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COMMENTARY

The acid test for pH-dependent gating in cloned H_V1 channels

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Cells interact with their milieu by means of cell membranes, and several ingenious mechanisms have evolved to mediate these interactions. Ion channels occupy a privileged position among these mechanisms, being responsible for regulated ion flux in response to changes in membrane voltage, membrane tension, and ligand binding, among other signaling mechanisms. The revelations of the diversity of ion channel structures and molecular mechanisms do not seem to have reached a steady state yet, and it seems likely that more surprises are just around the corner. Two papers by DeCoursey and coworkers (Cherny et al. and Thomas et al.) in this issue of the *Journal of General Physiology* are a case in point; they describe the unusual pH-dependent gating properties of a proton channel from the snail *Helisoma trivolvis*.

The peculiar voltage-dependent ion channels involved in the transport of protons were first described in neurons of freshwater and terrestrial snails (Thomas and Meech, 1982; Byerly et al., 1984). Voltage-gated proton channels play important roles in intracellular pH regulation, immune cell responses, sperm motility, and mucus secretion in epithelia, to name just a few of their functions (DeCoursey, 2013). These curious proteins were given a molecular identity when Ramsey et al. (2006) and Sasaki et al. (2006) cloned the human and mouse H_V1 proteins, respectively.

The H_V1 protein has the same structural fold as voltage-sensing domains (VSDs) found in classic or canonic voltage-gated ion channels (Takeshita et al., 2014; Randolph et al., 2016). The proton channel, at least in humans, is formed by a dimer of two VSDs. It is thought that each VSD acts as both a voltage sensor and the proton-permeable pore, although the allosteric coupling between these two processes is not understood (Tombola et al., 2008). One of the quirky features of H_V1 channels is that the range of voltages over which their activation can occur is strongly modulated by the difference between the extracellular (pH_o) and intracellular (pH_i) pH, which is known as ΔpH.

In most H_V1 channels, this dependence has evolved to allow a voltage-dependent proton flux from the inside to the outside of the cell, thus allowing these channels to serve as efficient regulators of intracellular pH. In human H_V1, both natively and heterologously expressed, the voltage of half maximal activation ($V_{1/2}$, or other measures of the activation range) shifts −40 mV for every 1 unit of ΔpH. This is true whether the channels are dimeric or forced to be monomeric, and even holds for several H_V1 channels from different organisms and tissues, suggesting that ΔpH-dependent gating may be a property of the VSD.

When pH is involved in modulation of voltage gating, two mechanisms come to mind. Protons can screen surface charges in the membrane near the channel or in the channel protein itself (Hille, 1968), giving rise to biasing potentials that can shift the voltage dependence. Another plausible mechanism is direct protonation of a titratable amino acid residue and a consequent conformational change in the voltage sensor. Because ΔpH gating is not compatible with a surface charge–dependent mechanism, given the symmetry of the pH effect, it has been proposed that protonation plays an important role in explaining ΔpH-sensitive gating (Cherny et al., 1995). However fundamental, the mechanism by which proton gradients regulate voltage-dependent gating of H_V1 remains obscure. No clear protonatable sites have been found so far, and no alternative mechanisms to protonation have been explored (Ramsey et al., 2010).

As often happens, new evidence from comparative physiology experiments published in this issue of the *Journal of General Physiology* may help to sniff out a solution to this riddle. Two papers by DeCoursey and coworkers (Cherny et al., 2018; Thomas et al., 2018) show that a newly cloned H_V1 channel from the freshwater snail *H. trivolvis* exhibits uncommon properties, including ΔpH-dependent gating. This could help to illuminate the molecular mechanisms involved in ΔpH gating. The proton channel from *H. trivolvis* (HtH_V1) preserves key properties common to proton-permeable channels from other species; it activates steeply as a function of voltage and shows almost perfect proton selectivity. It is also inhibited by the divalent ions Zn^{2+} and Cd^{2+} in a similar fashion to that found in other H_V1 channels, albeit with reduced sensitivity. However, these channels activate almost 10 times faster than the human H_V1 (hH_V1) channel. Another rather surprising finding is that the activation time course is monoexponential, not sigmoidal as is common in hH_V1. Sigmoidal activation disappears when hH_V1 is forced to be a monomer by deletion

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of the C terminus, and it has been suggested that it reflects cooperativity between dimers during gating. Exponential activation in a channel that seems to preserve the dimerization domain suggests either that activation of HtH_V1 is not cooperative or that dimers and monomers gate through the same mechanism.

