PERSPECTIVES

It's the proton also in ClC-2

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In mammals, nine genes belong to the family of CLC anion transporters with several of them involved in genetic diseases (Jentsch, 2008). The family comprises passive channels, in which Cl^- ions diffuse down their electrochemical gradient, and active transporters in which the flux of Cl^- in one direction is stoichiometrically coupled to the movement of H^+ in the opposite direction (Zifarelli & Pusch, 2007). These different transport mechanisms are embodied by a protein architecture that is similar among the known CLC members. Of special importance is a conserved glutamate ('gating glutamate'). In ClC-ec1, a bacterial CLC transporter, the gating glutamate occupies one of the Cl^- binding sites blocking access to the external space (see Fig. 1). Upon protonation it is displaced and allows ion flow (Zifarelli & Pusch, 2007).

In this issue of *The Journal of Physiology*, Niemeyer *et al.* (2009) explore the role of protons in relation to the gating of ClC-2, a ubiquitously expressed Cl^- channel. ClC-2 is activated by hyperpolarisation, cell swelling and intracellular Cl^-, but also by extracellular pH (pH_e). Its modulation by pH_e is complex: currents increase at moderate acidic pH but are reduced at more acidic pH. This observation was explained by postulating the presence of two independent proton binding sites with opposite effects (Arreola *et al.* 2002).

The activating site has been previously identified by Sepúlveda's group as E207, the gating glutamate of ClC-2 (Niemeyer *et al.* 2003). Neutralisation of E207 abolished H^+ induced potentiation without influencing inhibition by more acidic pH.

In their new work, Niemeyer *et al.* (2009) further expand our knowledge of the relation between ClC-2 gating and protons. As a first major result, they identify H532 as the H^+ sensor for channel block at acidic pH. Mutating histidine 532 into phenylalanine selectively removes channel inhibition at acidic pH, leaving a pure H^+ induced activation. According to the structure of ClC-ec1, H532 is exposed extracellularly (Fig. 1*A*), consistent with the independence of H^+ block from voltage. The residue is located distantly from the pore (Fig. 1*B*). It appears therefore that protonation of this residue affects channel properties allosterically.

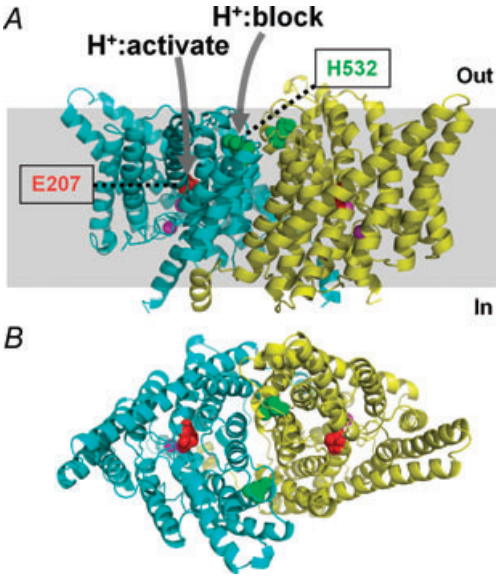

Figure 1. Location of residues mediating activation and inhibition of ClC-2 by extracellular protons
The figure shows the structure of the bacterial ClC-ec1 (pdb entry 1OTS) with different colours for the two subunits. The residues corresponding to E207-ClC-2 (E148 in CLC-ec1) and H532-ClC-2 (L421 in ClC-ec1) are coloured in red and green, respectively. Chloride ions are shown in pink. In *A* the view is approximately from within the membrane; in *B* the view is from the extracellular side. (Figure prepared with pymol.)

Another major breakthrough of this work is to provide mechanistic insight about the activation of ClC-2 by hyperpolarisation. Elimination of the H^+ induced block (using mutant H532F) allowed the authors to concentrate on the activating mechanism mediated by the protonation of E207. They show that the voltage dependence of activation is conferred by the voltage-dependent protonation of the gating glutamate. As E207 is located approximately half-way across the conduction pathway (Fig. 1*A*), protons have to traverse some distance across the membrane electric field and therefore the rate of protonation is increased (and/or the rate of deprotonation is decreased) by negative voltages. In principle, part of the voltage dependence of activation could be due to a competition of Cl_i^- with the side chain of E207 for a Cl^- binding site. However, the authors could rule out this possibility as Cl_i^- does not act in a voltage-dependent manner.

Extracellular H^+ also activate the *Torpedo* channel ClC-0 through protonation of the gating glutamate, but only in a weakly voltage-dependent manner. On the other hand, the activation mechanism of ClC-2 bears some resemblance with the effect of intracellular protons on ClC-0: it has been recently shown that a major part of the voltage dependence of ClC-0 gating arises from a voltage-dependent protonation of the gating glutamate from the inside by a proton originating from water dissociation (Zifarelli & Pusch, 2009).

Several important questions remain open. What is the mechanism by which protonation of a histidine residue located away from the pore effects channel closing? What is the relationship between the two pH-dependent gates? A link between these two gates has been suggested by the finding

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that the inhibitory effect of low pH can occur only from the closed state (Arreola *et al.* 2002). What is the relationship of the H532 mediated pH effect with the gate that acts on both protopores in CLC channels? It will be important to find out if the protonation or the deprotonation reaction of E207 (or both) are voltage dependent. Furthermore, the molecular basis for the different pH and Cl^- dependencies of the homologous channels ClC-0, ClC-1 and ClC-2 is unclear.

From a physiological point of view a detailed knowledge of the dependence of ClC-2 on pH and $[Cl^-]$ is highly relevant. This is because based on results from knock-out studies, it seems that ClC-2 is important for the transport of Cl^- and/or the regulation of the membrane potential in cells with a restricted extracellular space in which large concentration changes of ions can be expected (Jentsch, 2008). In this respect, the new results of Niemeyer *et al.* (2009) represent important progress.

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