Molecular basis for differential modulation of BK channel voltage-dependent gating by auxiliary γ subunits

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Large conductance Ca2+- and voltage-activated potassium (BK) channels are comprised of pore-forming α subunits and various regulatory auxiliary subunits. The BK channel auxiliary γ (BKγ) subunits are a newly identified class of proteins containing an extracellular leucine-rich repeat domain (LRRD), a single transmembrane (TM) segment, and a short cytoplasmic C-terminal tail (C-tail). Although each of the four BKγ proteins shifts the voltage dependence of BK channel activation in a hyperpolarizing direction, they show markedly different efficacies, mediating shifts over a range of 15–145 mV. Analyses of chimeric BKγ subunits created by swapping individual structural elements, and of BKγ deletion and substitution mutants, revealed that differential modulation of BK gating by the four BKγ subunits depends on a small region consisting of the TM segment and the adjacent intracellular cluster of positively charged amino acids. The γ1 and γ2 TM segments contributed approximately −100 mV, and the γ1 and γ3 C-tails contributed approximately −40 mV, to shifting the voltage dependence of BK channel activation, whereas the γ3 and γ4 TM segments and the γ2 and γ4 C-tails contributed much less. The large extracellular LRRDs were mainly functionally interchangeable, although the γ1 LRRD was slightly less effective at enhancing (or slightly more effective at attenuating) the shift in BK channel voltage-dependent gating toward hyperpolarizing potentials than those of the other BKγ subunits. Analysis of mutated BKγ subunits revealed that juxta-membrane clusters of positively charged amino acids determine the functions of the γ1 and γ3 C-tails. Therefore, the modulatory functions of BKγ subunits are coarse- and fine-tuned, respectively, through variations in their TM segments and in the adjacent intracellular positively charged regions. Our results suggest that BK channel modulation by auxiliary γ subunits depends on intra- and/or juxta-membrane mechanisms.

INTRODUCTION

The large conductance Ca2+- and voltage-activated potassium (BK) channel is widely expressed and plays important roles in many physiological processes, including neuronal firing and neurotransmitter release (Gribkoff et al., 2001), and frequency tuning of auditory hair cells (Ramanathan et al., 1999). The BK channel features exceptionally large single-channel conductance and dual voltage- and calcium-sensing properties. The BK channel α subunits are a group of homotetrameric pore-forming voltage- and calcium-sensing α subunits (BKα) alone or in association with regulatory tissue-specific auxiliary β or γ subunits. The newly identified BK channel auxiliary γ (BKγ) subunits are a group of leucine-rich repeat (LRR)-containing membrane proteins, called γ1 (LRRC26), γ2 (LRRC52), γ3 (LRRC55), and γ4 (LRRC38) (Yan and Aldrich, 2010, 2012). The four BKγ subunits have different tissue-specific mRNA expression and may broadly modulate BK channels in different tissues, including brain, which expresses the γ1 and γ3 mRNAs (Yan and Aldrich, 2012). The as yet limited studies on the mechanisms and physiological functions of BKγ subunits have mainly focused on γ1, which has been reported to regulate BK channels in prostate cancer (Gessner et al., 2006; Yan and Aldrich, 2010), salivary gland cells (Almassy and Begenisich, 2012), and also likely in airway epithelial cells (Manzanares et al., 2014) and arterial smooth muscle cells (Evanston et al., 2014). The mouse γ2 subunit was found to function as an accessory subunit of the sperm-specific mouse Slo3 channels (Yang et al., 2011). The BKγ subunits are structurally unrelated to the double-membrane–spanning BK channel β subunits. The four BKγ subunits are similar to each other in terms of their overall protein sequences (Fig. 1), and they all contain an N-terminal signal peptide, a relatively large
extracellular LRR domain (LRRD), a single transmembrane (TM) segment, and a short intracellular C-terminal tail (C-tail) (Figs. 1 and 2). Unlike the complex effects and mechanisms of different BK channel β (BKβ) subunits on many aspects of BK channel gating (Wallner et al., 1999; Brenner et al., 2000; Meera et al., 2000; Xia et al., 2000; Zeng et al., 2003; Savalli et al., 2007; Contreras et al., 2012; Sun et al., 2012), the action of the γ1 subunit is remarkable in its mechanistic simplicity and modulatory magnitude (Yan and Aldrich, 2010; Zhang and Yan, 2014). An analysis of the γ1 subunit’s effects on different BK channel gating parameters within the framework of an allosteric Horrigan and Aldrich (HA) model (Horrigan and Aldrich, 2002) suggested that this subunit modulates the BK channel mainly by an ~20-fold enhancement in the allosteric coupling factor between voltage sensors and the channel pore (Yan and Aldrich, 2010). The γ1 subunit also exhibited an “all-or-none” regulatory effect on BK channels independent of the molar ratio of injected BKα/γ1 RNA in Xenopus laevis oocytes (Gonzalez-Perez et al., 2014). This action is fundamentally different from that of BKβ subunits, which regulate the voltage dependence of BK channel activation in a titration-dependent mode (Wang et al., 2002).

In spite of the above interesting structural and functional features of the BKγ subunits, the molecular basis and mechanisms underlying BK channel regulation by BKγ subunits are largely unknown. The four BKγ subunits have distinct capabilities in shifting the voltage dependence of BK channel activation toward hyperpolarizing voltages by ~140 mV (γ1), 100 mV (γ2), 50 mV (γ3), and 20 mV (γ4) in the absence of calcium (Yan and Aldrich, 2010, 2012). Identification of key structural elements underlying BKγ subunits’ different modulatory effects on BK channels could provide important insight into the molecular basis and mechanisms underlying BK channel regulation by BKγ subunits. In this study, we generated chimeric BKγ proteins by swapping individual structural elements among the four BKγ subunits. Together with deletion and point mutations, we systematically analyzed the functional contributions of different structural elements to the BKγ subunits’ modulatory effects on BK channel voltage-dependent gating. We found that the differential modulatory functions of BKγ subunits are mainly determined by a small region consisting of the TM segment and its adjacent intracellular cluster of positively charged residues. Our results suggest involvement of major intra- and/or juxtamembrane mechanisms in the auxiliary γ subunits’ activating effects on BK channels.

**MATERIALS AND METHODS**

**Expression of BKα and BKγ proteins in human embryonic kidney (HEK)-293 cells**

Recombinant cDNA constructs of human BKα (hSlo), γ1 (LRRC26), γ2 (LRRC32), γ3 (LRRC55), and γ4 (LRRC38) subunits were used for heterologous expression studies in HEK-293 cells. To divide extracellular LRR domain (LRRD), a single transmembrane (TM) segment, and a short intracellular C-terminal tail (C-tail) (Figs. 1 and 2).

