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Modulation of BK Channel Function by Auxiliary Beta and Gamma Subunits

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Abstract

The large-conductance, Ca^{2+} - and voltage-activated K^+ (BK) channel is ubiquitously expressed in mammalian tissues and displays diverse biophysical or pharmacological characteristics. This diversity is in part conferred by channel modulation with different regulatory auxiliary subunits. To date, two distinct classes of BK channel auxiliary subunits have been identified: β subunits and γ subunits. Modulation of BK channels by the four auxiliary β ($\beta 1$ – $\beta 4$) subunits has been well established and intensively investigated over the past two decades. The auxiliary γ subunits, however, were identified only very recently, which adds a new dimension to BK channel regulation and improves our understanding of the physiological functions of BK channels in various tissues and cell types. This chapter will review the current understanding of BK channel modulation by auxiliary β and γ subunits, especially the latest findings.

1. INTRODUCTION

The large-conductance, calcium- and voltage-activated potassium (BK, also known as MaxiK, Slo1, or $\text{K}_{\text{Ca}1.1}$) channel is a unique member of the potassium channel family. The BK channel has exceptionally large single-channel conductance (200–300 pS) that is 10–20 times larger than most other K^+ channels. BK channel activity is dually regulated by two independent physiological signals, membrane voltage, and intracellular free Ca^{2+} ($\text{Ca}^{2+}_{\text{i}}$), and therefore has a powerful integrative role in regulating cellular excitability and calcium signaling in electrically excitable cells (Ghatta, Nimmagadda, Xu, & O'Rourke, 2006; Salkoff, Butler, Ferreira, Santi, & Wei, 2006). BK channels are ubiquitously expressed in most types of mammalian tissues and cells and are critically involved in various physiological processes. In central nervous system neurons, BK channels mediate the repolarization and fast afterhyperpolarization of action potentials (Shao, Halvorsrud, Borg-Graham, & Storm, 1999; Womack & Khodakhah, 2002), shape dendritic Ca^{2+} spikes (Golding, Jung, Mickus, & Spruston, 1999), and regulate neurotransmitter release at presynaptic terminals (Hu et al., 2001; Raffaelli, Saviane, Mohajerani, Pedarzani, & Cherubini, 2004; Samengo, Curro, Barrese, Taglialatela, & Martire, 2014; Xu & Slaughter, 2005; see Chapter “BK Channels in Neurons” by Barth and Contet). Neuronal BK channels are involved in motor coordination (Sausbier et al., 2004), learning and memory (Matthews & Disterhoft, 2009; Springer, Burkett, & Schrader, 2014; Typlt et al., 2013; Ye, Jalini, Mylvaganam, & Carlen, 2010), the brain's intrinsic rhythmicity of the circadian clock

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(Farajnia, Meijer, & Michel, 2015; Meredith et al., 2006; Montgomery, Whitt, Wright, Lai, & Meredith, 2013; Pitts, Ohta, & McMahon, 2006) and respiration (Onimaru, Ballanyi, & Homma, 2003; Zavala-Tecuapetla, Aguileta, Lopez-Guerrero, Gonzalez-Marin, & Pena, 2008; Zhao, Hulsmann, Winter, Dutschmann, & Richter, 2006), frequency tuning of the cochlear hair cell (Fettiplace & Fuchs, 1999), pain modulation (Cao, Chen, Li, & Pan, 2012; Chen, Cai, & Pan, 2009; Waxman & Zamponi, 2014; Zhang, Mok, Lee, Charbonnet, & Gold, 2012), and neuroprotection in pathological conditions (Mancini et al., 2014; Runden-Pran, Haug, Storm, & Ottersen, 2002; Shen, Kishimoto, Linden, & Sapirstein, 2007; Zhang, Xie, et al., 2009). Defects or dysregulation in human neuronal BK channels can cause epilepsy and paroxysmal dyskinesia (Brenner et al., 2005; Du et al., 2005) and are implicated in mental retardation (Deng et al., 2013; Higgins, Hao, Kosofsky, & Rajadhyaksha, 2008), autism (Laumonnier et al., 2006), and schizophrenia (Zhang, Li, Zhou, & Xing, 2006). BK channels also control contractile tone of almost all types of smooth muscle cells, playing a central role in the regulation of vascular blood pressure (Brenner, Perez, et al., 2000), urinary bladder function (Meredith, Thorneioe, Werner, Nelson, & Aldrich, 2004), and erectile function (Werner, Zvara, Meredith, Aldrich, & Nelson, 2005).

