$$\textbf{I.}\ \mathrm{GLN} \Longrightarrow \mathrm{GLU} + \mathrm{NH_3}$$

$$\textbf{II.}\ \mathrm{Glu\text{-}tRNA^{Gln}} + \mathrm{ATP} \Longrightarrow \gamma\mathrm{P\text{-}Glu\text{-}tRNA^{Gln}} + \mathrm{ADP}$$

$$\textbf{III.}\ \gamma\mathrm{P\text{-}Glu\text{-}tRNA^{Gln}} + \mathrm{NH_3^+} \Longrightarrow \mathrm{Gln\text{-}tRNA^{Gln}} + \mathrm{P_i}$$

Figure 1. Pathway of Gln-tRNA^{Gln} Synthesis by AdT Enzymes

Reaction **I** takes place in a distinct active site associated with the D subunit of GatDE, and the NH_3 generated must be transferred across the heterodimeric enzyme to the tRNA active site located on the E subunit, where reactions **II** and **III** are thought to occur.

address other important questions. For example, while both active sites of GatDE share similarities with other previously characterized families of enzymes, the extent to which the tRNA dependence of the reaction might give rise to unique mechanistic features remains largely unknown. Another fundamental unresolved question for any AdT concerns the identification of the important tRNA nucleotides that allow efficient recognition of misacylated tRNAs only. Finally, it is of great interest to appreciate the structure-function relationships of AdTs in the context of the broader metabolism of the organisms in which they are found, as well as with respect to their phylogeny (Feng et al., 2004). Although a distinguishing phylogenetic feature of AdTs has been the absence of the canonical tRNA synthetase in the genome, there are in fact a remarkable variety of combinations in which GlnRS, AsnRS, GatDE, and GatCAB are variously present or absent in a given organism. Hence, the continued study of AdTs should provide a fascinating window on the detailed characteristics of an RNA modification reaction as well as on the molecular evolution of the three domains of life.

John J. Perona
Department of Chemistry and Biochemistry
University of California, Santa Barbara
Santa Barbara, California 93106

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One Channel: Open and Closed

Kuo et al. (2005) report structural information about the prokaryotic KirBac3.1 inward rectifier family K^+ channel from *Magnetospirillum magnetotacticum*. These results from two-dimensional electron cryomicroscopy (EM) shed light on the gating mechanism of members of the Kir channel family.

K^+ channels are large transmembrane proteins that control and regulate the passage of K^+ ions across the cell membrane. Significant conformational changes of the protein are expected to underlie the process, referred to as "gating," by which a channel opens and closes its passageway to ions. To understand the specific gating mechanism for a given family of K^+ channels, it is essential to determine the three-dimensional (3D) structure of at least two functional states of the same protein.

In this issue of *Structure*, Kuo et al. (2005) report EM data for two conformational states of KirBac3.1 at 9 Å resolution, in which they observe a substantial increase in pore size from less than 6 Å to greater that 11 Å. The EM data provide a projection view of the KirBac3.1 channel in the natural environment of a lipid bilayer membrane. Depending on the experimental conditions, two different crystal forms were observed: one obtained in the presence of Mg^{2+}, in which the channel structure appears tightly closed, and another in the absence of Mg^{2+}, in which the channel structure is more expanded and putatively open. Using calculations of cross correlation values between the 2D EM projection data and the 3D atomic structure of Kirbac1.1 (a homolog of Kirbac3.1) that was previously determined by X-ray crystallography (Kuo et al., 2003), the authors were able to determine a structural model that fits well to the expanded state density. Although there is no direct evidence as to the functionality of this expanded conformation, all intuition suggests that this may be an open state of the channel.

The analysis reveals two distinct conformational states for the same channel protein. To date, structural comparisons between open and closed states have

been made only between channel families but not within: KcsA (Doyle et al., 1998) and KirBac1.1 (Kuo et al., 2003) for the closed state, and MthK (Jiang et al., 2002) for the open state. While those comparisons reveal the overall nature of the conformational change underlying channel closing and opening—the inner helices form a constriction point that blocks the pore entrance in the closed state of the channel, whereas they are bent and splayed outward at a highly conserved glycine hinge residue in the open state—the structures provided by Kuo et al. allow the examination of this general K^+ channel gating mechanism within the reference frame of a single channel type. In particular, these results put important constraints on the gating mechanism for members of the inward rectifier family of K^+ channels (Kir).