![Figure 1](image_url) - Protein sequence alignment of BKγ subunits in humans. The corresponding amino acid sequences for different domains or segments are assigned and used for the generation of chimeric proteins. Cluster (++) refers to the cluster of positively charged amino acids. The key residues of the consensus sequence (LxxLxLxxN) in each LRR unit are marked by black squares below. Four cysteine pairs for potential disulfide formation are indicated. The two residues in γ3 used for point mutation analyses, D269 and K304, are outlined in blue.
each BKv subunit into four structural elements for generation of chimeric BKv proteins, we defined the N-terminal sequence as the N-terminal sequence before the first conserved disulfide-forming Cys residue of the LRRNT unit, the TM segment as a relatively hydrophilic region of 29-aa residues flanked by charged amino acid residues, LRRD as the sequence located between N-terminal peptide and the TM segment, and C-tail as the sequence on the C-terminal side of the TM segment (Fig. 1). Synthetic cDNA sequences of chimeric BKv subunits were subcloned into the mammalian expression vector of pCDNA6, with V5 tags attached at their C termini. As described previously (Yan and Aldrich, 2010, 2012), fusion cDNA constructs, which encode precursor fusion proteins of human BKα and C-terminal–tagged BKv proteins, were generated with the pCDNA6 vector and used to facilitate the co-translational assembly of BKα–BKv protein complexes after endogenous cleavage by peptidases at the linker (signal peptide) region in the mature proteins. This previously established co-translational assembly strategy produced comparably reproducible results as the other strategies that generated over- or super-expression of BKα relative to BKv in a single HEK-293 cell using either an IRES (internal ribosome entry site)-based single bicistronic expression method or BKv-stable cell line method (Yan and Aldrich, 2010, 2012). HEK-293 cells were obtained from ATCC, transfected with plasmids using Lipofectamine 2000 (Invitrogen), and subjected to electrophysiological assays 16–72 h after transfection.

Electrophysiology

To record the BK channel currents, we used patch-clamp recording in excised inside-out patches of HEK-293 cells with symmetric internal and external solutions of 136 mM KMeSO3, 4 mM KCl, and 20 mM HEPES, pH 7.20. The external solution was supplemented with 2 mM MgCl2, and the internal solution was supplemented with 5 mM HEDTA without Ca2+ to create a virtually Ca2+-free solution. To record the BK channel currents, we used patch-clamp recording in excised inside-out patches of HEK-293 cells with symmetric internal and external solutions of 136 mM KMeSO3, 4 mM KCl, and 20 mM HEPES, pH 7.20. The external solution was supplemented with 2 mM MgCl2, and the internal solution was supplemented with 5 mM HEDTA without Ca2+ to create a virtually Ca2+-free solution. Steady-state activation was expressed as the normalized conductance (G/Gmax) calculated from the relative amplitude of the tail currents (deactivation at −120 mV). The voltage of half-maximal activation (V1/2) and the equivalent gating charge (z) were obtained by fitting the relations of G/Gmax versus voltage with the single-Boltzmann function G/Gmax = 1/(1 + e^(z(V1/2/V−V))}).

Table 1

Boltzmann-fit parameters of the voltage-dependent BK channel activation in the presence of auxiliary BKv wild types, chimeras, and mutants

| Expression | Boltzmann fit parameters |
|------------|--------------------------|
|            | mV | z | n |
| BKα alone  | 167 ± 2 | 1.26 ± 0.07 | 8 |
| +γ1        | 22 ± 4 | 1.75 ± 0.09 | 7 |
| +γ2        | 61 ± 3 | 1.17 ± 0.07 | 8 |
| +γ3        | 115 ± 2 | 1.36 ± 0.05 | 6 |
| +γ4        | 154 ± 3 | 1.27 ± 0.07 | 7 |
| +γ1/γ4-signal | 15 ± 3 | 1.49 ± 0.06 | 4 |
| +γ3/γ1-signal | 121 ± 5 | 1.37 ± 0.06 | 3 |
| +γ4/γ1-signal | 155 ± 1 | 1.32 ± 0.03 | 4 |
| +γ1/γ2-LRRD | −3 ± 3 | 1.38 ± 0.04 | 6 |
| +γ1/γ5-LRRD | 6 ± 3 | 1.52 ± 0.09 | 8 |
| +γ1/γ4-LRRD | −12 ± 3 | 1.66 ± 0.05 | 11 |
| +γ1/γ2-TM&tail | 90 ± 2 | 1.28 ± 0.15 | 4 |
| +γ1/γ3-TM&tail | 151 ± 3 | 1.48 ± 0.07 | 6 |
| +γ1/γ2-tail | 143 ± 3 | 1.26 ± 0.05 | 4 |
| +γ1/γ3-tail | 120 (25%) | 1.06 | 3 |
| +γ1/γ4-tail | 155 ± 4 | 1.40 ± 0.07 | 3 |
| +γ2/γ1-TM | 17 ± 2 | 1.86 ± 0.10 | 3 |
| +γ2/γ5-tail | 21 ± 2 | 1.67 ± 0.12 | 4 |
| +γ2/γ4-tail | 68 ± 2 | 1.12 ± 0.08 | 5 |
| +γ3/γ1-tail | 120 ± 2 | 1.81 ± 0.20 | 3 |
| +γ3/γ2-tail | 141 ± 5 | 1.66 ± 0.20 | 3 |
| +γ3/γ4-tail | 156 ± 4 | 1.40 ± 0.07 | 3 |
| +γ4/γ1-tail | 32 ± 3 | 1.40 ± 0.07 | 5 |
| +γ1/γ2-TM | 135 ± 2 | 1.24 ± 0.02 | 3 |
| +γ1/γ4-TM | 155 ± 4 | 1.30 ± 0.03 | 5 |
| +γ2/γ1-TM | 155 ± 4 | 1.33 ± 0.09 | 6 |
| +γ2/γ3-TM | 155 ± 5 | 1.54 ± 0.07 | 4 |
| +γ2/γ4-TM | 158 ± 7 | 1.46 ± 0.10 | 4 |
| +γ3/γ1-TM | 2 ± 5 | 1.14 ± 0.05 | 5 |
| +γ3/γ2-TM | 119 | 1.11 | 1 |
| +γ3/γ4-TM | 138 ± 6 (36%) | 1.12 ± 0.10 | 7 |
| +γ2/γ3-TM | 62 ± 3 (64%) | 1.11 ± 0.05 | 5 |
| +γ3/γ5-TM | 117 ± 3 | 1.56 ± 0.11 | 5 |
| +γ3/γ2-TM | 26 ± 2 | 1.44 ± 0.06 | 5 |
| +γ3/γ4-TM | 160 ± 4 | 1.38 ± 0.06 | 4 |
| +γ4/γ2-TM | 115 ± 4 | 1.15 ± 0.11 | 4 |
| +γ4/γ3-TM | 72 ± 3 | 1.27 ± 0.06 | 5 |
| +γ4/γ4-TM | 29 ± 4 | 1.40 ± 0.09 | 4 |
| +γ1/ΔtailC280–313 | 168 ± 3 | 1.22 ± 0.11 | 5 |
| +γ2/ΔtailC280–313 | 73 ± 3 | 1.12 ± 0.04 | 7 |
| +γ3/ΔtailC280–313 | 63 ± 2 | 1.28 ± 0.09 | 4 |
| +γ3/ΔtailN291–321 | 166 ± 6 | 1.16 ± 0.01 | 5 |
| +γ5/ΔtailN291–321 | 109 ± 3 | 1.56 ± 0.12 | 4 |
| +γ3/ΔtailN324–351 | 180 ± 3 | 1.26 ± 0.12 | 5 |
| +γ5/ΔtailN324–351 | 144 ± 2 | 1.54 ± 0.12 | 4 |

n values are the number of recorded excised inside-out patches from different HEK-293 cells.

