Paul F. Cranefield Award to Merritt C. Maduke

The Journal of General Physiology

The late Paul F. Cranefield, MD, PhD, was the Editor of *The Journal of General Physiology* for 30 years, from 1966 to 1995. During his editorship, Dr. Cranefield worked tirelessly to advance the mission of the Journal: to promote and publish original research of the highest quality that elucidates basic biological, chemical, or physical mechanisms of broad physiological significance, and provides insight into fundamental mechanisms that govern biological function at all levels.

When Dr. Cranefield stepped down as Editor, the Council of the Society of General Physiologists created the Paul F. Cranefield Award to recognize his enduring contributions to the Journal and the Society, and to carry on his vision of excellence. The award was to be given to a young, independent investigator who in the preceding year published an article of exceptional quality in the Journal. The award would be given at the Annual Meeting and Symposium of the Society in Woods Hole, Massachusetts. It was also decided that the award would only be given to a candidate who met stringent criteria, with the result that it has not been given every year. In 2009 no award was made. The announcement of the 2008 award was inadvertently delayed during the transition of editors at the Journal.

In 2008 the leadership of the Society selected Merritt C. Maduke of Stanford University for the Cranefield Award. Dr. Maduke graduated with a BS in chemistry summa cum laude from Wheaton College in 1989. She received her PhD in chemistry and biochemistry from UC San Diego in 1995 in the laboratory of Dr. David Roise, where she established that a certain mitochondrial presequence could be moved across lipid bilayers in the absence of protein translocation pore. Dr. Maduke then did postdoctoral research in the laboratory of Chris Miller at Brandeis, where she discovered ClC homologues in prokaryotic genomes and went on to clone and functionally express and otherwise characterize the first such protein, ClC-ec1 from *Escherichia coli* (Maduke et al., 1999). This seminal investigation revealed that ClC-ec1 is homodimeric and chloride-selective in its transport function. Dr. Maduke took a position as assistant professor in molecular and cellular physiology at Stanford in 2001, and was promoted to the rank of associate professor in 2009.

The Cranefield Award was given to Dr. Maduke for a pair of papers characterizing chloride and voltage-dependent fast gating of ClC-0 channels; the papers were published in the October 2007 issue of the Journal. ClC-0 channels belong to a family of transport proteins whose individual members may function either as Cl^- channels or as Cl^-/H^+ antiporters. ClC-0, expressed in the electric ray *Torpedo*, was the first ClC to be discovered and characterized (White and Miller, 1979). Humans ubiquitously express nine distinct ClCs that are essential for normal cardiovascular, muscular, neuronal, and epithelial function (Jentsch, 2008), and genetic defects in ClCs give rise to serious diseases of muscle, kidney, bone, and brain (Planells-Cases and Jentsch, 2009). In the first of the two papers, Maduke and coworkers Anita Engh and José Faraldo-Gomez (Engh et al., 2007a) performed an extensive analysis of the fast gating of ClC-0 channels expressed in oocytes and exposed to different external concentrations of chloride. They also constructed a homology model of ClC-0 based on the x-ray crystal structure of ClC-ec1 (Dutzler et al., 2003) and used a Poisson-Boltzmann theoretical framework to derive electrostatic-binding energies and transmembrane potentials for the model with various states of chloride occupancy. Fitting of the model to the data revealed that their results and previously published results could be well characterized as a four-state system in which chloride binding during gating is voltage dependent, involving one or more of three known chloride-binding sites that must be situated within at least 10% of the transmembrane field.

In the second paper, Maduke and coworkers (Engh et al., 2007b) characterized the changes in voltage- and chloride-dependent gating of ClC-0 channels with a series of mutations in the strictly conserved residue K149. Previous work had shown that the K149C mutation produces a +70-mV shift in the voltage dependence of ClC-0 fast gating. Application of the model developed in the first paper to the data pinpointed the major effect of mutations of K149 to be on the depolarization-activated gating step that follows the binding of extracellular

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chloride. K149 does not directly contribute to the electrostatic field in the pore, alter the chloride-binding sites, or constitute part of the gating charge. Rather, K149 makes a critical contribution to the barrier for the reaction $Channel_{closed} \cdot Cl \rightarrow Channel_{open} \cdot Cl$ in part through its effect on the packing of neighboring residues: K149 mutations affect this step by weakening chloride binding to one of two internal binding sites and/or by altering chloride movement between these sites.

Dr. Maduke's laboratory continues to investigate ClCs, with a focus on understanding common mechanisms of both channel and antiporter subtypes. The larger focus has contributed to the development and testing of hypotheses about shared functional features of the family members and the evolution of the channel members of the family from antiporters. Miller (2006) proposed a unifying hypothesis for the ClC family, the "degraded transporter model." According to this model, the ClC family members that function as chloride channels should retain the proton permeation pathway requisite to the antiporter function of the other family members. Single-channel recordings have provided critical insight into antiporter-like behavior of ClC channels. Single ClC-0 channels show asymmetry in the fast and slow gating observable in patch recordings. The chloride conductance paths of the two homodimeric units of ClC-0 can open or close independently. Once the common slow gate is open, the channel can "flicker" rapidly (fast gating) between three conductance states (0, 1, and 2 fast gates open), but the channel often remains completely closed for longer stretches (slow gating). The asymmetry is that common gate closure occurs nearly exclusively from a state in which one fast gate is open, whereas channel opening after common gate closure occurs primarily to the state in which both fast gates are open: transitions $1 \rightarrow 0 \rightarrow 2 \rightarrow 1$ are much more frequent than transitions $1 \rightarrow 2 \rightarrow 0 \rightarrow 1$. This asymmetry violates microscopic reversibility and requires an energy source that drives the slow-gating cycle. By varying chloride and pH gradients and analyzing their contributions to the asymmetry, Lísal and Maduke (2008) revealed the underlying energy source to be the proton gradient, rather than the chloride gradient as previously proposed. This important insight provides a new link between the channels and Cl^-/H^+ antiporters in the ClC family, and draws fresh attention to the proton permeation pathway. Recent evidence from the Maduke laboratory obtained with 19F NMR spectroscopy of ClC-ec1 (which can be obtained in large quantities) also points to shared conformation changes of ClC channels and antiporters, as hopefully will the laboratory's discovery of the only known inhibitors of ClC antiporters.

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