

Engineered Kir6.2 mutations that correct the trafficking defect of K_{ATP} channels caused by specific SUR1 mutations

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K_{ATP} channels consisting of Kir6.2 and SUR1 couple cell metabolism to membrane excitability and regulate insulin secretion. The molecular interactions between SUR1 and Kir6.2 that govern channel gating and biogenesis are incompletely understood. In a recent study, we showed that a SUR1 and Kir6.2 mutation pair, E203K-SUR1 and Q52E-Kir6.2, at the SUR1/Kir6.2 interface near the plasma membrane increases the ATP-sensitivity of the channel by nearly 100-fold. Here, we report the finding that the same mutation pair also suppresses channel folding/trafficking defects caused by select SUR1 mutations in the first transmembrane domain of SUR1. Analysis of the contributions from individual mutations, however, revealed that the correction effect is attributed largely to Q52E-Kir6.2 alone. Moreover, the correction is dependent on the negative charge of the substituting amino acid at the Q52 position in Kir6.2. Our study demonstrates for the first time that engineered mutations in Kir6.2 can correct the biogenesis defect caused by specific mutations in the SUR1 subunit.

Introduction

The pancreatic ATP-sensitive potassium (K_{ATP}) channel is a hetero-octamer composed of four Kir6.2 subunits and four sulfonylurea receptor 1 (SUR1) subunits.¹ K_{ATP} channels play a key role in coupling cell metabolism with membrane excitability to regulate insulin secretion.^{2–4} Dysfunction of K_{ATP} channels rendered by mutations in the SUR1 and Kir6.2 genes underlies a spectrum of insulin secretion disorders.³

It is well recognized that both Kir6.2 and SUR1 contribute to channel biogenesis and gating.^{1,5} When expressed individually, neither subunit traffics to the cell surface owing to the presence of an -RKR- ER retention/retrieval motif.⁶ When co-expressed and co-assembled into an octameric complex, the RKR motifs are concealed to allow channels to traffic from the endoplasmic reticulum (ER) to the plasma membrane.⁶ In the functional channel complex, Kir6.2 forms the pore and mediates ATP inhibition,^{7,8} whereas SUR1 modulates Kir6.2 gating by conferring the stimulatory effect of MgATP/ADP,^{9–11} increasing the open probability of Kir6.2^{8,12–14} and enhancing channel sensitivity to ATP inhibition.⁸ An outstanding question remains as to how SUR1 and Kir6.2 interact at the structural level to govern channel biogenesis and gating.

A structural domain that has emerged as important in physical and functional coupling between SUR1 and Kir6.2 is the first transmembrane domain of SUR1,^{12,14,15} designated TMD0 (see Fig. 1A). TMD0 alone can assemble with Kir6.2 to form channels that have the high open probability resembling WT channels. In addition, the cytoplasmic loop L0 immediately following TMD0 interacts with the N-terminal cytoplasmic domain of Kir6.2 to modulate channel gating.^{12,17–20} Recently, we identified an engineered interaction between SUR1-E203K and Kir6.2-Q52E (denoted as E203K//Q52E; hereinafter “//” separates mutations in SUR1 and Kir6.2, and “/” separates mutations within the same subunit) that increased the channel-s sensitivity to ATP by nearly 100-fold.²¹ E203

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Abbreviations: SUR1, sulfonylurea receptor 1; Kir6.2, inward rectifier potassium channel 6.2; TMD0, transmembrane domain zero; K_{ATP} , ATP-sensitive potassium channel