*The indicated percentage in parentheses refers to the portion of the channels’ subpopulation that was obtained from a double-Boltzmann function fit.
or with the double-Boltzmann function $G/G_{\text{max}} = a/(1 + e^{-\frac{V-V_{1/2}}{z_1}R T}) + (1-a)/(1 + e^{-\frac{V-V_{1/2}}{z_2}R T})$. Experimental values are reported as the mean ± the SEM.

**RESULTS**

Differential modulation of BK channel voltage-dependent gating by the BKγ subunits

We observed that the four BKγ subunits, when heterologously expressed in HEK-293 cells, produced distinct shifts toward hyperpolarizing potentials in the voltage dependence of BK channel activation (Fig. 3, A and B), which is similar to the findings of a previous report (Yan and Aldrich, 2012). The half-activation voltages ($V_{1/2}$) of BK channels were shifted by 145 ± 5 mV by γ1 ($V_{1/2} = 22 ± 4$), 107 ± 4 mV by γ2 ($V_{1/2} = 61 ± 3$ mV), 52 ± 3 mV by γ3 ($V_{1/2} = 115 ± 2$ mV), and 13 ± 4 mV by γ4 ($V_{1/2} = 154 ± 3$ mV), compared with the BKα channel alone ($V_{1/2} = 167 ± 2$ mV) in the virtual absence of [Ca$^{2+}$]. The equivalent gating charge ($z$) of BK channel voltage gating was largely unaffected by the γ2 ($z = 1.17 ± 0.07$ e), γ3 ($z = 1.36 ± 0.05$ e), and γ4 ($z = 1.27 ± 0.07$ e) subunits but markedly increased by the γ1 subunit ($z = 1.75 ± 0.09$ e), compared with the BKα channel alone ($z = 1.26 ± 0.07$ e) in the virtual absence of [Ca$^{2+}$]. For comparison, the Boltzmann fit parameters of the voltage-dependent BK channel activation in the presence of auxiliary BKγ wild types, as well as all chimeras and mutants discussed below, are given in Table 1.

We first determined whether the BKγ subunits’ distinct capabilities in modulating BK channel voltage-dependent gating are intrinsic properties of their mature proteins or are caused by differences in their signal peptide functions. We generated chimeric BKγ proteins of the γ3/γ1-signal and γ4/γ1-signal by replacing the N-terminal signal peptide sequences in γ3 and γ4 with that of γ1. Similarly, we generated chimeric BKγ proteins of the γ1/γ3-signal by using the γ4 N-terminal signal peptide sequence to guide the expression of γ1 protein. We observed that swapping the N-terminal signal peptide regions, which elicited changes of <10 mV in the BK channel
LRRD does not determine the modulatory functions of different BKγ subunits

The relatively large LRRD comprises six tandem LRR structural units (LRR1–6) and two cysteine-rich modules, LRRNT and LRRCT, which are capped on the N- and C-terminal sides, respectively (Fig. 1). We found that substituting the LRRD of γ1 with those of the other three BKγ subunits resulted in no reduction but instead slight to moderate gain-of-function in the γ1 subunit’s modulatory effect on BK channel voltage-dependent gating (Fig. 3, A and D). Compared with the unaltered γ1 protein ($V_{1/2} = 22 \pm 4$ mV), the resultant LRRD-swapping chimeras of $\gamma_1/\gamma_2$-LRRD ($V_{1/2} = -3 \pm 3$ mV), $\gamma_1/\gamma_3$-LRRD ($V_{1/2} = 6 \pm 3$ mV), and $\gamma_1/\gamma_4$-LRRD ($V_{1/2} = -12 \pm 3$ mV) caused further shifts of 25, 16, and 34 mV, respectively, in the voltage dependence of BK channel activation toward hyperpolarizing potentials (Fig. 3 D). Because the other BKγ subunits are weaker modulators than γ1 in terms of shifting the voltage dependence of BK channel activation, this result contradicts what one would expect if the LRRD were a determinant of the subunits’ different modulatory functions. In contrast, replacing the TM and C-tail regions of γ1 with those of γ2 or γ3 caused a 47% reduction ($V_{1/2} = 90 \pm 2$ mV with $\gamma_1/\gamma_2$-TM&tail) or a 89% loss ($V_{1/2} = 151 \pm 3$ mV with $\gamma_1/\gamma_3$-TM&tail) of the γ1 subunit’s modulatory function (Fig. 3 E), which suggests that the TM and C-tail regions determine the BKγ subunits’ modulatory functions. The modulatory effects of the $\gamma_1/\gamma_2$-TM&tail and $\gamma_1/\gamma_3$-TM&tail chimeras were even smaller than those of the unaltered γ2 and γ3 subunits, respectively. These results suggest that compared with the LRRDs of other BKγ subunits, the γ1 LRRD plays a slightly larger inhibitory or less stimulatory role in shifting the BK channel voltage-dependent gating toward hyperpolarizing potentials (Fig. 2).

C-tails partially contribute to the difference in modulatory functions among BKγ subunits

The short C-tails of the four BKγ subunits have different lengths and very low sequence similarities. According to our assignments of the C-terminal borders of the neighboring TM segments, the $\gamma_1$, $\gamma_2$, $\gamma_3$, and $\gamma_4$ C-tails have 44, 40, 12, and 18 amino acid residues, respectively (Fig. 1). Substituting the C-tail of $\gamma_2$ with those of the other three BKγ subunits produced fully functional chimeric proteins (Fig. 4 A). The modulatory effect of the $\gamma_2/\gamma_4$-tail chimera ($V_{1/2} = 68 \pm 2$ mV) was nearly the same as that of unaltered $\gamma_2$, which suggests that the $\gamma_4$ C-tail functions similarly as the $\gamma_2$ C-tail. In contrast, both the $\gamma_1$ and $\gamma_3$ C-tails potentiated the $\gamma_2$ acceptor subunit’s modulatory function by approximately −40-mV shifts in the BK channel $V_{1/2}$ ($17 \pm 2$ mV with $\gamma_2/\gamma_1$-tail and $21 \pm 2$ mV with $\gamma_2/\gamma_3$-tail), which became more like that produced by γ1. These modifications in $V_{1/2}$ were accompanied by an increase in the steepness of the GV
relationship curve (\( z = 1.86 \pm 0.1 \) e with \( \gamma^2/\gamma^1\)-tail and \( z = 1.67 \pm 0.12 \) e with \( \gamma^2/\gamma^3\)-tail, as compared with \( z = 1.17 \pm 0.07 \) e with \( \gamma^2 \)), which approached that of \( \gamma^1 \) (\( z = 1.75 \pm 0.09 \) e). These results suggest that the \( \gamma^1 \) and \( \gamma^3 \) C-tails are similarly effective, whereas the \( \gamma^2 \) and \( \gamma^4 \) C-tails are similarly less effective or ineffective, in their contributions to the BK\( _Y \) subunits’ modulatory functions.