BK channels are homotetramers of the pore-forming, Ca^{2+} -, and voltage-sensing α subunits ($\text{BK}\alpha$). The $\text{BK}\alpha$ (rv130 kDa) contains seven-transmembrane (TM) segments (S0–S6), a short extracellular N-terminus, and a large cytosolic C-terminus composed of two RCK (regulating conductance of K^+) domains (Fig. 1A). Similar to other voltage-gated K^+ channels, the S1–S4 TM segments form the voltage-sensor domain (VSD), and the S5 and S6 TM segments form the pore-gate domain (PGD). Ca^{2+} and Mg^{2+} sensitivity is conferred by the two RCK domains in the cytosolic C-terminus. The RCK2 domain contains a well-defined Ca^{2+} -binding site called the Ca^{2+} bowl, formed by a string of negatively charged residues (Schreiber & Salkoff, 1997; Xia, Zeng, & Lingle, 2002; Yuan, Leonetti, Hsiung, & MacKinnon, 2012; Yusifov, Savalli, Gandhi, Ottolia, & Olcese, 2008). The RCK1 domain is believed to be involved in the formation of another Ca^{2+} -binding site (Xia et al., 2002; Zhang et al., 2010) and a Mg^{2+} -binding site which also involves residues located in the S0–S1 linker (D99) and S2–S3 loop (N172) (Shi et al., 2002; Xia et al., 2002; Yang et al., 2008). The electric (voltage) and chemical (Ca^{2+} or Mg^{2+} binding) energies are converted to mechanical forces through their sensory domains to induce conformational change in the PGD, which toggles between the “closed state” and “open state” to control K^+ flux (Horrigan & Aldrich, 1999; Horrigan, Cui, & Aldrich, 1999).

Unlike many other mammalian potassium channels, all $\text{BK}\alpha$ subunits are encoded by a single gene (*Slo1*, *KCNMA1*). Native BK channels exhibit a wide range of biophysical, pharmacological, and functional properties that differ among various cell types (Ding, Li, & Lingle, 1998; Gessner et al., 2005; Jones, Gray-Keller, Art, & Fettiplace, 1999; Xia, Ding, & Lingle, 1999), at different stages of development (Carvalho-de-Souza, Varanda, Tostes, & Chignalia, 2013), and in different physiological or pathological conditions (Hu et al., 2011; Manzanares et al., 2015; Tao et al., 2015). BK channels with slightly different biophysical properties can be produced by alternative splicing of *KCNMA1* precursor mRNA at several different sites (Glauser, Johnson, Aldrich, & Goodman, 2011; Ramanathan, Michael, Jiang, Hiel, & Fuchs, 1999; Saito, Nelson, Salkoff, & Lingle, 1997; Schreiber, Yuan, & Salkoff,

