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Lipids driving protein structure?:

Evolutionary adaptations in Kir channels

Nazzareno D'Avanzo[1,2], **Wayland W.L Cheng**[1,2], **Shizhen Wang**[1,2,3], **Decha Enkvetchakul**[3], and **Colin G. Nichols**[1,2,*]

[1]Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MI USA

[2]Center for Investigation of Membrane Excitabiltiy Diseases, Washington University School of Medicine, St. Louis, MI USA

[3]Department of Pharmacological and Physiological Science, Saint Louis University School of Medicine, St. Louis, MI USA

Abstract

Many eukaryotic channels, transporters and receptors are activated by phosphatidyl inositol bisphosphate (PIP_2) in the membrane, and every member of the eukaryotic inward rectifier potassium (Kir) channel family requires membrane PIP_2 for activity. In contrast, a bacterial homolog (KirBac1.1) is specifically inhibited by PIP_2. We speculate that a key evolutionary adaptation in eukaryotic channels is the insertion of additional linkers between trans-membrane and cytoplasmic domains, revealed by new crystal structures, that convert PIP_2 inhibition to activation. Such an adaptation may reflect a novel evolutionary drive to protein structure,; one that was necessary to permit channel function within the highly negatively charged membranes that evolved in the eukaryotic lineage.

Keywords

inward rectifier; gating; structure; K_{ATP}; PIP_2; KCNJ; KirBac

Perhaps the most universally recognized regulators of ion channel gating, after membrane voltage, are the phosphorylated phosphatidyl inositols, the archetype being $PI(4,5)P_2$ (PIP_2). In many eukaryotic channels, transporters and receptors, including voltage-gated K channels,[1–4] epithelial Na channels,[5] the transient receptor potential (TRP) family of channels,[6] the Na^+-Ca^{2+} exchanger,[7] and P2X receptor channels,[8] increased PIP_2 in the membrane stimulates activity. Likewise, every member of the eukaryotic inward rectifier potassium channel (Kir or KCNJ) family requires membrane PIP_2 for activity.[9,10] How, and —teleologically—why, PIP_2 activates these channels and transporters has been difficult to assess at the biochemical level, partly because of the complexity of cell-based systems typically used to study them. Over the past five years, the ability to express and purify active bacterial homologs of inward rectifier channels (KirBacs) has allowed us to study regulation of pure channel protein in lipid bilayers of defined composition, and has led to the surprising realization that, in contrast to the above, these cousins of eukaryotic Kir channels are specifically *inhibited* by PIP_2.[11,12]

*Correspondence to: Colin G. Nichols; cnichols@wustl.edu.

We have suggested that this paradoxical behavior might be the result of missing key residues in the KirBac structure that are crucially involved in PIP_2 binding and transduction in eukaryotic Kir channels.[11] These key residues are located in two short linker regions that connect the large cytoplasmic domain to the transmembrane (TM) pore-forming region of the channel. Alignments of KirBac and eukaryotic Kir sequences (Fig. 1) reveal that each of these linkers is longer by three residues in the eukaryotic Kirs. Additionally, the second linker, between TM2 and the cytoplasmic domain, contains two charged residues which, when mutated, invariably causes activation loss of PIP_2[13,14] and loss of PIP_2 binding.[15] These three residue insertions are predicted to displace the cytoplasmic domain away from the membrane surface, and as the new structure of the chicken Kir2.2 channel reveals,[16] this is indeed the case (Fig. 2).

How does this displacement convert inhibition of KirBacs into activation PIP_2 of eukaryotic Kir channels? Interactions between the slide helix and the cytoplasmic domain of Kir channels have been suggested to play a key role in channel gating. Mutations which disrupt this interaction can destabilize the open state and favor channel closure, although the ability of these proteins to bind PIP_2 remains intact.[17] Thus, we can speculate that the shorter linkers in KirBacs energetically favor interactions between the slide helix and the cytoplasmic domain, leading to opening of the channel in the absence of PIP_2 (Fig. 3) Binding of PIP_2 to KirBacs may act to destabilize this interaction, separating the cytoplasmic domain from the slide helix, leading to channel closure (Fig. 3). In eukaryotic Kirs, the longer linker would minimize the interaction between the slide helix and the cytoplasmic domain, and thereby keep the channel closed in membranes that lack PIP_2. However, the PIP_2 head-group can extend up to 17 Å from the surface of the bilayer,[18,19] and PIP_2 binding may pull the cytoplasmic domain back towards the membrane, restoring its interaction with the slide helix to drive channel opening (Fig. 3).