Consistent with the above inference, the \( \gamma^1 \) C-tail did not cause a significant (i.e., \( \pm 25 \) mV in \( V_{1/2} \)) change in the \( \gamma^3 \) acceptor subunit's function (\( V_{1/2} = 120 \pm 2 \) mV with \( \gamma^3/\gamma^1\)-tail). In contrast, the \( \gamma^2 \) and \( \gamma^4 \) C-tails both markedly reduced the \( \gamma^3 \) acceptor subunit's modulatory function (\( V_{1/2} = 141 \pm 2 \) mV with \( \gamma^3/\gamma^2\)-tail and \( V_{1/2} = 156 \pm 4 \) mV with \( \gamma^3/\gamma^4\)-tail) to levels similar to that of the weaker \( \gamma^4 \) (\( V_{1/2} = 154 \pm 3 \) mV) (Fig. 4 B). The \( \pm 40\) mV difference between the pair of the \( \gamma^1 \) and \( \gamma^3 \) C-tails and the pair of the \( \gamma^2 \) and \( \gamma^4 \) C-tails in their influences on the \( \gamma^2 \) or \( \gamma^3 \) acceptor's modulatory functions agrees well with the differences between \( \gamma^1 \) and \( \gamma^2 \) and between \( \gamma^3 \) and \( \gamma^4 \) in their abilities to shift the voltage dependence of BK channel activation. These results indicate that although the C-tail region is not a major determinant of the overall large difference in modulatory functions across the four BK\( _Y \) subunits, it is very likely a key determinant of the functional differences between \( \gamma^1 \) and \( \gamma^2 \), and between \( \gamma^3 \) and \( \gamma^4 \).

\( \gamma^1 \) TM segment and other BK\( _Y \) subunits’ C-tails are incompatible

The above results with C-tail–swapped chimeric BK\( _Y \) proteins showed that the C-tails of \( \gamma^1 \) and \( \gamma^3 \) function similarly in the presence of the main bodies (i.e., the LRRDs and TM segments) of \( \gamma^2 \) and \( \gamma^3 \). However, upon replacement of the \( \gamma^1 \) C-tail with the \( \gamma^3 \) C-tail, the resultant \( \gamma^1/\gamma^3\)-tail chimera caused the G-V relationships of BK channels to be fitted to at least two populations of BK channels in all recorded 11 excised patches (Fig. 4 C). In three patches, the G-V relationships were best fitted to a major population (\( \sim 75\% \)) of low voltage–activated channels (\( V_{1/2} \) of \( \pm 25 \) mV), similar to those associated with the unaltered \( \gamma^1 \) subunit (BK\( _\alpha/\gamma^1 \)) and to a minor population (\( \sim 25\% \)) of high voltage–activated channels more similar to those of BK\( _{\alpha} \) alone. In the other eight patches, the G-V relationships were best fitted to a minor population (16\%) of low voltage–activated BK\( _\alpha/\gamma^1\)-type channels and to a major population (84\%) of high voltage–activated BK\( _\alpha\)-type channels. These two distinct G-V relationships for different membrane patches have been observed in the same batch of transfected cells. These results suggest that the \( \gamma^3 \) C-tail did function as the \( \gamma^1 \) C-tail in the \( \gamma^1/\gamma^3\)-tail chimera in about half of the recorded BK channels. However, in the other half of the recorded BK channels, the \( \gamma^3 \) C-tail caused a drastic reduction (72–88\%) in the \( \gamma^1 \)’s modulatory function. Moreover, both the \( \gamma^2 \) and \( \gamma^4 \) C-tails caused an \( \pm 85\% \) loss of the \( \gamma^1 \) acceptor subunit’s modulatory function (\( V_{1/2} = 143 \pm 3 \) mV with \( \gamma^1/\gamma^2\)-tail and \( V_{1/2} = 147 \pm 3 \) mV with \( \gamma^1/\gamma^4\)-tail) (Fig. 4 D). Given that the \( \gamma^1 \) C-tail in the presence of \( \gamma^2 \) and \( \gamma^3 \) TM segments produced only a limited contribution (i.e., \( \pm 40 \) mV) to the shift of the BK channel \( V_{1/2} \), the \( \gamma^1 \) C-tail is unlikely a sole determinant of \( \gamma^1 \)’s modulatory function. The drastic reduction in the \( \gamma^1 \) subunit’s modulatory function upon replacement of its C-tail with those of the other \( \gamma \) subunits was likely caused by some structural or functional incompatibility (i.e., antagonistic effect) between the other \( \gamma \) subunits’ C-tails and other regions of the \( \gamma^1 \) subunit.

Although both \( \gamma^1 \) and \( \gamma^2 \) modulate the BK channel very effectively, the \( \gamma^2/\gamma^1\)-TM chimera, which resulted from \( \gamma^1 \)’s TM segment being in the presence of \( \gamma^2 \)’s LRRD and C-tail, was ineffective (i.e., the resultant \( V_{1/2} \) is within \( \pm 15 \) mV of that produced by BK\( _\alpha \) alone) in modulating BK channel voltage gating (\( V_{1/2} = 155 \pm 4 \) mV) (Fig. 5 A), suggesting that incompatibility exists between the \( \gamma^1 \) TM segment and either the \( \gamma^2 \) LRRD or the \( \gamma^2 \) C-tail. Given that the \( \gamma^2 \) LRRD was fully compatible (i.e., no obvious antagonistic effect) with the rest of \( \gamma^1 \) in the \( \gamma^1/\gamma^2\)-LRRD chimera (Fig. 3 D) and that the \( \gamma^1/\gamma^2\)-tail chimera was only slightly effective (i.e., the resultant \( V_{1/2} \) is within \( \pm 5–30 \) mV of that produced by BK\( _\alpha \) alone) in modulating the BK channel (Fig. 4 D), this incompatibility must exist between the \( \gamma^1 \) TM segment and the \( \gamma^2 \) C-tail.

Unexpectedly, replacing the TM segment of \( \gamma^3 \) with that of \( \gamma^1 \) created a \( \gamma^3/\gamma^1\)-TM chimera that completely failed to potentiate \( \gamma^3 \)’s modulatory function and instead caused a loss of \( \gamma^3 \)’s modulatory function in most recorded excised patches (\( V_{1/2} = 165 \pm 5 \) mV, \( n = 5; V_{1/2} = 119 \) mV, \( n = 1 \)) (Fig. 5 A and Table 1). This outcome suggests that in addition to the partial incompatibility that exists between the \( \gamma^1 \) TM segment and the \( \gamma^3 \) C-tail, functional incompatibility exists between the \( \gamma^1 \) TM segment and the \( \gamma^3 \) LRRD. \( \gamma^3 \) has a negatively charged residue, D269, which in \( \gamma^1 \) is a positively charged residue, R260, located immediately on the N-terminal side of the TM segment (Fig. 1). We arbitrarily included these residues’ positions in the LRRDs to construct chimeric BK\( _Y \) proteins (Fig. 1). The D269R mutation itself had no effect on \( \gamma^3 \)’s modulatory function (Fig. 5 A), but the \( \gamma^1 \) TM segment became partially effective in potentiating the \( \gamma^3/\gamma^1\)-TM chimera that could be fitted with a double-Boltzmann function to a major population (64\%) of low voltage–activated channels (\( V_{1/2} = 62 \) mV, \( z = 1.1 \) e) and to a minor population (36\%) of high voltage–activated channels (\( V_{1/2} = 138 \) mV, \( z = 1.1 \) e) (Fig. 5 A and Table 1). Therefore, in the presence of the \( \gamma^3 \) C-tail, the \( \gamma^1 \) TM segment is also incompatible with the extracellular \( \gamma^3 \) juxta-membrane charged residue D269. Overall, the \( \gamma^1 \) subunit has a special TM segment that is
fully compatible with the γ1 C-tail, partially compatible with the γ3 C-tail, and fully incompatible with the γ2 and γ4 C-tails.