1999; Yu, Upadhyaya, & Atkinson, 2006). BK channel function is differently and potentially modulated by auxiliary subunits. To date, four types of beta (β) auxiliary subunits ($\beta 1$ – $\beta 4$, encoded by the *KCNMB1–4* genes) (Behrens et al., 2000; Brenner, Jegla, Wickenden, Liu, & Aldrich, 2000; Knaus, Folander, et al., 1994; Meera, Wallner, & Toro, 2000; Orio, Rojas, Ferreira, & Latorre, 2002; Wallner, Meera, & Toro, 1999; Xia et al., 1999; Xia, Ding, & Lingle, 2003; Xia, Ding, Zeng, Duan, & Lingle, 2000; Zeng, Xia, & Lingle, 2008) and four types of gamma (γ) auxiliary subunits ($\gamma 1$ – $\gamma 4$, encoded by the *LRRC26*, *LRRC52*, *LRRC55*, and *LRRC38* genes) (Yan & Aldrich, 2010, 2012) have been identified (Table 1). These auxiliary subunits affect nearly all aspects of the BK channel's biophysical and pharmacological properties, including the apparent voltage dependence (Cox & Aldrich, 2000; Wang & Brenner, 2006; Yan & Aldrich, 2010, 2012) and Ca^{2+} sensitivity (Brenner, Jegla, et al., 2000; McManus et al., 1995; Nimigean & Magleby, 1999; Xia et al., 1999), gating kinetics, inactivation (Brenner, Jegla, et al., 2000; Xia et al., 1999, 2000), ion current rectification (Xia et al., 2000; Zeng, Xia, & Lingle, 2003), and sensitivity to extracellular modulators (Almassy & Begenisich, 2012; Valverde et al., 1999). Regulation by auxiliary subunits is therefore a key mechanism of BK channel functional diversity that adapts the channel for diverse needs in different mammalian tissues and cell types (Table 1). The modulation of BK channels by β subunits has been extensively investigated and comprehensively reviewed elsewhere (Sun, Zaydman, & Cui, 2012; Torres, Granados, & Latorre, 2014; Torres, Morera, Carvacho, & Latorre, 2007). This chapter will review the literature of BK channel modulation by auxiliary β and γ subunits, including a comparison of these two different types of auxiliary subunits, specifically their modulatory effects and underlying mechanisms.

2. DISCOVERY

Four different β subunits ($\beta 1$ – $\beta 4$) have been cloned and identified in mammals. The first β subunit was identified as a binding partner of $\text{BK}\alpha$ in the BK channel complexes purified from bovine tracheal smooth muscle by extensive conventional chromatography together with sucrose gradient centrifugation or by immunoprecipitation (Garcia-Calvo et al., 1994; Knaus, Eberhart, Kaczorowski, & Garcia, 1994; Knaus, Folander, et al., 1994; Knaus, Garcia-Calvo, et al., 1994). In these early experiments, charybdotoxin (ChTX), which is a peptide blocker of BK channels, was radiolabeled as a tool for BK channel complex or protein detection, and ChTX was found to be attached to a β subunit upon cross-linking. This smooth muscle-specific auxiliary protein was later named the $\beta 1$ subunit. With advancements in molecular biology, the other three family members were discovered thereafter by sequence similarity and molecular cloning (Behrens et al., 2000; Brenner, Jegla, et al., 2000; Meera et al., 2000; Uebele et al., 2000; Wallner et al., 1999; Xia et al., 1999).

The first representative of the γ subunit family was identified relatively more recently. An unusual type of K^+ current was initially noticed in lymph node carcinoma of prostate (LNCaP) cells, which showed a Kv -like low half-activation voltage ($V_{1/2}$) of ~ 30 mV in the absence of intracellular calcium ($[\text{Ca}^{2+}]_i$) but had many characteristics of BK channels (Gessner et al., 2005). These BK-like features included large single-channel conductance, activation by Ca^{2+} and Mg^{2+} , and sensitivity to specific BK channel activator and blockers

(Gessner et al., 2005). The range of voltages needed for channel activation for this endogenous BK-like channel in LNCaP cells was shifted to the hyperpolarization direction by more than 120 mV compared with human BK α channels expressed in HEK-293 cells. Therefore, it was concluded that the LNCaP cell contains a special BK channel or a BK-like K⁺ channel which was designated as BK_L (Gessner et al., 2005). Later, it was demonstrated by another research group that the LNCaP cells did express BK α at the protein level, as detected by the BK α antibody, and the BK α existed in a normal zero-splicing form (NCBI accession NP_002238), according to reverse transcriptase *PCR* and sequencing of mRNA (Yan & Aldrich, 2010). A proteomic approach was then used to immunopurify the channel complex, and mass spectrometry was used to identify potential novel interacting partners that may drastically modify the BK channel's gating property (Yan & Aldrich, 2010). A 35-kDa leucine-rich repeat-containing protein, LRRC26, was specifically identified in the BK α pull-down components. Knockdown of this protein in LNCaP cells resulted in a complete loss of the BK channel's property of being activated at low voltage in the absence of calcium. Meanwhile, overexpression of LRRC26 in another prostate cancer cell line, PC3, which lacks endogenous LRRC26 expression, converted the endogenous typical BK α channels into the low-voltage-activated LNCaP-type BK channels. In addition, it was shown in a heterologous expression system (HEK-293 cells) that LRRC26 was specifically associated with BK α , as detected by reciprocal coimmunoprecipitation, and shifted the conductance–voltage (*G*–*V*) relationship of BK channels to the hyperpolarization direction by ~140 mV, as was seen in the LNCaP cells. LRRC26 is structurally and functionally distinct from the four β subunits and thus was considered a new type of BK channel auxiliary subunit. Later, three other structurally related leucine-rich repeat-containing proteins, LRRC52, LRRC55, and LRRC38, were also reported as able to modulate BK channels when coexpressed heterologously with BK α in HEK-293 cells (Yan & Aldrich, 2012). These proteins have also been shown to produce marked shifts in the ranges of voltages needed for channel activation in the hyperpolarizing direction, although the shifts are smaller than those produced by LRRC26.