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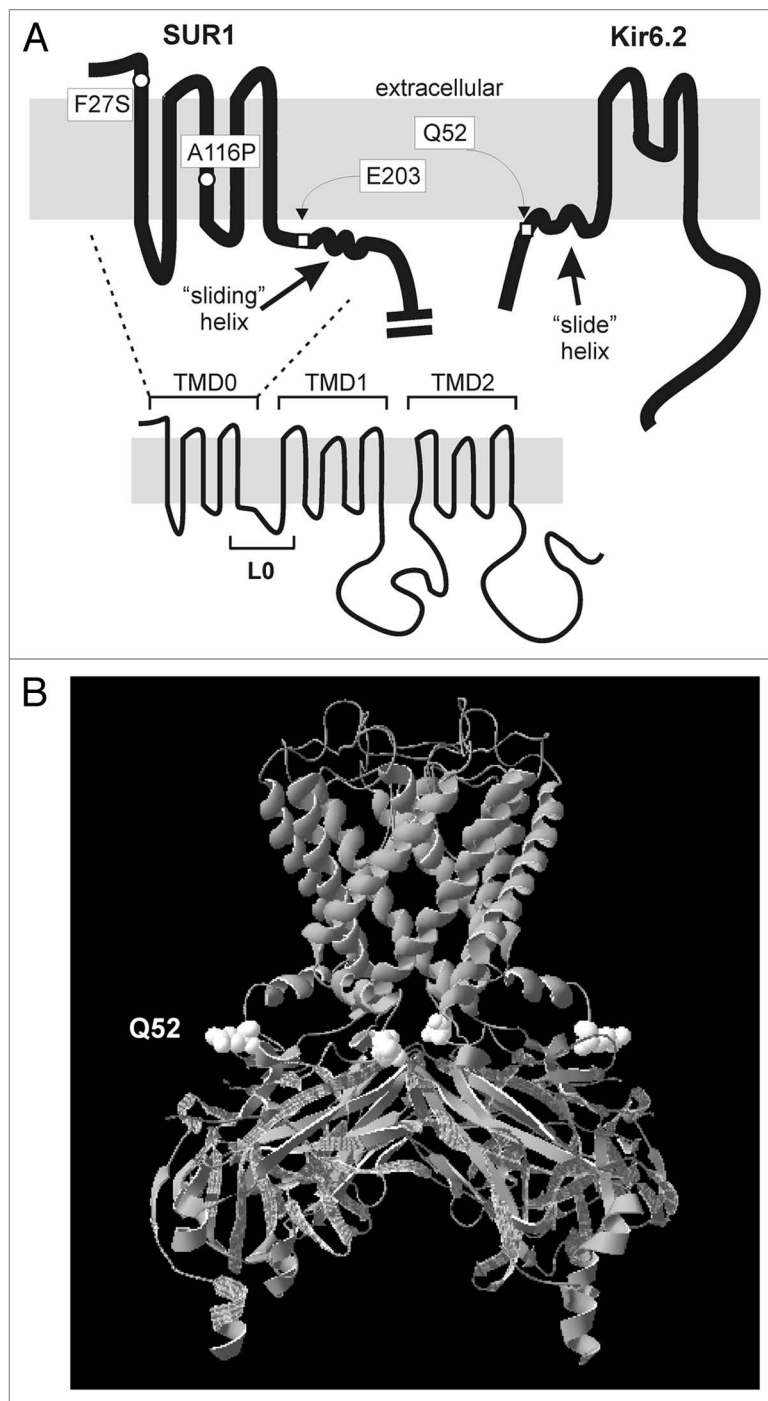


Figure 1. (A) Schematic of SUR1 and Kir6.2 proteins, highlighting the TMD0 portion of SUR1. Positions of SUR1-E203 and Kir6.2-Q52 residues (open squares) as well as the two TMD0 trafficking mutations F27S and A116P (open circles) are indicated. (B) A homology model of Kir6.2 tetramer marking the position of the Q52 residue (white). The model was made with Modeler and Chimera using a chicken Kir2.2 channel crystal structure (PDB ID: 3JYC)¹⁶ as the template. Amino acids 30–352 of Kir6.2 which correspond to amino acids 42–369 of chicken Kir2.2 based on sequence alignment were modeled.

of SUR1 is located at the beginning of L0 close to the plasma membrane just downstream of TMD0 and near the beginning of a predicted amphipathic, so called,

“sliding” helix. Q52 of Kir6.2 is also close to the plasma membrane just N-terminal to the amphipathic “slide” helix (Fig. 1A) and is predicted to be exposed to

the surface available for interaction with SUR1 in the Kir6.2 tetramer homology model (Fig. 1B). These studies highlight the importance of TMD0 and the nearby SUR1-Kir6.2 interface close to the plasma membrane in regulating channel gating.^{21–23} Interestingly, many mutations in TMD0 of SUR1 cause channel biogenesis defects, resulting in loss of channel expression at the cell surface and the disease congenital hyperinsulinism.^{24,25} One hypothesis is that these mutations disrupt the conformation of TMD0-SUR1 necessary for interaction with Kir6.2 during channel biogenesis. In this work, we tested whether the aforementioned engineered SUR1-Kir6.2 interaction could overcome channel biogenesis and trafficking defects caused by TMD0 mutations.

