applying our ED protocol to different number of subunits (SUs). Our simulations showed that the movement of a single SU is not sufficient to open the activation gate. But by moving three SUs by ED simulations, the activation gate of the KirBac1.1 channel opens to the same extent as in the four SUs ED simulation protocol. These findings are in line with fluorescence detection studies, which showed that the SUs act cooperatively during gating (Blunck et al. 2008).

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Gating Motions of KirBac1.1 Cytoplasmic Domain with Respect to Transmembrane Domain Revealed by FRET
Shizhen Wang1,2, Sarah Heyman1, Decha Enkvetchakul3, Colin G. Nichols1. 1Washington University School of Medicine, Saint Louis, MO, USA, 2Department of Pharmacological and Physiological Sciences, Saint Louis University, Saint Louis, MO, USA, 3KirBac1.1 is a bacterial inward rectifier potassium (Kir) channel, which, contrary to its eukaryotic homologues, is strongly inhibited by phosphatidylinositol-4,5-bisphosphate (PIP2). The most recent crystal structures of eukaryotic Kir2.2 in complex with PIP2 indicate that the TM-CD linker forms a short a-helix in the presence of PIP2. As a result, the cytoplasmic domain of Kir2.2 moves about 6 angstrom towards the membrane surface. However, the ‘KKR’ motif in the TM-CD linker of Kir2.2, which directly interacts with PIP2, is absent in KirBac1.1 and the question arises: how does KirBac1.1 cytoplasmic domain move in response to PIP2 inhibition? In the present work, we have made KirBac1.1 tandem tetramer constructs and purified KirBac1.1 tandem proteins successfully. Reconstituted liposome flux assays indicate that the KirBac1.1 tandem protein remains functional, and retains sensitivity to PIP2 inhibition. We introduced two cysteine into the KirBac1.1 tandem tetramer, one at the extracellular loop of subunit B (loop 20) and one in the cytoplasmic domain of subunit 2 (A273). We labeled these cysteines with a FRET dye pair (Alexa-Fluor 488 and DABCYL) and measured FRET efficiencies in protein samples reconstituted into liposomes, in the absence and presence of PIP2. Our results indicate that the KirBac1.1 cytoplasmic domain moves ~2-3 angstrom away from the transmembrane domain in the presence of PIP2 – opposite the direction suggested from eukaryotic Kir2.2 crystal structures in the presence and absence of PIP2. Reversed PIP2-dependent motions of the cytoplasmic domain with respect to the transmembrane domain between prokaryotic and eukaryotic Kir channels may explain their differential response to PIP2 modulation.

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Simulations of the Helix Bundle Crossing Gate Opening in Kir Channels
Xuanyu Meng, Meng Cui, Diomedes E. Logothetis. Virginia Commonwealth University, Richmond, VA, USA. Inwardly rectifying K+ (Kir) channels are gated by the phospholipid PIP2. Along the ion permeation pathway, there are two major regions of the selectivity filter -SF, the inner helix bundle crossing (HBC), and the intracellular G-loop. The SF is the narrowest part of the channel and serves as the gate to control ion permeation. A crystal structure of a Kir3.1 chimera [Nishida et al., 2007] captured the cytosolic G-loop gate in "closed/constricted" or "open/dilated" conformations. 100 nm Molecular Dynamics (MD) simulations studying the PIP2-driven Kir channel activation of the Kir3.1 chimera led us to propose a molecular mechanism of the G-loop gate opening [Meng et al., 2012]. However, opening of the HBC gate was not observed throughout this simulation. Mutagenesis and single-channel recording studies in our lab showed that a proline mutation on the inner helix of the Kir3.4 channel dramatically increased the open probability of the channel [Jin et al., 2002]. We introduced the corresponding M170P mutation on the Kir3.1 chimera structure and ran 100 ns long simulations of four mutant channel systems: dilated and constricted M170P Kir3.1 chimera in the presence (holo) and absence (apo) of PIP2, using the GROMACS program [Hess et al., 2008]. Phosphatidyl inositols present in the SF passed through the HBC gate in the system of the holo dilated M170P Kir3.1 channel with the 100 ns simulation time. Minimal distance measurements indicated that the HBC gate was able to open only when PIP2 was present and the G-loop gate was stabilized in the open state. Principal component analysis revealed coupled conformational changes in the SF helix, DE- and LM-loops, possibly related to the opening of the HBC gate. Moreover, unique residue interactions within the transmembrane domains were observed in the dilated holo system. Predictions of these models are being tested experimentally.