3. STRUCTURAL CHARACTERISTICS

BK channel β subunits are a family of small double-pass membrane proteins (20–30 kDa) (Behrens et al., 2000; Brenner, Jegla, et al., 2000; Uebele et al., 2000). Four types of BK β genes (*KCNMB1-4*) have been cloned in mammals (Marty, 1981; Orio et al., 2002; Xia et al., 2003, 2000; Zeng et al., 2008). Among the four β subunits, β 1 shares 53% similarity with β 2 in amino acid sequence similarity, and 37% with β 3, whereas less than 20% with β 4 (Behrens et al., 2000; Wallner et al., 1999; Xia et al., 2000) (Fig. 2A). The four β subunits display similar topology containing short N- and C-termini both on the intracellular side, two TM helices, and a large extracellular loop (116–128 amino acid residues) connecting the two TM segments (Orio et al., 2002) (Figs. 1B and 2A). The loop has three or four putative glycosylation sites and multiple pairs of conserved disulfide-forming cysteine residues (Fig. 2A).

The four γ subunits have similar molecular weights of about 35 kDa. They are type I single-span membrane proteins containing a classic N-terminal cleavable signal peptide for extracellular localization of the N-terminal LRR domain in the mature proteins (Fig. 1C).

The signal peptide region was found to be absent in the mature protein. Mutations in this region caused the signal peptide to be retained in the expressed protein and led to a loss of modulatory function in the $\gamma 1$ subunit, suggesting that proper maturation guided by the signal peptide region is critical for the function of γ subunits (Yan & Aldrich, 2012). The mature proteins of the four γ subunits all contain a single-transmembrane domain, an N-terminal extracellular LRRD (rv240 amino acids), and a short C-terminal tail (Fig. 1C). The four γ subunits share an overall sequence similarity of 35–40%, which is comparable with that of the four β subunits (Fig. 2B).

The LRR domain in many proteins is known to provide a structural framework for protein–protein interactions, typically through the concave β -sheet side (Kobe & Kajava, 2001). The LRR domains of γ subunits all contain six LRR units and two cysteine-rich regions, a small one called LRRNT, capped on the N-terminal side, and a large one called LRRCT, capped on the C-terminal side. As in many other LRR-containing proteins, each LRR unit in the γ subunits consists of 24 residues and has a classic consensus sequence of LxxLxLxxN (where x can be any amino acid). Based on structural modeling (Yan & Aldrich, 2012), the LRR domain is a banana-shaped structure with a curved parallel β -sheet lining the inner circumference and small helices or turns flanking the convex circumference, formed by six LRR units stacked together in the middle (Fig. 3A). Each LRR unit forms a β -strand lining the concave face and a short α -helix connected by loops flanking the outer circumference. The hydrophobic core of the LRR domain is tightly packed by the parallel inward-pointing leucine residues, shielded by the LRRCT and LRRNT caps on the N- and C-terminal ends. Both LRRNT and LRRCT contain two pairs of fully conserved cysteine residues that in total potentially form four disulfide linkages in the favorable oxidizing extracellular environment. Consistent with their predicted extracellular location, the LRR domains of the γ subunits all contain single or multiple consensus N-glycosylation sites: Asn-Xaa-Ser/Thr, where Xaa is not a proline. For the $\gamma 1$ subunit, N147Q mutation and enzymatic removal of the N-linked glycan by PNGase F resulted in the disappearance of an upper glycosylated-mass band on SDS-PAGE (Yan & Aldrich, 2012).