The TM segment is a key determinant of BKγ subunits’ different modulatory functions. The TM segment of γ2, unlike that of γ1, was fully compatible with other BKγ subunits’ C-tails. Upon being swapped with other BKγ subunits, the γ2 TM segment fully potentiated the γ3 and γ4 subunits’ modulatory functions (V1/2 = 26 ± 2 mV with γ3/γ2-TM and V1/2 = 72 ± 3 mV with γ4/γ2-TM) to levels nearly as high as those of γ1 and γ2, respectively, but caused little change in γ1’s function (V1/2 = 32 ± 3 mV with γ1/γ2-TM) (Fig. 5 B). This observation indicates that in the presence of the γ1 or γ3 C-tail, the γ2 TM segment is as effective as the γ1 TM segment in modifying BK channel gating. However, replacing the TM segments of γ1 and γ2 with those of γ3 and γ4 resulted in a 78–92% reduction of γ1 and γ2’s modulatory functions, which became more similar to those of γ3 and γ4 (Fig. 5, C and D). The resultant BK channel V1/2 values were 135 ± 2 mV with γ1/γ3-TM, 155 ± 4 mV with γ1/γ4-TM, 155 ± 5 mV with γ2/γ3-TM, and 158 ± 7 mV with γ2/γ4-TM (Table 1). The smaller modulatory effect of the γ1/γ3-TM chimera compared with that of γ3 was likely caused by the functional difference between the γ1 and γ3 LRRDs. Therefore, the γ3 and γ4 TM segments are similarly ineffective in modifying BK channel voltage gating in the presence of the γ2 or γ4 C-tails, as indicated by similarly high V1/2 values with γ4 and chimeras of γ2/γ3-TM, γ2/γ4-TM, and γ3/γ4-tail. Nevertheless, in the presence of the γ1 or γ3 C-tail, the γ3-TM segment appeared to be slightly more effective than the γ4-TM segment. There was approximately −20-mV difference in the resultant BK channel V1/2 between γ1/γ3-TM and γ1/γ4-TM. Likewise, replacing the TM segment of γ3 with that of γ4 caused the functional conversion of γ3 to be more like that of γ4 (160 ± 4 mV with γ3/γ4-TM) in four of the eight examined excised patches (Fig. 5 D). Collectively, the above results suggest that the TM segment is a key determinant of the difference in the modulatory functions between the potent modulators γ1 and γ2 and the much weaker modulators γ3 and γ4.

Effects of the TM segment, C-tail, and LRRD on BK channel voltage-dependent gating
To thoroughly compare the contributions of the LRRD, C-tail, and TM segment to the BKγ proteins’ modulatory functions on BK channels, we grouped and plotted the shifts in V1/2 (ΔV1/2) of the BK channels caused by all wild-type and chimeric BKγ proteins, except those containing the incompatible γ1-TM segment and other BKγ subunits’ C-tails (Fig. 6). The BK channel V1/2 values are first determined by the TM segment. The differences between the γ1- and γ2-TM segments in their effects on BK channel V1/2 were indistinguishable (i.e., <10 mV) or small (i.e., 10–20 mV), i.e., −9 mV between γ1 and γ1/γ2-TM and −20 mV between γ1/γ2-LRRD and γ2/γ1-tail (Fig. 6). As noted above, the differences between the γ3- and γ4-TM segments in their effects on the BK channel V1/2 in the presence of the γ1 or γ3 C-tail were limited.

Figure 5. Effects of swapping TM segments on the modulatory functions of BKγ subunits. Voltage dependence of BK channel activation in the presence of BKγ subunit chimeras, whose main bodies were from different γ subunits and whose TM segments were from γ1 (A), γ2 (B), γ3 (C), and γ4 (D). Thick dot lines are used for the major wild-type BKγ protein(s) intended for comparison. Error bars represent ± SEM.
However, the difference between these two pairs of TM segments (γ1 and γ2 vs. γ3 and γ4) in their effects on the BK channel $V_{1/2}$ was very large (~100 mV). The differences between the γ1- and γ3-TM segments in their effects on the BK channel $V_{1/2}$ were more than −110 mV, for example, a −112-mV difference between γ1 and γ1/γ3-TM and a −114-mV difference between γ1/γ3-LRRD and γ3/γ1-tail. Similarly, the average difference between the γ2- and γ3-TM segments in their effects on BK channel $V_{1/2}$ was about −95 mV, with differences of −103 mV between γ1/γ2-TM and γ1/γ3-TM, −89 mV between γ3/γ2-TM and γ3, and −94 mV between γ2 and γ2/γ3 TM. The differences between the γ2- and γ4-TM segments in their effects on the BK channel $V_{1/2}$ were −97 mV between γ2 and γ2/γ4-TM and −82 mV between γ4/γ2-TM and γ4.

The shifts in BK channel $V_{1/2}$ values were second determined by the C-tail regions (Fig. 6). The differences between the γ1 and γ3 C-tails and between the γ2 and γ4 C-tails in their effects on the BK channel $V_{1/2}$ were nearly indistinguishable (i.e., <10 mV) in the presence of the γ2-TM segment; for example, the differences between γ2/γ1-tail and γ2/γ3-tail and between γ2 and γ2/γ4-tail were −4 and −7 mV, respectively. Nevertheless, the differences between these two pairs of C-tails in their effects on BK channel $V_{1/2}$ were at least as high as 45 mV, including differences of −58 mV between γ1/γ2-TM and γ1/γ2-TM and C-tail (γ1 C-tail vs. γ2 C-tail); −45 mV between γ2/γ1 C-tail and γ2 (γ1 C-tail vs. γ2 C-tail), and −48 mV between γ2/γ3-tail and γ2/γ4-tail (γ3 C-tail vs. γ4 C-tail). In the presence of the γ3 TM segment, the differences between the C-tails of individual subunits within each subunit pair (i.e., γ1 and γ3; γ2 and γ4) in their effects on the BK channel $V_{1/2}$ were either small or indistinguishable (e.g., 5 mV between γ3/γ1-tail and γ3 and −16 mV between γ1/γ3-TM and γ1/γ3-TM and C-tail for the difference between the γ1 and γ3 C-tails, and −15 mV between γ3/γ2-tail and γ3/γ4-tail for the difference between the γ2 and γ4 C-tails). The differences between these two pairs of C-tails in their effects on the BK channel $V_{1/2}$ were still significant (~20–40 mV) in the presence of the γ3- or γ4-TM segments (e.g., −41 mV between γ3 and γ3/γ4-tail for the difference between γ3 and γ4 C-tails, −21 mV between γ3/γ1-tail and γ3/γ2-tail for the difference between the γ1 and γ2 C-tails, and −26 mV between γ3 and γ3/γ2 C-tail for the difference between the γ3 and γ2 C-tails), although overall they were smaller than those in the presence of the γ2-TM segment. Therefore, in the presence of different BKγ (i.e., γ2, γ3, and γ4) TM segments, we observed a similar trend: the C-tails of individual subunits within each subunit pair (i.e., γ1 and γ3; γ2 and γ4) had more similar functionality, whereas the two pairs themselves differed significantly in functionality.