Results and Discussion

To test if the interaction between E203K-SUR1 and Q52E-Kir6.2 affects the biogenesis of channels with previously identified SUR1-TMD0 trafficking mutations,^{25,26} we placed several such mutations on the E203K//Q52E background and assessed channel processing efficiency by western blots. SUR1 in the assembled channel complex undergoes complex glycosylation in the Golgi to yield a higher molecular weight band distinguishable from the core-glycosylated SUR1 found in the ER. Of the three TMD0 mutations tested, F27S and A116P showed a clear upper band in addition to the lower immature band in the E203K//Q52E background; by contrast, the same trafficking mutations placed in the background without the E203K//Q52E mutations only exhibited the lower band (Fig. 2), indicating the proteins were retained in the ER as reported previously.^{25,26} Another TMD0 mutation, E128K, as well as three other previously identified, congenital hyperinsulinism-causing SUR1 trafficking mutations outside of TMD0 (R495Q, F686S and L1350Q),²⁵ however, showed no improvement in their processing efficiency when combined with E203K//Q52E (data not shown). These results led us to conclude that the E203K//Q52E mutations can overcome the folding and trafficking defects caused by some but not all TMD0 mutations.

E203K//Q52E has been found to increase channel sensitivity to ATP inhibition by nearly 100-fold in our recent study.²¹ The markedly increased ATP sensitivity is likely due to close electrostatic interactions between the two oppositely charged mutant residues. Mutation of Q52E-Kir6.2 alone only increased ATP-sensitivity by ~5-fold whereas E203K-SUR1 did not change ATP-sensitivity significantly. Close physical proximity of the two residues is further supported by the observation that in inside-out patch-clamp recording of E203C-SUR1//Q52C-Kir6.2 channels, application of the oxidizing reagent H₂O₂ to induce disulfide bond formation locked the channels in a closed state that was reversible by the reducing agent dithiothreitol.²¹ Given this, we considered the possibility that cross-linking of E203C//Q52C may rescue the folding/assembly defect caused by F27S- or A116P-SUR1 by stabilizing the mutant SUR1-Kir6.2 interface at this location. We attempted to test this hypothesis by treating cells expressing F27S/E203C//Q52C with H₂O₂. While we were able to observe a crosslinked SUR1-Kir6.2 species on immunoblots within 10 min of H₂O₂ exposure, no significant correction of the F27S processing defect was detected even after 30 min or overnight H₂O₂ exposure (data not shown). These results could indicate that forced interaction between the two residues at this subunit interface is insufficient to overcome the channel biogenesis defect. However, the negative results could also be explained by experimental parameters such as incomplete crosslinking or oxidative stress caused by H₂O₂ exposure.

Next, we tested the role of individual E203K-SUR1 or Q52E-Kir6.2 mutations in F27S-SUR1 mutant processing. Surprisingly, while the E203K-SUR1 mutation had little effect on F27S-SUR1 processing, co-expression of F27S-SUR1 with Q52E-Kir6.2 was sufficient to increase the upper F27S-SUR1 band, and surface F27S-SUR1 detected by surface biotinylation was nearly as abundant as F27S/E203K//Q52E (Fig. 3A). Moreover, we found that while Q52D-Kir6.2 similarly improved the processing and surface expression of F27S-SUR1, Q52K-Kir6.2 did not (Fig. 3B). Similar observations were made for the A116P mutation (data

