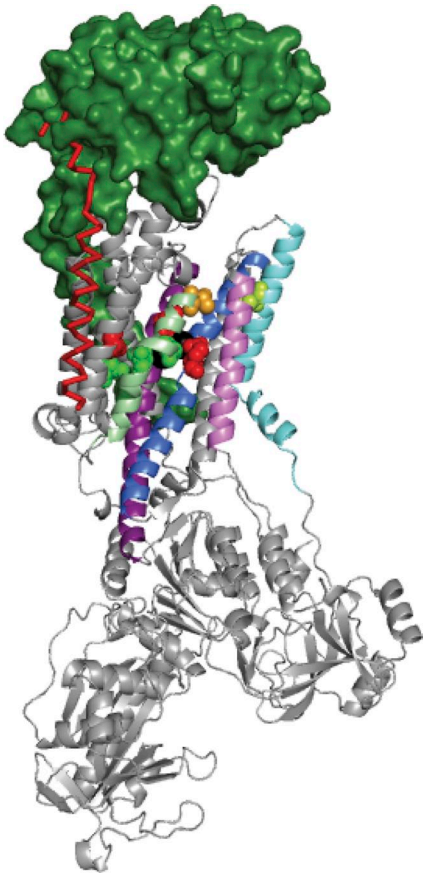
Generally Physiological

Of pumps, protons, chloride gradients, and microvesicles at the immunological synapse

This month's installment of *Generally Physiological* concerns the identification of the Na^+/K^+ pump as a hybrid transporter, investigation of the mechanisms whereby the neuronal chloride gradient is set, and the discovery that T cells send messages to antigen-presenting cells (APCs) via microvesicles.

A proton pathway through the sodium pump

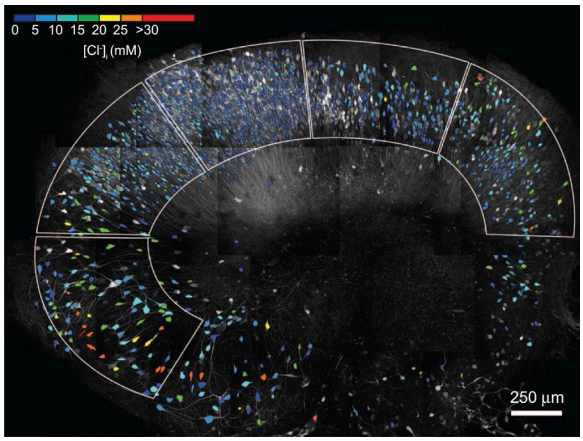
Transport proteins are usually identified by their substrates and co- or counter-transported species. But a small, though growing, number of them are turning out to perform two different jobs, involving distinct substrates. In this issue, Vedovato and Gadsby identify one of the most well-known transporters, the Na^+/K^+ ATPase—familiarly known as the Na^+ pump—as a hybrid transporter that also allows proton entry under physiological conditions. The Na^+/K^+ ATPase exports three Na^+ and imports two K^+ with every cycle, thereby generating an outward current that is abolished by the Na^+/K^+ ATPase inhibitor ouabain. A pH- and membrane potential–dependent ouabain-sensitive inward proton current mediated by the Na^+/K^+ ATPase in the absence of extracellular Na^+ and K^+ has been viewed as an artifact of these highly unphysiological conditions. Vedovato and Gadsby (2014) now show that this proton import is present at physiological concentrations of Na^+ and K^+ and resting potentials, identifying the Na^+/K^+ ATPase as a hybrid transporter (see Hilgemann in this issue for a thoughtful and entertaining discussion). The proton current, which is energetically downhill and depends on the reversibility of conformational changes associated with release of extracellular Na^+, flows through a route distinct from the pathway whereby Na^+ and K^+ are transported and is not required for the transport of Na^+ and K^+. The authors speculate that it may, however, have physiological or pathophysiological consequences under conditions in which extracellular pH is low, such as in skeletal muscle during vigorous exercise or during ischemia in the heart or brain.



Structure of the Na^+/K^+ ATPase. (From Vedovato and Gadsby, 2014; Protein Data Bank accession no. 2ZXE, Shinoda et al. 2009. *Nature*. 459:446–450.)