Collectively, the shifts in BK channel $V_{1/2}$ values were least affected by differences in LRRDs (Fig. 6). An analysis of six pairs of comparable chimeras formed with a TM segment and C-tail from the same BKγ subunit indicated that the LRRDs of γ2, γ3, and γ4 had little difference in their influences on the BK channel $V_{1/2}$ values, eliciting an average change of only 10 mV. A slightly larger difference of 18 mV was observed only between the γ3 and γ4 LRRDs in the presence of the γ1-TM segment and C-tail (i.e., γ1/γ3-LRRD vs. γ1/γ4-LRRD). However, compared with the LRRDs of the other three BKγ subunits, the γ1 LRRD caused a larger shift in the BK channel $V_{1/2}$ of 14–36 mV (mean, 23 mV) toward depolarizing voltages among seven pairs of comparable chimeras. Therefore, LRRDs are not major determinants of the BKγ subunits’ different modulatory functions, and they instead may slightly alleviate the functional difference between γ1 and other BKγ subunits.

![Figure 6. Shifts in BK channel $V_{1/2}$ ($ΔV_{1/2}$) values caused by different wild-type, chimeric, or mutant BKγ proteins. The names of the BKγ proteins appear above the graph, and the corresponding compositions of the TM and C-tail regions from different BKγ subunits appear below the graph. Each LRRD is indicated by a different color. The $ΔV_{1/2}$ values with BKγ wild types are indicated by dotted gray lines. Error bars represent ± SEM.](image_url)
Juxta-membrane positively charged residues determine the C-tail functions of γ1 and γ3

Each of the four BKγ subunits' C-tails contains a cluster of positively charged residues that is adjacent to the C terminus of the TM segment (Fig. 1). This residue cluster might be involved in the proper insertion and orientation of the TM segment, which follows the general "positive-inside rule" (White and von Heijne, 2004). The juxta-membrane cluster of positively charged amino acids in γ1 is heavily charged with six arginine residues within a peptide sequence of eight residues (RARRRRRLR298), whereas the clusters in γ2 and γ3 contain only one arginine, one lysine, and one histidine in short sequences of RKK297 (γ2) and HRWSK304 (γ3). To determine whether these clusters are major contributors to the modulatory functions of C-tails, we performed deletion and substitution mutations in the C-tail regions and assessed the mutated C-tails' effects on BK channel voltage-dependent gating. Deleting most or all residues on the C-terminal side of the clusters in γ1 and γ3 did not significantly change the subunits' modulatory functions (V1/2 = 29 ± 4 mV with γ1ΔtailC302–334 and V1/2 = 109 ± 3 mV with γ3ΔtailC309–311) (Fig. 7 A). However, similar to a previous report (Yan and Aldrich, 2010), deletion of the polyarginine region in γ1 caused a 100% loss of γ1's modulatory function (V1/2 = 168 ± 3 mV with γ1ΔtailC201–298). These results suggest that the juxta-membrane positively charged region is more than an important contributor to the γ1-induced shift in BK channel voltage-dependent gating; rather, the region is essential for the whole γ1 protein function, presumably by determining the protein's proper folding, surface expression, or assembly with BKα. Similarly, deletion of the positively charged region HRWSK304 in γ3 resulted in a loss of γ3's modulatory function, yielding a V1/2 (180 ± 3 mV with γ3ΔtailC308–314) close to that of BKα alone (Fig. 7 A). Neutralization of the last lysine residue (K304) in this cluster also reduced γ3's modulatory effect on the BK channel V1/2 by nearly 30 mV (V1/2 = 144 ± 2 mV with γ3K304N mutant vs. 115 ± 2 mV with wild type) (Fig. 7 A).

For the γ2 C-tail, the deletion of either the three-residue cluster RKK297 or the rest of the C-tail region to the C-terminal end alone did not significantly alter γ2's modulatory function (V1/2 = 73 ± 3 mV with γ2ΔtailN277–279 and V1/2 = 63 ± 2 mV with γ2ΔtailC280–313). However, the deletion of both regions resulted in a 100% loss of γ2's modulatory function (V1/2 = 166 ± 6 mV with γ2ΔtailC277–313) (Fig. 7 B). These results suggest that some amino acid residues on the C-tail—not necessarily the positively charged residues immediately following the TM segment—are still needed for γ2 to be functional. There are multiple positively charged and negatively charged residues on the C-terminal side (residues 280–313) of the three-residue cluster RKK297 in γ2's C-tail region.

**DISCUSSION**

In this study, by swapping structural elements among BKγ subunits and by performing deletion and substitution mutations, we were able to systematically analyze the functional contributions of the BKγ subunits' individual structural elements to the subunits' different modulatory functions. We identified the TM segment and the adjacent intracellular clusters of positively charged amino acids as key determinants of the distinct effects that different BKγ subunits have on BK channel voltage gating. Our results suggest that BK channel modulation by BKγ subunits involves major intra- and/or juxta-membrane mechanisms. Our findings also reveal a strategy in which the modulatory functions of regulatory membrane proteins of voltage-gated ion channels can be coarse- and fine-tuned, respectively, through variations in their TM segments and adjacent intracellular positively charged region.