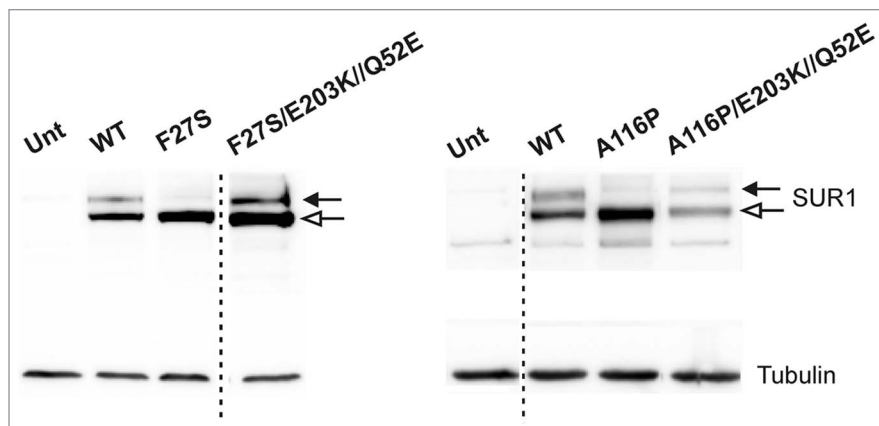


Figure 2. The E203K//Q52E mutation pair suppresses the processing defect caused by the F27S or A116P SUR1 mutation. Western blots of SUR1 protein in COSm6 cells transfected with cDNA encoding WT or mutant channels. The complex glycosylated form of SUR1 is indicated by the solid arrow and the core-glycosylated SUR1 is indicated by the open arrow. Tubulin was detected for each sample as a loading control. Dashed lines indicate spliced lanes from the same blot.

not shown). Note in the case of Q52K-Kir6.2, the pairing with E203 residue in SUR1 would represent a reverse-switch of charge at the two positions in relation to the E203K//Q52E mutation pair, and yet unlike E203K//Q52E, E203//Q52K failed to correct the trafficking defect caused by F27S and A116P. These results suggest that correction of the trafficking defects of F27S and A116P in the E203K//Q52E background is unlikely a consequence of electrostatic interactions between amino acids at the 203-SUR1 and 52-Kir6.2 positions, and that a negatively charged amino acid at position 52 of Kir6.2 is the major driving factor for expression rescue.

Little is known about how SUR1 and Kir6.2 interact with one another during subunit translation and assembly to ensure formation of a stable, functional channel complex. Our study identifies several mutations, including E203K//Q52E, Q52E-Kir6.2 and Q52D-Kir6.2, that can significantly improve the processing and surface expression of channels harboring specific TMD0 mutations. To our knowledge, this is the first report of such trafficking defect “suppressor” mutations in K_{ATP} channels. Analysis of the contribution from individual mutations revealed that the processing defect caused by F27S-SUR1 is little affected by E203K-SUR1 but is significantly alleviated by the Q52E and D mutations in Kir6.2 alone. In fact, the Q52E- or Q52D-Kir6.2 mutations alone were nearly as effective as the

E203K//Q52E mutation pair in correcting the processing defect of F27S and A116P. This scenario differs somewhat from that observed for gating regulation whereby E203K-SUR1 does not affect channel ATP-sensitivity and Q52E-Kir6.2 increases ATP-sensitivity by ~5-fold but E203K//Q52E increases ATP-sensitivity by ~100-fold.²¹ Moreover, while crosslinking of E203C//Q52C induces channel closure²¹ it does not appear to rescue the trafficking defect caused by F27S, at least under the experimental conditions we have tested. Together these observations argue that the electrostatic interactions between E203K//Q52E or crosslinking between E203C//Q52C needed to observe a profound change in gating are not required for the trafficking defect rescue.

That mutation in Kir6.2 can override the trafficking defect caused by a SUR1-TMD0 mutation is remarkable and provides important insight into the channel assembly process. It suggests that interactions between SUR1 and Kir6.2 may occur early in the channel biogenesis process, perhaps co-translationally while both subunits are being folded, before mutant SUR1 is deemed incapable of reaching a correctly folded state and targeted for degradation. Although the precise mechanism underlying our findings remains to be determined, the charge-dependence of the effect of Q52-Kir6.2 mutation on F27S-SUR1 processing leads us to speculate that the negative charge at this position may