From an evolutionary perspective, the differential PIP_2 regulation of prokaryotic and eukaryotic Kir channels may provide a fascinating illustration of the interplay of ligands and the evolution of protein structure. It is noteworthy that bacterial membranes typically do not contain PIP_2 or other phosphoinositide lipids. Instead, the dominant lipids are phosphatidylethanolamines (PE), and phosphatidylglycerols (PG),[20] in which KirBac channels are active.[11,21] As eukaryotic organisms evolved, PIP_2 and other acidic lipids became increasingly concentrated in plasmalemmal membranes. The unwonted inhibitory effect of PIP_2 on KirBac1.1 activity is such that at the predicted PIP_2 concentrations in mammalian membranes (~1% of phospholipids),[22,23] KirBac-based channel activity would be completely suppressed.[11] By contrast, the requirement for PIP_2 for activity would render eukaryotic Kir channels inactive in bacterial membranes and in intracellular membranes of the ER and Golgi, which also lack PIP_2. It is tempting to speculate that the 3 residue insertions in the cytoplasmic domain-TM domain linkers evolved to allow eukaryotic Kir channels to (i) be functionally active in membranes that evolved to contain PIP_2 for other critical cellular functions and/or (ii) take advantage of differences in membrane composition of the various cellular compartments, thereby protecting cells from undesirable channel activity during the trafficking process. Given the breadth of eukaryotic membrane proteins that are sensitive to PIP_2, this may be a more generally observable evolutionary mechanism. As more genomes are sequenced and advanced lipidomics are employed to resolve the compositions of specific membranes, this hypothesis can be rigorously examined.

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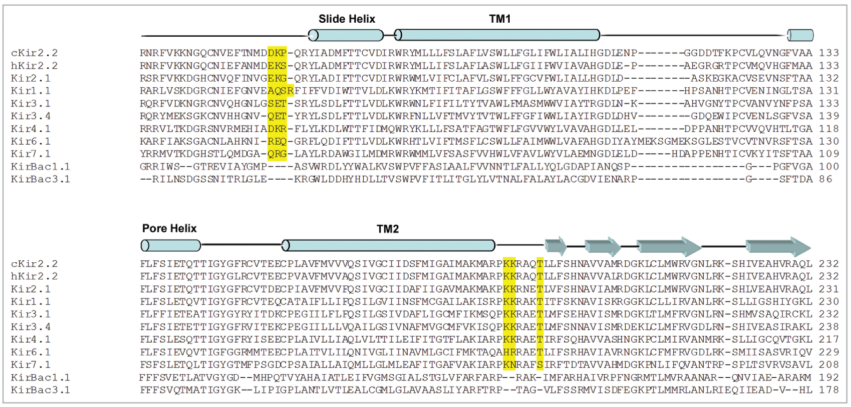

Figure 1.

Sequence Alignment of Eukaryotic and Bacterial Inward Rectifier K^+ Channels. Eukaryotic Kirs have a prominent 3 residue insertion (highlighted in yellow) in both the N- and C-terminal linkers that link the cytoplasmic domain to the transmembrane domains. These insertions, which include key residues for PIP_2 activation of eukaryotic Kir channels, are predicted to displace the cytoplasmic domain away from the membrane surface.