A previous study showed that deletion of a signal peptide, TM segment, or C-tail, or even a single LRR unit of the LRRD, resulted in a nearly 100% loss of the γ1's modulatory function (Yan and Aldrich, 2010), which could be complicated by disruptions in the subunit's 3-D structure. To maintain structural integrity, in this study, we systematically swapped individual structural elements among the four BKγ subunits to identify the molecular determinants of their different modulatory functions.
functions. Of the 37 BKγ chimera or mutants we examined, 20 effectively modulated BK channels and produced G-V curves that could be fitted by a single Boltzmann function with a normal slope (z > 1) and more than 30-mV shifts in V1/2 compared with that formed by BKα alone. According to the previously described “all-or-none” gating shift for the γ1 subunit (Gonzalez-Perez et al., 2014), the property of effective shift in V1/2 and the resultant normal G-V slope is a good indication that these 20 BKγ chimera or mutants formed functional complexes with BKα that reflect full stoichiometric assembly. Indeed, four of the other chimera and mutants resulted in two populations of channels, one with a significant shift in voltage-dependent gating and the other with a voltage dependence more similar to the channel formed by BKα alone. Similar variable ratio of two populations of BK channels, whose G-V curves were either shifted to a full extent or unchanged (“all-or-none”), was reported when the molar ratio of the injected BKα/γ1 RNA to Xenopus oocytes was varied (Gonzalez-Perez et al., 2014). Therefore, these proteins were likely partially defective in folding, surface expression, or proper assembly with BKα. For three of these four chimera and mutants, we unexpectedly observed some patch-to-patch variation that resulted in two distinct G-V relationships; e.g., the recorded BK channels were dominated by a low V1/2 population in three excised membrane patches but by a high V1/2 population in another eight patches in the presence of γ1/γ3-tail (Table 1). This variation might arise from patch-to-patch variation caused by differences in cell membrane composition (such as the presence or absence of lipid rafts) and/or cell-to-cell variation in protein trafficking machinery. The remaining 13 BKγ chimera or mutants resulted in V1/2 values within 30 mV of that produced by the BKα alone; these proteins may be only slightly effective in modifying the BK channel voltage gating or may have failed to fold, express on the cell surface, or assemble with BKα properly. Because we designed the present study to identify key determinants of the apparent differential modulatory effects of the four BKγ subunits on BK channels, we consider these non- or less-modulatory BKγ chimera or mutants, regardless of the causes, still useful when comparing with other chimeras or mutants for the apparent differential modulatory effects.

The BKγ subunits are type I single-pass TM proteins. Similar to the reported essential role of the N-terminal signal peptide in γ1 function (Yan and Aldrich, 2012), we expect that those of other BKγ subunits also play a key role in the subunits’ maturation and proper surface expression. An ineffective signal peptide can cause a defect in cellular localization or protein folding, leading to a dysfunctional protein. The sequences of the four BKγ subunits’ N-terminal signal peptide regions have very little similarity (Fig. 1). By swapping distinct signal peptide regions among BKγ proteins, we first demonstrated that the BKγ proteins’ different modulatory functions are intrinsic properties of the proteins and are independent of the proteins’ signal peptide sequences. The four BKγ subunits are closely related because of their LRRDs, which share a considerable level of sequence identity (31–37%) or similarity (43–48%) and have been predicted to have overall similar 3-D structures (Yan and Aldrich, 2012) (Figs. 1 and 2). We initially expected all four BKγ subunits’ LRRDs to play a critical role in determining their subunits’ differential modulatory functions, but to our surprise, only the γ1 LRRD was slightly different from the other LRRDs in function in BKγ chimeric proteins. The γ1 LRRD may play a slightly inhibitory role, or the other BKγ subunits’ LRRDs may play a slightly stimulatory role, in BK channel voltage-gated activation.

On the basis of the results of our functional analyses of the LRRDs, TM segments, and C-tails in the BKγ subunit chimeras, we concluded that the TM and the C-tail regions are the main determinants of the four BKγ subunits’ differential modulatory functions. As seen in Fig. 6, the differences in the different γ subunit–induced shifts of the BK channel V1/2 are first determined by the TM segments, in which the γ1 and γ2 TMs produced low V1/2 BK channels, whereas the γ3 and γ4 TM segments all resulted in high V1/2 channels. The differences between the TM segments of γ1 and γ2 and between those of γ3 and γ4 in their effects on BK channel V1/2 were small, but the difference between the TM segments of the two BKγ subunit pairs themselves was large (~100 mV). The C-tails further adjust the modulatory functions of the four BKγ subunits. Although the differences between the C-tails of γ1 and γ3 and between those of γ2 and γ4 in their effects on BKγ modulatory functions were small, the difference between the two pairs’ C-tails in their effects on BK channel V1/2 could be big (~40–50 mV), which could be largely accounted for by the different modulatory functions between γ1 and γ2 and between γ3 and γ4. We also found that the functions of the γ1 and γ3 C-tails are determined by the juxta-membrane clusters of positively charged amino acids. On the basis of these findings, we generalized the relative contributions of the four BKγ subunits’ individual structural elements to the subunits’ modulatory functions in BK channel voltage-dependent gating (Fig. 2).

Our findings demonstrate that the TM segments of γ1 and γ2 play a major role in setting these subunits’ high modulatory efficacies, and that the γ1 and γ3 subunits’ juxta-membrane clusters of positively charged amino acids also significantly contribute to these subunits’ modulatory functions. It was reported previously that only the TM segment is critical for the physical association of γ1 with BKα (Yan and Aldrich, 2010). Therefore, the physical association or interaction between the BKγ TM segments and BKα may be a main mechanism by which the γ1 and γ2 subunits modulate BK channel
voltage-dependent gating. In principle, this possibility is consistent with the previous notion that γ1 mainly affects the allosteric coupling (D-factor) between the voltage-sensor domain and the pore (Yan and Aldrich, 2010), which likely occurs in the membrane. The other BKγ subunits, particularly γ2, whose TM segment is similar to that of γ1 in function, may have a similar mechanism underlying their BK channel modulation. According to the HA allosteric model (Horrigan and Aldrich, 2002), an increase in D-factor may slightly steepen the slope of the G-V relationship curve, as reflected by an increased z value obtained from a Boltzmann fit. We found that, compared with other BKγ subunits, γ1 increased the z value of the BK channels to a greater degree. When the C-tail of γ2 was replaced with that of γ1 or γ3, γ2 elicited V1/2 and z values approaching those of γ1, suggesting that the C-tail has a large impact on the slope of the G-V curve. However, because a slight modification in the voltage-sensing parameters Zf or JfF can have a marked effect on the slope of the G-V curve, de-convolving the G-V curve to identify contributions from different HA model gating parameters to its slope is difficult.

The way in which the BKγ subunits’ C-tails contribute to the subunits’ modulatory functions remains unclear. The C-tails may contribute to the modulatory functions of BKγ subunits through two distinct mechanisms. First, the C-tail or its juxta-membrane cluster of positively charged amino acids may be required for BKγ to be functional, presumably by ensuring the proper insertion or orientation of the TM segment in accordance with the positive-inside rule (White and von Heijne, 2004). This possibility would explain why the deletion of C-tails or even the juxta-membrane clusters of positively charged amino acids led to a nearly 100% loss of the modulatory function of γ1 and γ3 in the present study. Second, through one or more unknown mechanisms, the juxta-membrane positively charged residues may directly affect BK channel gating independently of their influence on the TM segments. This would account for the additional shift in the BK channel V1/2 (approximately −40 mV) and the increase in the steepness of the G-V curve caused by the γ1 and γ3 C-tails as compared with the γ2 and γ4 C-tails in the presence of γ2’s LRRD and TM segments. Alternatively, the juxta-membrane clusters of positively charged amino acids in γ1 and γ3 may indirectly affect BK channel gating by interacting with the TM segments. This indirect mechanism might be similar to the positive-inside rule mechanism in that it alters the structure of the TM segment to enhance the function of the BKγ subunit, which would be consistent with our observation that the effects of individual C-tails on BK channel voltage gating somehow varied in a TM segment–dependent manner. The functional incompatibility of γ1’s TM segment with other BKγ subunits’ C-tails suggests their strong interactions. However, a precise delineation of the juxta-membrane border between the TM segment and intracellular C-tail will be needed in future studies to clearly define their separate modulatory functions and determine their interactions.