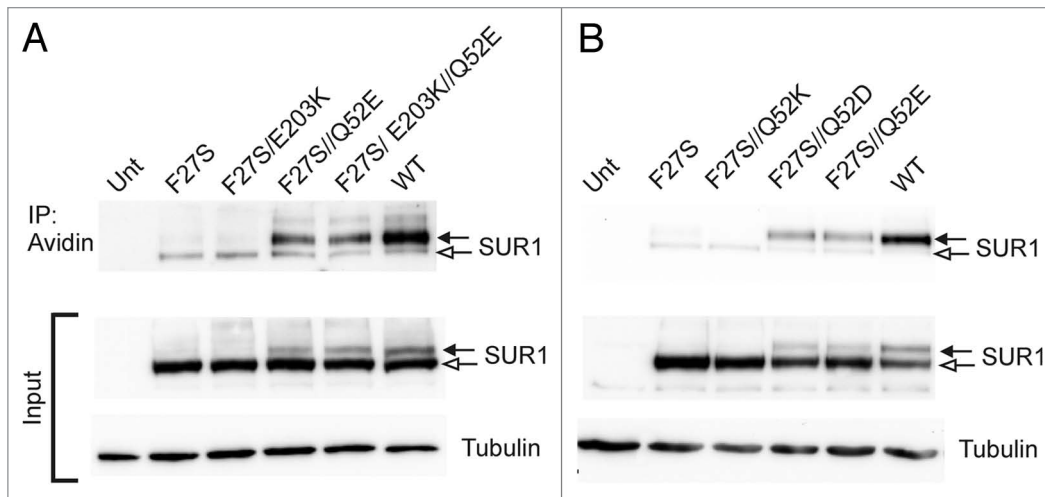


Figure 3. Substituting Q52 of Kir6.2 with negatively charged amino acids rescues surface expression of trafficking-impaired SUR1 mutant F27S. Surface SUR1 was detected using surface protein biotinylation followed by immunoprecipitation and immunoblotting. COSm6 cells were transiently transfected with cDNA for WT or mutant SUR1 and WT or mutant Kir6.2, then subjected to surface biotinylation. **(A)** The role of E203K-SUR1 or Q52E-Kir6.2 in F27S-SUR1 rescue was compared. **(B)** The rescue effect on F27S-SUR1 is dependent on the negative charge of the substituting amino acid at the Q52 position of Kir6.2. The upper complex-glycosylated band (indicated by solid arrow) was pulled down by the NeutrAvidin beads (top panel). Total SUR1 detected in whole-cell lysate in the corresponding samples are also shown with tubulin as loading controls (middle and lower panels). IP, immunoprecipitation. Note a small amount of lower SUR1 band was detected in the surface biotinylation blot either due to non-specific binding to the NeutrAvidin beads or due to penetrance of the biotinylation agent into unhealthy cells during surface biotinylation.

stabilize or facilitate membrane integration of the mutant TMD0 and/or protect it from being recognized and targeted for degradation. Our findings also raise an interesting possibility that the severity of K_{ATP} channel trafficking defects caused by certain SUR1 mutations identified in congenital hyperinsulinism could be modified by mutations or polymorphisms in Kir6.2 to affect disease presentation. In summary, our studies identify a SUR1-Kir6.2 interface near the plasma membrane that is important for determining the gating as well as folding and assembly of the channel complex.

Materials and Methods

Molecular biology. Rat Kir6.2 cDNA and hamster SUR1 cDNA constructs were in pCDNA1/Amp and pECE plasmids, respectively. Site-directed mutagenesis was performed using the QuikChange kit from Stratagene, and mutations were confirmed by direct sequencing as described in reference 21.

Immunoblotting and surface biotinylation. COSm6 cells were transfected with SUR1 and Kir6.2 cDNA using FuGene6. Cells were lysed 48–72 h post-transfection in a lysis buffer containing 50 mM

Tris-HCl, pH 7.0, 150 mM NaCl and 1% TritonX-100, with Complete^{TR} protease inhibitors. Cell lysates were run on SDS-PAGE, exposed to anti-SUR1 sera and visualized by enhanced chemiluminescence as described previously.²³ For surface biotinylation, cells were placed on ice and incubated in 1 mg/ml EZ-Link Sulfo-NHS-SS-Biotin (Pierce) in DPBS (Thermo Scientific) for 30 min. Cells were lysed immediately in the lysis buffer and biotinylated proteins were pulled down by incubation with NeutrAvidin-agarose beads (Pierce) and processed for immunoblotting.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

