Liu, X., Wang, Q., Haydar, T.F., and Bordey, A. (2005). Nat. Neurosci. *8*, 1179–1187.

Overstreet Wadiche, L.S., Bromberg, D.A., Bensen, A.L., and Westbrook, G.L. (2005). J. Neurophysiol., in press. Published online July 20, 2005. 10.1152/jn.00633.2005

Owens, D.F., and Kriegstein, A.R. (2002). Nat. Rev. Neurosci. *3*, 715–727.

Seri, B., Garcia-Verdugo, J.M., Collado-Morente, L., McEwen, B.S., and Alvarez-Buylla, A. (2004). J. Comp. Neurol. *25*, 359–378.

Schwab, M.H., Bartholomae, A., Heimrich, B., Feldmeyer, D., Druffel-Augustin, S., Goebbels, S., Naya, F.J., Zhao, S., Frotscher, M., Tsai, M.J., and Nave, K.A. (2000). J. Neurosci. *20*, 3714–3724.

Wang, L.-P., Kempermann, G., and Kettenmann, H. (2005). Mol. Cell. Neurosci. *29*, 181–189.

DOI 10.1016/j.neuron.2005.08.029

The Art of Dissecting the Function of a Potassium Channel

The essential function of potassium channels is to selectively facilitate the passage of K^+ ions across the cell membrane by lowering the energy barrier that opposes this process. In this issue of *Neuron*, Chatelain et al. report the results from an elegant study that sheds new light on the microscopic factors that modulate the function of these important membrane channels.

By using a powerful genetic screen, electrophysiology, and protein engineering, Chatelain et al. (2005) identified and characterized mutants of the inwardly rectifying potassium channels Kir2.1 that are functional, ion selective, and resistant to block by barium ions and that have minimally altered conductance properties. Unexpectedly, the mutants displaying resistance to barium block (T141K) bear a positively charged residue at the first position of the highly conserved potassium channel selectivity filter signature sequence, TTxGYG, near the COO-terminus of the pore helices. Yet, those channels have minimally altered conductance properties. These observations are counterintuitive. Could it be that electrostatic perturbations near the internal side of the selectivity filter have only minimal effects on the energetics of ion permeation?

The identification of mutant channels displaying resistance to barium block is particularly informative for dissecting potassium channel pore properties because this divalent cation has an ionic radius that is very similar to that of monovalent potassium. Barium binds preferentially to the innermost site of the four binding sites of the narrow selectivity filter, near the COO-terminus of the pore helices (Jiang and MacKinnon, 2000). It has been proposed that the interactions between the permeant ion and dipoles from the pore helices serve to lower the electrostatic destabilization that would otherwise occur upon transfer of an ion into the central cavity of the channel (Roux and MacKinnon, 1999). Given this model, it is disturbing to find that mutants, which should effectively cancel the stabilization afforded by the pore helix by placing formal positive charges at its negative end, pass the selection and are functional. How could four formally positively charged residues exist in proximity within the channel lumen? Further amino acid substitutions reveal that some charge compensation and interactions are required to enable the presence of the positively charged side chain near the entrance of the selectivity filter. In particular, a negatively charged residue D172, residing on the channel lumen face of the pore-lining cavity, must be present; the single mutant D172N is functional, but the double mutant T141K/D172N is not.

The results do show unambiguously that the inner vestibule of the potassium channel selectivity filter can withstand significant changes in local electrostatics while remaining able to maintain a high potassium throughput. Chatelain et al. then performed continuum electrostatic calculations, by using the inwardly rectifying bacterial potassium channel KirBAC as a template, to better understand how the T141K mutation could confer barium resistance and yet not impact ion permeation significantly. The results of these computations, which are summarized in Figure 6 of their paper, clearly show that both potassium and barium can bind to the site S_4 of the wild-type channel. In contrast, the binding of barium is highly destabilized by the double A109K/I138D mutation (corresponding to positions 141 and 172 of Kir2.1, respectively), whereas potassium binding is not significantly affected. The overall accord with the data supports an electrostatic origin for the observed experimental effects. Despite the limitations in the atomic structural models and the approximations underlying continuum electrostatic calculations (Allen et al., 2004), the good agreement suggest that one should be able to understand the factors that are at play in this system by using the results from the computations.

According to continuum electrostatics, the free energy to transfer an ion from the bulk to a particular binding site is (Roux and MacKinnon, 1999)

$$\Delta G = \frac{1}{2}AQ^2 + BQ + C.$$

In the context of this simple approximation, valence selectivity is largely controlled by the value of the optimal charge that yields the largest (most favorable) free energy, that is, $Q_{opt} = -B/A$. According to the calculations of Chatelain et al. done on the KirBAC channel (Table S1 of their paper) the optimal charge for occupying the site S_4 is 1.6*e* for the wt channel, while it is 0.8*e* for the A109K/I138D mutant (corresponding to positions 141 and 172 in Kir2.1, respectively). Therefore, in the simplest terms, it appears that the mutations effectively shift the optimal charge, resulting in an increased selectivity for the monovalent cation over the divalent cation. The basis for barium resistance in the mutant channel is best illustrated by plotting the value of the electrostatic transfer free energy as a function of the charge *Q* (see Figure 1). Because of the quadratic rise in ΔG as a function of the charge *Q*, a divalent cation can be significantly destabilized energetically while the monovalent cation is not. On a methodological note, it should be emphasized that such simple continuum

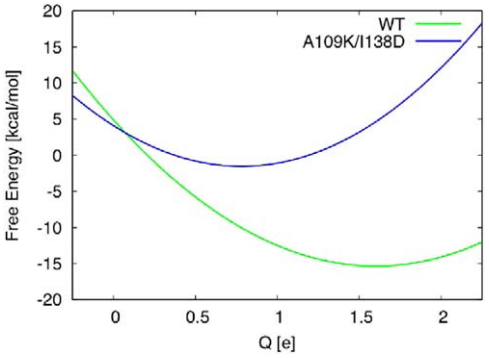

Figure 1. Calculated Electrostatic Transfer Energy from Bulk to the Binding Site S_4 in KirBAC

Figure taken from Table S1 of Chatelain et al. (2005).

electrostatic calculations with a fixed rigid protein structure are yielding qualitatively meaningful insight here only because barium and potassium have nearly the same ionic radius and, thereby, approximately the same coordination shell. In contrast, the flexibility and atomic fluctuations of the selectivity filter must be taken into account for a meaningful estimate of the relative free energy of potassium relative to sodium (Noskov et al., 2004).

The experimental results of Chatelain et al. beautifully underscore the utility of using genetic selection approaches as an unbiased way to identify and study channel elements that are important for the energetics of ion channel-blocker interactions. The study also illustrates that considerations based on electrostatic principles provide a sound basis to understand how the functional properties of ion channels are modulated.

Benoît Roux
Physiology Department
Weill Medical College of Cornell University
1300 York Avenue
New York, New York 10021

Selected Reading

Allen, T.W., Andersen, O.S., and Roux, B. (2004). J. Gen. Physiol. *124*, 679–690.

Chatelain, F.C., Alagem, N., Xu, Q., Pancaroglu, R., Reuveny, E., and Minor, D.L. (2005). Neuron *47*, this issue, 833–843.

Jiang, Y., and MacKinnon, R. (2000). J. Gen. Physiol. *115*, 269–272.

Roux, B., and MacKinnon, R. (1999). Science *285*, 100–102.

Noskov, S.Y., Bernèche, S., and Roux, B. (2004). Nature *431*, 830–834.

DOI 10.1016/j.neuron.2005.08.031