The protein sequences in the LRR domains of γ subunits are closely related but become divergent in the transmembrane and intracellular C-terminal tail regions (Fig. 2B). The single-transmembrane segments of the γ subunits are well predicted from their hydrophobicity and the presence of charged residues on both sides, particularly multiple positively charged residues on the intracellular side following the general “positive-inside rule” for membrane insertion and orientation of membrane proteins. For the C-terminal tail regions, in addition to the cluster of positively charged residues adjacent to the transmembrane domain, it is interesting to note that the rest of the amino acid sequence is enriched in proline residues (11 out of 36 residues) for $\gamma 1$ and enriched in acidic residues for $\gamma 2$, $\gamma 3$, and $\gamma 4$.

In the absence of direct structural information, a group led by Marx and Karlin have used a biochemical method to engineer disulfide linkage to approximately localize the various structural parts of the β subunits relative to the different BK α TM helices within the BK α / β complex. It was found that the first TM helix (TM1) of $\beta 1$ was localized near the S1 and S2 helix of α subunits, and the second TM helix (TM2) near the S0 helix. This places the

extracellular ends of $\beta 1$ TM helices within the crevice formed by VSDs of two adjacent α subunits (Liu et al., 2010, 2008). Later, similar interacting patterns between $\beta 2$, $\beta 3$, or $\beta 4$ subunits with BK α subunits were also reported (Wu et al., 2009, 2013). Recently, the cytoplasmic ends of $\beta 1$ TM1 and TM2 were found to be adjacent and located between the S2–S3 loop of one α subunit and S1 of a neighboring α subunit, but not adjacent to S0, suggesting that the interaction between BK α and the $\beta 1$ subunit is more complex than previously thought (Fig. 3B) (Liu et al., 2015). As discussed later, it is believed that the extracellular loop of the β subunit is in proximity to the extracellular mouth of the BK α pore gate because of its effect on the ion flow through the channel's extracellular mouth (Fig. 1B) (Gruslova, Semenov, & Wang, 2012; Zeng et al., 2003). Currently, very little is known about the exact protein regions and residues in BK α that directly interact with β subunits to mediate channel modulation. There is no report yet on the structural location of the γ subunit in the BK α / γ complex. The structural information obtained from the biochemical studies on the location of the β subunits in the BK α / β channel complex might provide an initial biochemical basis from which to decode the molecular mechanisms of BK channel modulation by auxiliary subunits.

The BK α subunit is universally expressed across different species of animals including invertebrates *Drosophila melanogaster* and *Caenorhabditis elegans*. The BK channel β subunits are present in both mammal and non-mammal animals such as chicken and turtle. The γ subunits (LRRC26 and its three paralogs) belong to a previously grouped “Elron” subfamily of the “extracellular LRR”-containing proteins that are mainly present in mammals (Dolan et al., 2007), suggesting that in evolution, the acquisition of BK channel proteins occurred in the order of BK α , β , and γ subunits. Although the β and γ subunits belong to distinct protein families with no amino acid sequence similarity, they all share some common structural features. First, they are all single or double membrane-spanning proteins with a large extracellular domain and a relatively much smaller intracellular region. Our previous study showed that the TM domain in $\gamma 1$, as in the β subunits, is essential for the domain's modulatory function and physical association with BK α (Yan & Aldrich, 2010). Second, they all contain multiple pairs of disulfide-bond-forming cysteine residues on their extracellular sides. Third, as plasma membrane proteins with an extracellular soluble domain, they are all subject to modification by N-glycosylation. Both glycosylation and extra-cellular disulfide linkages in the β subunits were reported to directly affect the BK channels' voltage dependence (Hagen & Sanders, 2006) or ion flow through the channel's extracellular mouth (Zeng et al., 2003), respectively.