- Inagaki N, Gonoi T, Clement JP 4th, Namba N, Inazawa J, Gonzalez G, et al. Reconstitution of IKATP: an inward rectifier subunit plus the sulfonylurea receptor. *Science* 1995; 270:1166-70; PMID:7502040; <http://dx.doi.org/10.1126/science.270.5239.1166>
- Aguilar-Bryan L, Bryan J. Molecular biology of adenosine triphosphate-sensitive potassium channels. *Endocr Rev* 1999; 20:101-35; PMID:10204114; <http://dx.doi.org/10.1210/er.20.2.101>
- Ashcroft FM. ATP-sensitive potassium channelopathies: focus on insulin secretion. *J Clin Invest* 2005; 115:2047-58; PMID:16075046; <http://dx.doi.org/10.1172/JCI25495>
- Nichols CG. KATP channels as molecular sensors of cellular metabolism. *Nature* 2006; 440:470-6; PMID:16554807; <http://dx.doi.org/10.1038/nature04711>
- Aguilar-Bryan L, Nichols CG, Wechsler SW, Clement JP 4th, Boyd AE 3rd, González G, et al. Cloning of the beta cell high-affinity sulfonylurea receptor: a regulator of insulin secretion. *Science* 1995; 268:423-6; PMID:7716547; <http://dx.doi.org/10.1126/science.7716547>
- Zerangue N, Schwappach B, Jan YN, Jan LY. A new ER trafficking signal regulates the subunit stoichiometry of plasma membrane K(ATP) channels. *Neuron* 1999; 22:537-48; PMID:10197533; [http://dx.doi.org/10.1016/S0896-6273\(00\)80708-4](http://dx.doi.org/10.1016/S0896-6273(00)80708-4)
- Shyng S, Ferrigni T, Nichols CG. Control of rectification and gating of cloned KATP channels by the Kir6.2 subunit. *J Gen Physiol* 1997; 110:141-53; PMID:9236207; <http://dx.doi.org/10.1085/jgp.110.2.141>
- Tucker SJ, Gribble FM, Zhao C, Trapp S, Ashcroft FM. Truncation of Kir6.2 produces ATP-sensitive K⁺ channels in the absence of the sulphonylurea receptor. *Nature* 1997; 387:179-83; PMID:9144288; <http://dx.doi.org/10.1038/387179a0>
- Gribble FM, Tucker SJ, Ashcroft FM. The essential role of the Walker A motifs of SUR1 in K-ATP channel activation by Mg-ADP and diazoxide. *EMBO J* 1997; 16:1145-52; PMID:9135131; <http://dx.doi.org/10.1093/emboj/16.6.1145>
- Gribble FM, Tucker SJ, Haug T, Ashcroft FM. MgATP activates the beta cell KATP channel by interaction with its SUR1 subunit. *Proc Natl Acad Sci U S A* 1998; 95:7185-90; PMID:9618560; <http://dx.doi.org/10.1073/pnas.95.12.7185>

