cell current traces of HEK293T cell expressing ClC-4 with all amino acids between 13 and 19 mutated to alanine. **C)** Current-voltage curves of ClC-$4_{13\text{-}19A}$ (n = 6).



Supplementary Figure S4. A) Voltage protocol used to estimate the kinetics of gating charge mobilization at +90 mV for the three isoforms ClC-3, ClC-4 and ClC-5. **B)** Representative recording of ClC-$3_{13\text{-}19A}$ E281Q using the stimulus protocol in A).

Supplementary Methods

Endosomal pH model - We assumed a vesicle with established pH gradient ΔpH across the enclosing membrane. The electrochemical gradient for protons ΔG in this case can be expressed as:

$$\Delta G = -2.3RT\Delta pH + zFV \quad \text{(Eq. S1)},$$

where *R, T, F* and *V* are the universal gas constant, the temperature, the Faraday constant and the transmembrane voltage respectively (*2*). The value *z* represents the charge of the transported ion and in the case of protons equals +1 e_0. The electrochemical gradient is built up by electrogenic V-type ATP-ases, and is therefore limited by the energy that is released by the hydrolysis of one molecule ATP (ΔG_{ATP}=-32 kJ/mol (*2*)).

The capacitance of the vesicle *C* is given by the specific capacitance C_0 (0.5 μF/cm^2 or 1 μF/cm^2, (*2*)) multiplied by the surface area of the vesicle, or in the case of spherical vesicles:

$$C = 4\pi r^2 C_0 \quad \text{(Eq. S2)}.$$

The luminal concentration of free charges (m^{-3}), considering only the dissociation of water molecules equals:

$$A_{cap} = 1000\left(10^{-pH_i} - \frac{K_w}{10^{-pH_i}}\right) \quad \text{(Eq. S3, (2))},$$

Where K_w denotes the dissociation constant of water ($-\log_{10}K_w$=14) and pH_i is the luminal pH.

We then investigated the effects of the two functional characteristics of the ClC transporters, the nonlinear capacitance and coupled anion/proton exchange.

A) Nonlinear capacitor

To qualitatively describe the function of the ClC transporter as a capacitor, we assumed that no anions are transported by the ClC protein and that the luminal Cl^- concentration (Cl_i) equals the cytosolic Cl^- concentration (Cl_o):

$$Cl_i = Cl_o \quad \text{(Eq. S4)}.$$

Following the approach of Rybak et al. (*2*), we expressed the voltage across the vesicle membrane as:

$$V_{cap} = F\left(\frac{r}{3C_0}\right)A_{cap} \quad \text{(Eq. S5)},$$

where *r* represents the radius of the vesicle in decimeter.

Combining equations S1, S2, S3 and S5 allows directly calculating the maximal equilibrium pH that can be built up by the electrogenic ATP-dependent proton pump.

B) Anion/proton exchanger

The equilibrium condition for a Cl^-/H^+ exchanger with a 2:1 stoichiometry will be (*2*)(*3*):

$$0 = 2RT\ln\left(\frac{Cl_i}{Cl_o}\right) - RT\ln\left(\frac{H_i}{H_o}\right) - 3FV \qquad \text{(Eq. S6).}$$

Combining Eq. S6 with Eq. S1 and solving for Cl_i when using the luminal and cytosolic concentration of protons (H_i and H_o), one obtains:

$$Cl_i = \frac{H_o Cl_o}{H_i} e^{\left(\frac{-3\Delta G_{ATP}}{2RT}\right)} = \frac{e^{-pH_o} Cl_o}{e^{-pH_i}} e^{\left(\frac{-3\Delta G_{ATP}}{2RT}\right)} \qquad \text{(Eq. S7).}$$

The concentration of free charges and the transmembrane voltage will be:

$$A_{exch} = 1000\left(10^{-pH_i} - \frac{K_w}{10^{-pH_i}} - \frac{e^{-pH_o} Cl_o}{e^{-pH_i}} e^{\left(\frac{-3\Delta G_{ATP}}{2RT}\right)}\right), \text{ and } V_{exch} = F\left(\frac{r}{3C_0}\right)A_{exch} \qquad \text{(Eq. S8).}$$

The two cases were solved numerically in Matlab 6 (Mathworks) to obtain estimates of the luminal pH and chloride concentrations. A cytosolic Cl^- concentration (Cl_o) of 10 mM was assumed.

References

1. Zhao, Z., Li, X., Hao, J., Winston, J. H., and Weinman, S. A. (2007) The ClC-3 Chloride Transport Protein Traffics through the Plasma Membrane via Interaction of an N-terminal Dileucine Cluster with Clathrin, *Journal of Biological Chemistry 282*, 29022–29031.
2. Rybak, S. L., Lanni, F., and Murphy, R. F. (1997) Theoretical considerations on the role of membrane potential in the regulation of endosomal pH, *Biophys. J. 73*, 674–687.
3. Accardi, A., and Miller, C. (2004) Secondary active transport mediated by a prokaryotic homologue of ClC Cl- channels, *Nature 427*, 803–807.