Voltage gated proton channels - past, present and future

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To our knowledge, the first human being to imagine the existence of voltage gated proton channels (Hv1) was the late J. Woodland (Woody) Hastings who proposed that they trigger the flash of bioluminescent dinoflagellates (Fogel & Hastings, 1972). The first voltage-clamp recording of Hv1 currents was in snail neurons in 1982 by Roger Thomas and Bob Meech (Thomas & Meech, 1982). A decade later, Tom DeCoursey made the first direct recording in mammalian cells by patch clamping rat alveolar epithelial cells (DeCoursey, 1991). Many mechanistic details of the channel biophysics were elucidated subsequently, including the absolute selectivity for protons, voltage, pH and temperature dependence, deuterium isotope effects, and sensitivity to divalent metals, but not until 2006 when the gene was finally discovered simultaneously in mouse and human (Sasaki *et al.*, 2006; Ramsey *et al.*, 2006) did it become possible to probe structure-function relationships.

The protein shares many similarities to the voltage sensors of other ion channels having 4 transmembrane helices labeled S1-S4. It differs from other channels in lacking an explicit pore domain (and thus no S5-S6 helices/segments). The channel forms a dimer in plasma-membranes with each monomer containing a proton pathway that traverses the center of the voltage sensor. We have shown that the selectivity of the channel is governed by a single aspartate residue on S1 (D112 in the human channel) in the narrow part of the pore (Musset *et al.*, 2011). The aspartate can be moved to a location one turn of the helix outward on S1 and still maintain the channel's function and extreme selectivity, showing limited plasticity of the pore structure (Morgan *et al.*, 2013). The S4 helix contains 3 arginine residues (R205, R208, R211; Human) that sense voltage. Channel opening occurs when the S4 helix moves so that the middle arginine can hydrogen bond with the aspartate, allowing conduction. The C-terminus contains a long region that forms a coiled-coil structure that is believed to hold the channel together as a dimer. The N-terminus contains phosphorylation sites, in particular threonine 29, which alters channel gating when phosphorylated (Morgan *et al.*, 2007; Musset *et al.*, 2010).

The physiological functions of Hv1 are just beginning to be unraveled. The best studied function is its critical relationship to the respiratory burst in phagocytes in which it compensates for electron flow through NADPH oxidase and simultaneously regulates cytosolic pH (DeCoursey *et al.*, 2003; Morgan *et al.*, 2009). Hv1 also facilitates NADPH oxidase activity in B cells controlling signaling (Capasso *et al.*, 2010) and is involved in histamine release from basophils (Musset *et al.*, 2008). We recently confirmed Prof Hastings' hypothesis by discovering a proton channel in a dinoflagellate that has the properties required to mediate an action potential (Smith *et al.*, 2011). There is an emerging role for the proton channel in pathophysiology, suggesting it may have future potential as a marker or drug target in disease states. It has been shown to exacerbate neuronal damage in ischemic stroke, is highly expressed in advanced breast cancer tissues and we have found that a novel isoform of the channel is enriched in chronic lymphocytic leukemia cells (Capasso *et al.*, Submitted).

We have learned a great deal about this channel's form and function in the last 30 years but it feels like we have only scratched the surface.

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