11. Shyng S, Ferrigni T, Nichols CG. Regulation of KATP channel activity by diazoxide and MgADP. Distinct functions of the two nucleotide binding folds of the sulfonylurea receptor. *J Gen Physiol* 1997; 110:643-54; PMID:9382893; <http://dx.doi.org/10.1085/jgp.110.6.643>
12. Babenko AP, Bryan J. Sur domains that associate with and gate KATP pores define a novel gatekeeper. *J Biol Chem* 2003; 278:41577-80; PMID:12941953; <http://dx.doi.org/10.1074/jbc.C300363200>
13. Babenko AP, Gonzalez G, Aguilar-Bryan L, Bryan J. Sulfonylurea receptors set the maximal open probability, ATP sensitivity and plasma membrane density of KATP channels. *FEBS Lett* 1999; 445:131-6; PMID:10069387; [http://dx.doi.org/10.1016/S0014-5793\(99\)00102-7](http://dx.doi.org/10.1016/S0014-5793(99)00102-7)
14. Chan KW, Zhang H, Logothetis DE. N-terminal transmembrane domain of the SUR controls trafficking and gating of Kir6 channel subunits. *EMBO J* 2003; 22:3833-43; PMID:12881418; <http://dx.doi.org/10.1093/emboj/cdg376>
15. Schwappach B, Zerangue N, Jan YN, Jan LY. Molecular basis for K(ATP) assembly: transmembrane interactions mediate association of a K⁺ channel with an ABC transporter. *Neuron* 2000; 26:155-67; PMID:10798400; [http://dx.doi.org/10.1016/S0896-6273\(00\)81146-0](http://dx.doi.org/10.1016/S0896-6273(00)81146-0)
16. Tao X, Avalos JL, Chen J, MacKinnon R. Crystal structure of the eukaryotic strong inward-rectifier K⁺ channel Kir2.2 at 3.1 Å resolution. *Science* 2009; 326:1668-74; PMID:20019282; <http://dx.doi.org/10.1126/science.1180310>
17. Babenko AP, Bryan J. SUR-dependent modulation of KATP channels by an N-terminal KIR6.2 peptide. Defining intersubunit gating interactions. *J Biol Chem* 2002; 277:43997-4004; PMID:12213829; <http://dx.doi.org/10.1074/jbc.M208085200>
18. Babenko AP, Gonzalez G, Bryan J. The N-terminus of KIR6.2 limits spontaneous bursting and modulates the ATP-inhibition of KATP channels. *Biochem Biophys Res Commun* 1999; 255:231-8; PMID:10049691; <http://dx.doi.org/10.1006/bbrc.1999.0172>
19. Koster JC, Sha Q, Shyng S, Nichols CG. ATP inhibition of KATP channels: control of nucleotide sensitivity by the N-terminal domain of the Kir6.2 subunit. *J Physiol* 1999; 515:19-30; PMID:9925874; <http://dx.doi.org/10.1111/j.1469-7793.1999.019ad.x>
20. Reimann F, Tucker SJ, Proks P, Ashcroft FM. Involvement of the n-terminus of Kir6.2 in coupling to the sulphonylurea receptor. *J Physiol* 1999; 518:325-36; PMID:10381582; <http://dx.doi.org/10.1111/j.1469-7793.1999.0325p.x>
21. Pratt EB, Zhou Q, Gay JW, Shyng SL. Engineered interaction between SUR1 and Kir6.2 that enhances ATP sensitivity in KATP channels. *J Gen Physiol* 2012; 140:175-87; PMID:22802363; <http://dx.doi.org/10.1085/jgp.201210803>
22. Pratt EB, Shyng SL. ATP activates ATP-sensitive potassium channels composed of mutant sulfonylurea receptor 1 and Kir6.2 with diminished PIP2 sensitivity. *Channels (Austin)* 2011; 5:314-9; PMID:21654216; <http://dx.doi.org/10.4161/chan.5.4.16510>
23. Pratt EB, Tewson P, Bruederle CE, Skach WR, Shyng SL. N-terminal transmembrane domain of SUR1 controls gating of Kir6.2 by modulating channel sensitivity to PIP2. *J Gen Physiol* 2011; 137:299-314; PMID:21321069; <http://dx.doi.org/10.1085/jgp.201010557>
24. Pratt EB, Yan FF, Gay JW, Stanley CA, Shyng SL. Sulfonylurea receptor 1 mutations that cause opposite insulin secretion defects with chemical chaperone exposure. *J Biol Chem* 2009; 284:7951-9; PMID:19151370; <http://dx.doi.org/10.1074/jbc.M807012200>
25. Yan FF, Lin YW, MacMullen C, Ganguly A, Stanley CA, Shyng SL. Congenital hyperinsulinism associated ABCC8 mutations that cause defective trafficking of ATP-sensitive K⁺ channels: identification and rescue. *Diabetes* 2007; 56:2339-48; PMID:17575084; <http://dx.doi.org/10.2337/db07-0150>
26. Yan F, Lin CW, Weisiger E, Cartier EA, Taschenberger G, Shyng SL. Sulfonylureas correct trafficking defects of ATP-sensitive potassium channels caused by mutations in the sulfonylurea receptor. *J Biol Chem* 2004; 279:11096-105; PMID:14707124; <http://dx.doi.org/10.1074/jbc.M312810200>