A common finding in hH_V1 and H_V1 from other organisms is that, regardless of the side at which pH is changed, gating is influenced only by the absolute value of ΔpH, so changing either solution's pH value can alter ΔpH. When ΔpH is positive, and thus the driving force for protons is outward, the midactivation voltage of H_V1 shifts to negative voltages. This means that only outward proton currents are produced during a depolarization. Conversely, a negative ΔpH shifts activation to the positive range of voltages, again ensuring that protons are extruded from the cell while the membrane is depolarized. In almost all H_V1 channels, one finds that the $V_{1/2}$ shifts 40 mV per unit of ΔpH. The symmetry involved in this ΔpH gating phenomenology has led to quantitative models in which proton-binding sites with alternating accessibilities exist at both intra- and extracellular sides of the channel (Cherny et al., 1995).

The most interesting characteristic of the newly characterized HtH_V1 is that, although ΔpH-dependent gating is still functional, it shows properties that may be different from those found in hH_V1. Thomas et al. (2018) report a normal response to ΔpH when external pH is changed, but a smaller than expected response to internal pH change, even with the same ΔpH value. This finding suggests that there may indeed be an intracellular protonatable site in H_V1 channels, which in HtH_V1 might behave differently.

Through sequence comparisons, the authors of the accompanying paper (Cherny et al., 2018) identify a histidine residue in hH_V1, which in HtH_V1 is a glutamine. This residue had previously been implicated as an important determinant of channel kinetics in a sea urchin H_V1 channel (Sakata et al., 2016). Remarkably, mutation of this residue (H168) in hH_V1 to several other amino acids significantly impaired ΔpH gating by reducing the sensitivity of the mutants to changes in intracellular pH. This gating impairment occurred despite preserving the 40 mV/pH_o unit shift, much in the same way as was observed in the new snail HtH_V1. This result suggests that this histidine, which is likely located on an intracellular loop at the entrance of the internal water-filled cavity of the VSD (Randolph et al., 2016), might act as an intracellular titratable site that is coupled to gating. Intriguingly, the reverse mutation in HtH_V1 (Q to H) did not convert its ΔpH gating to the hH_V1 phenotype, indicating that the structural context is important for the composition of this putative protonation site.

If indeed protonation of discrete binding sites forms the basis for ΔpH gating in H_V1 channels, what might be the molecular mechanism? One could speculate that protonation sites at the entrance of the intracellular and extracellular cavities in the VSD would regulate the equilibrium position of its S4 helix and thus modulate the midpoint of voltage activation. Protonation of the intracellular site might alter the electrostatics of the VSD, facilitating its outward movement with voltage. By symmetry, the exact opposite would be expected from protonation of an extracellular location. Now, all we need to do is find an extracellular titratable site. Of course, alternative explanations for ΔpH gating are still possible. Protons most certainly can penetrate the water-filled cavities in the VSD and alter the charge–charge electrostatic interactions between pairs of negative and positive residues that are fundamental to the structure and function of voltage sensitive domains. Because VSDs have both external and internal cavities, protons have access from the inside and the outside. Such a mechanism would be compatible with gating models with symmetric proton accessibility, but incompatible with titratable sites.

Collectively, the results obtained from studying the H_V1 channel from *H. trivolvis* open a new avenue of research for the voltage-gated proton channel field and offer a glimpse of a possible mechanism for pH-regulated gating.

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