Maintaining your equilibrium

Although the neurotransmitter GABA, which activates a ligand-gated channel permeable to chloride (the $GABA_A$ receptor), has long been viewed as inhibitory, modest changes in intracellular chloride concentration ($[Cl^-]_i$) can shift the transmembrane chloride concentration gradient so that the $GABA_A$ receptor reversal potential is above threshold for action potential generation. Questioning the prevailing view that neuronal $[Cl^-]_i$ is determined by the cation-chloride cotransporters (CCCs) KCC2, a potassium-chloride symporter that extrudes chloride, and the Na-K-Cl cotransporter NKCC1, which imports chloride into the cell, Glykys et al. (2014) measured ($[Cl^-]_i$) in acute and organotypic brain slices of mice expressing the ratiometric fluorescent chloride indicator Clomeleon. In both neonatal and adult neurons



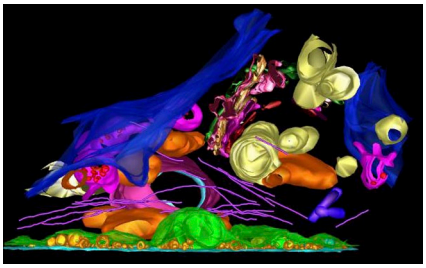
[Cl^-] distribution in neurons in an organotypic hippocampal slice from a mouse expressing Clomeleon. (Glykys et al. 2014. *Science*, 343:670–675. Reprinted with permission from AAAS.)

Identifying the Na^+/K^+ pump as a hybrid transporter, investigating the neuronal chloride gradient, and discovering that T cells send messages to APCs via microvesicles

(in which NKCC1 and KCC2 are, respectively, the primary transporter), the somatic $[Cl^-]_i$ was broadly distributed and inconsistent with the equilibrium concentrations predicted for either transporter. Moreover, pharmacological blockade of the two CCCs failed to alter $[Cl^-]_i$ in a manner consistent with their being the primary determinants of $[Cl^-]_i$, nor did their blockade affect subcellular $[Cl^-]_i$ gradients. Rather, analyses of the relationship between $[Cl^-]_i$ and impermeant intracellular and extracellular anions (nucleic acids and extracellular matrix glycoproteins), together with the effects of manipulating the intracellular and extracellular anion concentrations and osmotic balance, led to the conclusion that local impermeant anions are the primary determinants of $[Cl^-]_i$ and $[Cl^-]_o$, with the CCCs acting to maintain chloride homeostasis.

Packing up the TCR for special delivery

Adaptive cellular immunity depends on interactions between T cells and APCs, such as B cells, macrophages, and dendritic cells. T cell receptor (TCR) recognition of peptide-bearing major histocompatibility complex molecules (pMHC) on the APC stimulates formation of the immunological synapse. Curiously, the central region of the immunological synapse, where TCRs accumulate, shows little TCR signaling; moreover, TCR–pMHC complexes in this central region show a lack of mobility relative to the periphery of this highly organized structure. Choudhuri et al. (2014) used a combination of total internal reflection fluorescence microscopy, transmission electron microscopy, and electron tomography to investigate formation of the T cell immunological synapse in a model system in which $CD4^+$ T cells engage with pMHC and the adhesion ligand ICAM-1 in a supported lipid bilayer. Remarkably, they found that, after movement of TCR–pMHC complexes to the center of the immunological synapse, the center of the contact interface reorganized into an extracellular cavity containing numerous microvesicles. T cells transferred TCRs to B cells bearing cognate pMHC, a process inhibited by knockdown of TSG101, a protein required for TCR sorting into microvesicles. Moreover, isolated TCR-enriched microvesicles were capable of initiating signals in B cells. Thus, T cells appear to use microvesicles to transfer signals to APCs across the immunological synapse.



3-D model of microvesicles at an "immunological synapse" between T cells and a supported planar lipid bilayer. (Reprinted by permission from Macmillan Publishers, Ltd. K. Choudhuri, J. Llodrá, E.W. Roth, J. Tsai, S. Gordo, K.W. Wucherpfennig, L.C. Kam, D.L. Stokes, M.L. Dustin. 2014. *Nature*. http://dx.doi.org/10.1038/nature12951, copyright 2014.) To watch a video from which the image above was taken, see http://www.jgp.org/cgi/content/full/jgp.201411196/DC1.

Elizabeth M. Adler
Executive Editor, *JGP*
eadler@rockefeller.edu

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