There has been considerable debate about the conformational changes associated with Kir channel gating. While one hypothesis proposes an intracellular gate similar to that of the helical bundle seen in KcsA, a number of other studies have suggested that changes in the selectivity filter itself may act as the predominant gate in these channels (Bichet et al., 2003). Although the existence of additional gates remains possible, the previous closed state crystal structure of KirBac1.1, together with the new structural data by Kuo et al. (2005), clearly shows that there is an intracellular gate formed by the inner helices that move away from the pore in the expanded conformation.

The availability of models of KirBac3.1 (Kuo et al., 2003) in the closed and open states provides a unique opportunity to examine the importance of microscopic factors affecting channel function using computational methods. The authors have already assessed the stability of a similar open-state model of KirBac1.1 using molecular dynamics simulations (Domene et al., 2005) and found, satisfyingly, that it was very stable during extensively long simulations (138 ns). The energetics of ion permeation can also be explored using simple Poisson-Boltzmann (PB) calculations in which the solvent and the membrane are represented as continuum dielectrics. As an illustration, the electrostatic free energy profile of a K^+ ion along the pore axis was calculated for the closed and open structures of KirBac1.1 (using the open state model based on KirBac3.1 from Domene et al., 2005) and is shown in Figure 1. Clearly, the unfavorable energy barrier at the intracellular bundle crossing in the closed state is completely abolished and, in the open state, the profile is remarkably flat throughout the cytoplasmic domain up to the center of the cavity. These observations support the notion that the latter conformation can conduct ions at a high rate. Figure 1 also shows that a cation in the cavity is electrostatically destabilized in the open state relative to the closed state, a qualitative trend that is observed for many different K^+ channels (Jogini and Roux, 2005). In the future, it is anticipated that the availability of both the closed- and open-state conformations of KirBac3.1 will permit further computational studies that will help resolve current issues about the binding locations of multivalent cations (Mg^{2+}, polyamines), which characteristically cause intracellular blocks of outward ionic flow in inward rectifier channels (John et al., 2004; Shin and Lu, 2005).

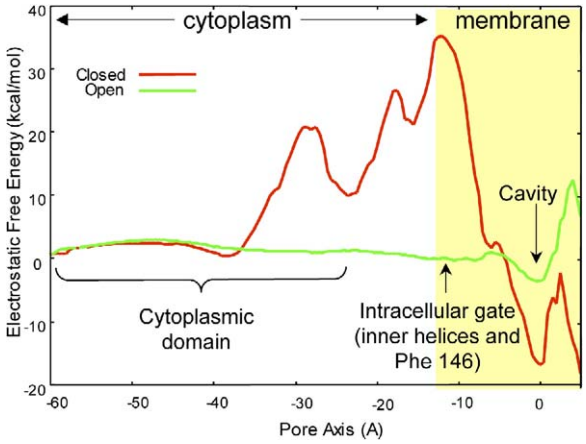

Figure 1. Electrostatic Free Energy Profile of K^+ along the Pore Axis of KirBac1.1 in the Closed and Open State

PB calculations were carried out for the closed KirBac1.1 structure (pdb:1p7b) and the open KirBac1.1 model based on the EM data of KirBac3.1 (Kuo et al., 2005; Domene et al., 2005). In the calculations, the channel structures are aligned with the pore axis along the Z-axis and positioned such that the extracellular side is at Z > 0 and the nonpolar vestibular cavity is at the center of the membrane (Z = 0). The calculations were performed using the PBEQ module of the CHARMM program; see Jogini and Roux (2005) for further details.

Perhaps the most intriguing aspect of the results of Kuo et al. (2005) concerns the observation that the presence of Mg^{2+} stabilizes the closed conformation of the channel in crystallization assays. Electrophysiological studies of Kir channels have long considered the intracellular block by Mg^{2+} and polyamines to occur through rapid open-state binding from the intracellular side. The location and specific binding of Mg^{2+} is not fully known, although there are multiple negatively charged residues along the channel pore that have been shown to be involved in binding and current blockade. The results from the work of Kuo et al. (2005) suggest that the conformation of KirBac3.1 could be directly affected by the presence of Mg^{2+}. The thought that Mg^{2+} binding could produce such large structural changes is surprising, but it is consistent with a previous study that suggested that spermine blockade in Kir2.1 could induce channel closing (Chang et al., 2003). Further investigation will be required to clarify whether the binding of Mg^{2+} triggers such a conformational change in the channels in their native environment, and, if so, by what mechanism.

Janice L. Robertson and Benoît Roux
Department of Physiology and Biophysics
Weill Medical College of Cornell University
New York, New York 10021

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