4. MODULATION OF THE BK CHANNEL'S BIOPHYSICAL PROPERTIES BY β SUBUNITS

The four β subunits display different and complex effects on apparent calcium and voltage sensitivities, macroscopic current kinetics, and pharmacological sensitivities, which involve multiple distinct mechanisms (Fig. 4). The $\beta 1$ and $\beta 2$ subunits overall induce slowing of the macroscopic kinetics and an increase in apparent calcium and voltage sensitivity (Behrens et al., 2000; Brenner, Jegla, et al., 2000; Contreras, Neely, Alvarez, Gonzalez, & Latorre, 2012; Orio & Latorre, 2005; Savalli, Kondratiev, de Quintana, Toro, & Olcese, 2007). The $\beta 2$ and

some splice variants of $\beta 3$ subunits also cause rapid inactivation through their intracellular N-termini (Uebele et al., 2000; Xia et al., 1999, 2000). In contrast with the classic shaker Kv inactivation peptide, the intrinsically disordered N-terminal peptide of $\beta 3$ subunit inactivated BK channels in two steps, which involves a stereospecific binding interaction that precedes blockade (Gonzalez-Perez, Zeng, Henzler-Wildman, & Lingle, 2012; Lingle et al., 2001; Xia et al., 2000). The $\beta 3$ subunits generate rectifying outward currents regulated by their extracellular loops (Xia et al., 2000; Zeng et al., 2003). The brain-specific $\beta 4$ subunit, in addition to greatly slowing activation and deactivation kinetics, reduces apparent calcium sensitivity in low Ca^{2+}_i conditions but increases apparent sensitivity in high $[\text{Ca}^{2+}]_i$ conditions (Behrens et al., 2000; Brenner, Jegla, et al., 2000). The $\beta 1$, $\beta 2$, and $\beta 4$ subunits were also found to modulate BK channels by altering $\text{BK}\alpha$ expression and trafficking. It was reported that when coexpressed in HEK-293 cells, $\beta 1$ could reduce the steady-state $\text{BK}\alpha$ surface expression and alter the diffused intracellular expression of $\text{BK}\alpha$ to a pattern of punctate cytoplasmic localization that overlapped with the $\beta 1$ expression (Toro et al., 2006). A similar effect on BK channel expression was also observed with the $\beta 2$ subunit (Lv et al., 2008; Zarei et al., 2007). Expression of $\beta 4$ subunit was also found to reduce surface expression of BK channels in mouse CA3 neurons which was mediated by a C-terminal ER retention sequence (Shruti et al., 2012). In contrast, the N-terminal domain of $\beta 1$ was reported to stimulate trafficking of VEDEC (a $\text{BK}\alpha$ C-terminal isoform) channels to the plasma membrane (Kim, Zou, Ridgway, & Dryer, 2007). A recent study showed that the palmitoylated $\beta 4$ subunit regulated surface expression of BK channels through masking a trafficking motif (REVEDEC) at the C-terminus of the $\text{BK}\alpha$ subunit (Chen et al., 2013).

With mathematical modeling and simulation, the mechanisms of action of different β subunits were investigated by multiple laboratories within the framework of the well-established BK channel allosteric HCA (Horrigan et al., 1999) or HA (Horrigan & Aldrich, 2002) models of voltage- and Ca^{2+} -dependent gating. According to the HA model (Fig. 5) (Horrigan & Aldrich, 2002), the activation or open probability (P_o) of BK channels is independently affected by voltage and Ca^{2+} , which can be calculated or described by eight gating parameters: L_0 and Z_L were referred to as equilibrium constant and associated gating charge for the channel pore's closed $\rightarrow$ open transition; J_0 and Z_J were referred to as equilibrium constant and associated gating charge for the voltage sensors' resting $\rightarrow$ activated transition; D was considered the allosteric coupling factors between the pore gate and the voltage sensors; C was designated as the allosteric coupling factor between the pore gate and the calcium sensors, and K_d as the elementary Ca^{2+} dissociation constant when the channel is closed and voltage sensors are not activated; E was referred to as the allosteric coupling factor between the voltage and the calcium sensors, which was considered to be very weak in the case of the BK channel (Horrigan & Aldrich, 2002). The $\beta 1$ subunit was found to affect multiple gating processes including the voltage-sensor activation (J_0), the intrinsic opening of pore gate (L_0), and the coupling between voltage sensors and pore gate (D) but with only minor effect on the Ca^{2+} sensor and its coupling the pore gate (C) (Bao & Cox, 2005; Cox & Aldrich, 2000; Orio & Latorre, 2005; Wang & Brenner, 2006). The apparent increase in sensitivity to Ca^{2+} is largely caused by modulation on other gating parameters (Ma et al., 2006; Orio & Latorre, 2005; Wang & Brenner, 2006; Yang et al., 2008). The $\beta 4$ subunit appears to be essentially similar to the $\beta 1$ subunit in reducing the intrinsic pore gate