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REFERENCES

Almassy, J., and T. Begenisich. 2012. The LRRC26 protein selectively alters the efficacy of BK channel activators. *Mol. Pharmacol.* 81:21–30. http://dx.doi.org/10.1124/mol.111.075294

Brenner, R., T.J. Jegla, A. Wickenden, Y. Liu, and R.W. Aldrich. 2000. Cloning and functional characterization of novel large conductance calcium-activated potassium channel beta subunits, hKCNMB3 and hKCNMB4. *J. Biol. Chem.* 275:6453–6461. http://dx.doi.org/10.1074/jbc.275.9.6453

Conteras, G.F., A. Neely, O. Alvarez, C. Gonzalez, and R. Latorre. 2012. Modulation of BK channel voltage gating by different auxiliary β subunits. *Proc. Natl. Acad. Sci. USA.* 109:18991–18996. http://dx.doi.org/10.1073/pnas.1216953109

Evanson, K.W., J.P. Bannister, M.D. Leo, and J.H. Jaggar. 2014. LRRC26 is a functional BK channel auxiliary γ subunit in arterial smooth muscle cells. *Circ. Res.* 115:423–431. http://dx.doi.org/10.1161/CIRCRESAHA.115.303407

Gessner, G., K. Schönherr, M. Soom, A. Hansel, M. Asim, A. Baniahmad, C. Derst, T. Hoshi, and S.H. Heinemann. 2006. BKγγ channels activating at resting potential without calcium in LNCaP prostate cancer cells. *J. Membr. Biol.* 208:229–240. http://dx.doi.org/10.1007/s00232-005-0830-z

Gonzalez-Perez, Y., X.M. Xia, and C.J. Lingle. 2014. Functional regulation of BK potassium channels by γ1 auxiliary subunits. *Proc. Natl. Acad. Sci. USA.* 111:4868–4873. http://dx.doi.org/10.1073/pnas.1322123111

Gribkoff, V.K., J.E. Starrett Jr., and S.I. Dworetzky. 2001. Maxi-K potassium channels: Form, function, and modulation of a class of endogenous regulators of intracellular calcium. *Neuroscientist.* 7:166–177. http://dx.doi.org/10.1177/107385840100700211

Gurnett, C.A., and K.P. Campbell. 1996. Transmembrane auxiliary subunits of voltage-dependent ion channels. *J. Biol. Chem.* 271:27975–27978. http://dx.doi.org/10.1074/jbc.271.45.27975

Horrigan, F.T., and R.W. Aldrich. 2002. Coupling between voltage sensor activation, Ca2+ binding and channel opening in large conductance calcium-activated potassium channels. *J. Gen. Physiol.* 120:267–305. http://dx.doi.org/10.1085/jgp.20028605

Manzanoa, D., M. Srinivasan, S.T. Salathe, P. Ivonnet, N. Baumlin, J.S. Dennis, G.E. Conner, and M. Salathe. 2014. IFN-γ-mediated reduction of large-conductance, Ca2+-activated, voltage-dependent K+ (BK) channel activity in airway epithelial cells leads to mucociliary dysfunction. *Am. J. Physiol. Lung Cell. Mol. Physiol.* 306:453–462. http://dx.doi.org/10.1152/ajplung.002472013

Meera, P., M. Wallner, and L. Toro. 2000. A neuronal beta subunit (KCNMB4) makes the large conductance voltage and Ca2+-activated K+ channel resistant to charybdotoxin and iberitoxin. *Proc. Natl. Acad. Sci. USA.* 97:5562–5567. http://dx.doi.org/10.1073/pnas.100118597

Ramanathan, K., T.H. Michael, G.J. Jiang, H. Hiel, and P.A. Fuchs. 1999. A molecular mechanism for electrical tuning of cochlear hair
cells. *Science*. 283:215–217. http://dx.doi.org/10.1126/science.283.5399.215

Savalli, N., A. Kondratiiev, S.B. de Quintana, L. Toro, and R. Olcese. 2007. Modes of operation of the BKCa channel β2 subunit. *J. Gen. Physiol*. 130:117–131. http://dx.doi.org/10.1085/jgp.200709805

Sun, X., M.A. Zaydman, and J. Cai. 2012. Regulation of voltage-activated K+ channel gating by transmembrane β subunits. *Front Pharmacol*. 3:63. http://dx.doi.org/10.3389/fphar.2012.00063

Wallner, M., P. Meera, and L. Toro. 1999. Molecular basis of fast inactivation in voltage and Ca2+-activated K+ channels: A transmembrane β-subunit homolog. *Proc. Natl. Acad. Sci. USA*. 96:4137–4142. http://dx.doi.org/10.1073/pnas.96.7.4137

Wang, Y.W., J.P. Ding, X.M. Xia, and C.J. Lingle. 2002. Consequences of the stoichiometry of Slo1 alpha and auxiliary beta subunits on functional properties of large-conductance Ca2+-activated K+ channels. *J. Neurosci*. 22:1550–1561.

White, S.H., and G. von Heijne. 2004. The machinery of membrane protein assembly. *Curr. Opin. Struct. Biol*. 14:397–404. http://dx.doi.org/10.1016/j.sbi.2004.07.003

Xia, X.M., J.P. Ding, X.H. Zeng, K.L. Duan, and C.J. Lingle. 2000. Rectification and rapid activation at low Ca2+ of Ca2+-activated, voltage-dependent BK currents: Consequences of rapid inactivation by a novel beta subunit. *J. Neurosci*. 20:4890–4903.

Yan, D., and S. Tomita. 2012. Defined criteria for auxiliary subunits of glutamate receptors. *J. Physiol*. 590:21–31. http://dx.doi.org/10.1113/jphysiol.2011.213868

Yan, J., and R.W. Aldrich. 2010. LRRC26 auxiliary protein allows BK channel activation at resting voltage without calcium. *Nature*. 466:513–516. http://dx.doi.org/10.1038/nature09162

Yan, J., and R.W. Aldrich. 2012. BK potassium channel modulation by leucine-rich repeat-containing proteins. *Proc. Natl. Acad. Sci. USA*. 109:7917–7922. http://dx.doi.org/10.1073/pnas.1205451109

Yang, C., X.H. Zeng, Y. Zhou, X.M. Xia, and C.J. Lingle. 2011. LRRC52 (leucine-rich-repeat-containing protein 52), a testis-specific auxiliary subunit of the alkalization-activated Slo3 channel. *Proc. Natl. Acad. Sci. USA*. 108:19419–19424. http://dx.doi.org/10.1073/pnas.1111104108

Zeng, X.H., X.M. Xia, and C.J. Lingle. 2003. Redox-sensitive extracellular gates formed by auxiliary beta subunits of calcium-activated potassium channels. *Nat. Struct. Biol*. 10:448–454. http://dx.doi.org/10.1038/nsb932

Zhang, J., and J. Yan. 2014. Regulation of BK channels by auxiliary γ subunits. *Front Physiol*. 5:401. http://dx.doi.org/10.3389/fphys.2014.00401