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Native Currents in Hepatocytes with Characteristic Properties of Kir2 Channels
Richard Masia1, Gary Yellen2. 1Massachusetts General Hospital, Boston, MA, USA, 2Harvard Medical School, Boston, MA, USA. We investigated the electrophysiological properties of parenchymal liver cells (hepatocytes) by using the perforated patch-clamp technique with Ampholyte B. We found that dissociated mouse hepatocytes exhibited native currents with characteristic properties of inward rectifier potassium (Kir) channels, subfamily B, which has not been previously reported. Currents were constitutively active and stable for longer than 30 min during recording, with a current density of −16.2 ± 1.7 pA/pF (V_m = −114 mV, current at [K^+]_o = 5 mM minus current at [K^+]_o = 0 mM, n=14 cells). Currents exhibited strong and “steep” inward rectification, with essentially no outward current at voltages more than −20 mV, as typically seen with Kir2 channels. The reversal potential approached the predicted E_G (−81.8 ± 0.4 mV, −84.4 mV, [K^+]_o = 134 mM and [K^+]_o = 5 mM). Varying [K^+]_o over a range of 5 to 144 mM showed that E_m was strongly dependent on [K^+]_o with a Nernstian slope of 58.8 mV/decade, demonstrating that the currents are highly selective for K^+ currents were fully blocked by external Ba^2+, with K_Ba = 2.7 ± 0.2 mM (V_m = −94 mV, [K^+]_o = 60 mM, n=6 cells). This K_Ba value is in close agreement with published data on Ba^2+ block of homomeric Kir2.1 channels (Liu GX et al. J Physiol 2001). The currents were not significantly inhibited by acidification of the bath solution or pipette solution, which argues against a contribution of pH-sensitive Kir2 subunits such as Kir2.2 or Kir2.3. We thus hypothesize that the molecular identity of the observed currents is Kir2.1. Additional experiments are underway to test this hypothesis and to elucidate the physiological role of native Kir2 channels in hepatocytes.

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Reduced PIP2 Binding to KCNJ2 (M307I) Channels is Linked to Type 1 Andersen-Tawil Syndrome
Bi-Hua Tan1,2, Sinisa Dovat1, Blaise Z. Peterson1, Chunhua Song1. 1Penn State University College of Medicine, Hershey, PA, USA, 2University of Wisconsin, Madison, WI, USA. Background: Inward rectifier potassium channels play a key role in setting and maintaining the resting membrane potential and regulating excitability in various tissues. Kir2.x subfamily members mediate the cardiac inward rectifier potassium current (IK1). KCNJ2 encodes Kir2.1, the pore-forming alpha subunit responsible for cardiac IK1, and the mutations in this gene are associated with type 1 Andersen-Tawil Syndrome (ATS1). A Kir2.1 missense mutation, M307I, has been identified in a Korean family with ATS1. We found that the ATS1-associated M307I mutation is a loss-of-function mutation in KCNJ2 that mediates a dominant-negative effect on wild-type (WT) channels. M307I is located in the intracellular C-terminal domain in a region known to be associated with putative phosphatidylinositol 4,5-bisphosphate (PIP2) binding and channel trafficking. Here we explored the mechanisms underlying the dominant-negative effect of the mutation. Methods and Results: Human Kir2.1 was subcloned into pFlag-CMV vector and pFlag-Kir2.1-M307I was generated by site-direct mutagenesis. The Flag-Kir2.1-WT and Flag-Kir2.1-M307I were expressed in HEK293 cells and affinity purified. PIP2 binding was assessed using a Lipid-bead-protein pull-down assay with cell lysate and Protein-lipid overlay assay with purified proteins. The electrophysiological data showed that the M307I mutant channel significantly reduces whole cell current densities when co-expressed with Kir2.1-WT channels. Immunofluorescence (IF) staining assays reveal that M307I channels exhibit normal membrane trafficking. PIP2 binding assays show that Flag-Kir2.1-M307I channels exhibit dramatically decreased binding to PIP2 compared to WT channels. Conclusions: M307I is an ATS1-associated, loss-of-function missense mutation in KCNJ2 that mediates a dominant-negative effect on WT channels. M307I is located in the intracellular C-terminal domain in a region known to be associated with putative phosphatidylinositol 4,5-bisphosphate (PIP2) binding and channel trafficking. Here we explored the mechanisms underlying the dominant-negative effect of the mutation.

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Targeting of Kir_2.1 and Downregulation of Inward Rectifier K+ Current by miR-212
Dana Goldoni, Janet M. Yarham, Mary K. McGahan, Anna O’Connor, Jasenka Guduric-Fuchs, Kevin Edgar, Denise M. McDonald, David A. Simpson, Anthony Collins. Queen’s University Belfast, Belfast, United Kingdom. Downregulation of inwardly rectifying K+ channels contributes to an increased risk of cardiac arrhythmia in heart failure and to impaired cerebral arterial dilatation in chronic alcohol consumption. The downregulation mechanism is unknown, although post-transcriptional regulation of gene expression by microRNAs is a strong possibility that has not been fully investigated. miR-212 is markedly upregulated in heart failure and chronic alcoholism, and is predicted by bioinformatic algorithms to target Kir_2.1, the predominant inward rectifier K+ channel expressed in heart and arterial smooth muscle. We developed a fluorescence-based assay for identifying microRNA targets, using mCherry red