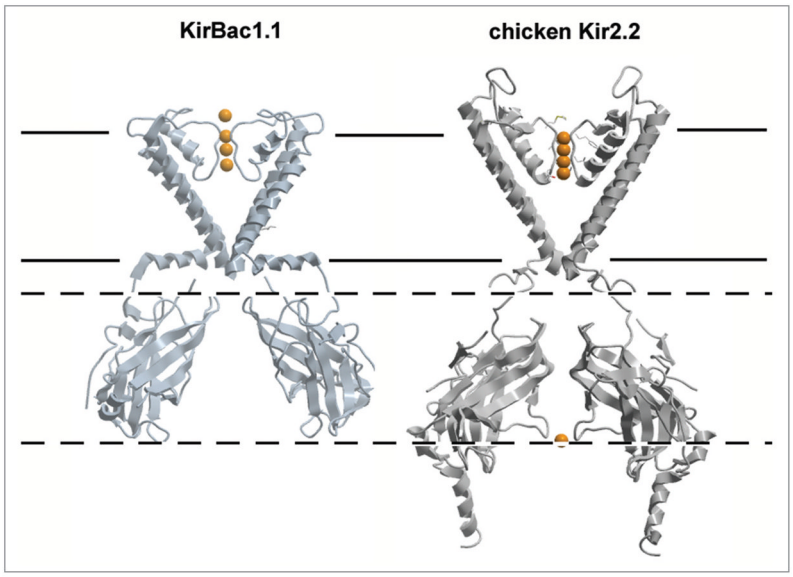

Figure 2.

Structural Comparison of Bacterial and Eukaryotic Kir channels. Closed-state structures of KirBac1.1 (PDB entry: 1P7B) and chicken Kir2.2 (PDB entry: 3YJC). For clarity, chain A and C TM domains, and chain B and D cytoplasmic domains are shown. Notably the tetrameric assembly of the chicken Kir2.2 soluble domain is rotated ~60° compared to the KirBac1.1 structure, and is displaced away from the cell membrane resulting in minimal interaction between the slide helix and the soluble domain in this structure.

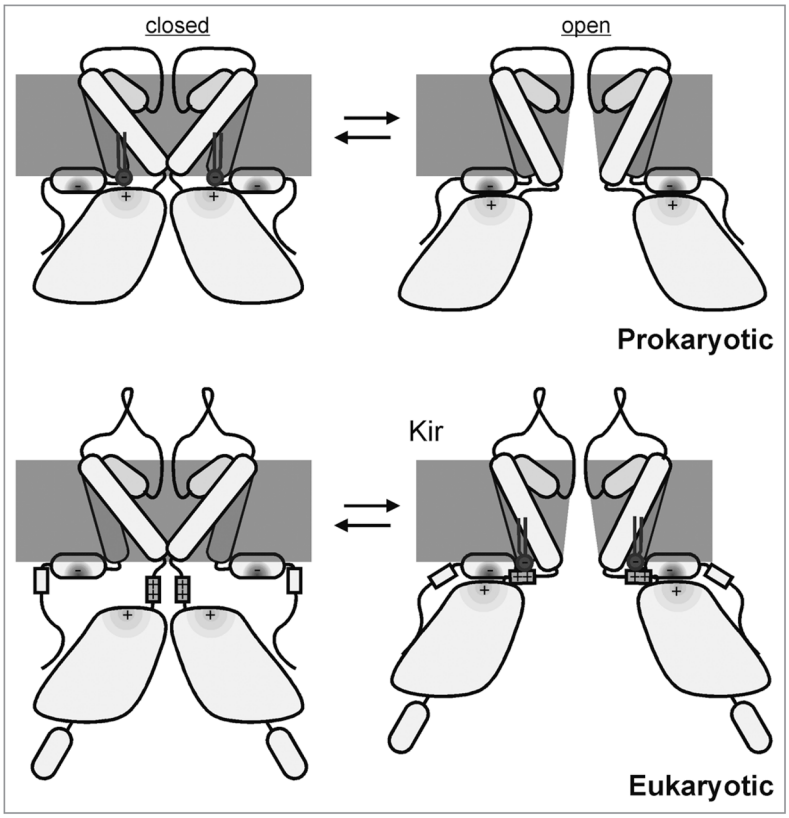

Figure 3.

Mechanism of $PI(4,5)P_2$ gating in Kir channels. In prokaryotic KirBac channels, short TM-cytoplasmic domain linkers may permit energetically favorable interactions between the slide helix and cytoplasmic domain to open the channel in the absence of PIP_2 (top right). The addition of PIP_2 to the membrane may act to destabilize this interaction, separating the cytoplasmic domain from the slide helix, leading to channel closure (top left). The longer linker in eukaryotic Kirs minimizes the interaction between the slide helix and cytoplasmic domain in the absence of PIP_2, keeping the channel in the closed state (bottom left). Binding of PIP_2 may recoil the cytoplasmic domain towards the membrane allowing for restored interaction with the slide helix to drive channel opening (bottom right).