opening and enhancing voltage-sensor activation, which ultimately results in a shift of the G - V relationship to the depolarizing direction at low Ca^{2+} but to the hyperpolarizing direction at high Ca^{2+} (approx. 10 μM) (Wang et al., 2006). By measuring gating currents, the $\beta 1$, $\beta 2$, and $\beta 4$ but not $\beta 3$ subunits were found to stabilize the BK voltage sensor in the active conformation (Contreras et al., 2012).

Over the past two decades, progress has been made in structurally and functionally identifying the potential protein regions and residues involved in BK channel modulation by β subunits. Studies of chimeric and mutant β subunits indicated that the cytosolic N- and C-termini of the BK $\beta 1$ subunit were crucial to altering the channel's intrinsic opening and voltage-sensor activation (Orio et al., 2006; Wang et al., 2006). It was recently found that substituting two lysine residues (ie, K3 and K4) in the N-terminus of $\beta 1$ can virtually abolish the effects of $\beta 1$ on voltage-sensor activation (Castillo et al., 2015). The N-termini of the $\beta 2$, $\beta 3a$, and $\beta 3c$ subunits contain an inactivating domain which blocks the entrance to the intracellular pore and inactivates the BK channels (Li et al., 2007; Wallner et al., 1999; Xia et al., 1999, 2003; Zeng et al., 2003; Zhang, Zeng, Xia, & Lingle, 2009; Zhang, Zhou, Ding, Xia, & Lingle, 2006). Different from the simple one-step occlusion mechanism for Kv channel blockade by N-terminal peptides which may mainly involve hydrophobic interactions with the channel pore, the BK channel inactivation by auxiliary β subunits involves two distinguishable kinetic steps (Lingle et al., 2001), a stereospecific binding interaction that precedes blockade (Gonzalez-Perez et al., 2012). Although the sites of stereospecific interactions between the β subunit N-terminus and BK channel pore have not been identified, it was proposed that the relatively larger inner pore of BK channels than Kv channels might require some stereospecific binding to achieve the affinity necessary for BK channel inhibition (Gonzalez-Perez et al., 2012). The extracellular loop and the disulfide bridges within the loop were found to be important in $\beta 3$ subunit-induced current rectification (Xia et al., 2000; Zeng et al., 2003). The lysine-rich ring (K137, K141, K147, and K150) on the extracellular loop of $\beta 2$ also was involved in causing the outward rectification (Chen et al., 2008). These results suggest close proximity between the extracellular loop of the β subunits and the extracellular mouth of the channel pore. Several residues (Y74, S104, Y105, and I106) on the extracellular loop of $\beta 1$, which are also conserved in other β subunits, were also found to be important in the $\beta 1$ subunit's modulatory effect on voltage-dependence activation of BK channel (Gruslova et al., 2012). It remains unclear whether the β subunit's extracellular loop can directly interact and modulate the BK channel or indirectly affect the channel properties by affecting the overall structure of the β subunit. Compared with the other regions, the function of the two TM segments in the β subunits has been relatively less studied. Their likely interposed location between VSDs of adjacent BK α subunits suggests a role in modulating voltage-sensor activation. The two TM segments in $\beta 1$ subunit were functionally replaceable by those from the $\beta 2$ subunit (Orio et al., 2006) but not from the $\beta 4$ subunit even replaced individually (Kuntamallappanavar, Toro, & Dopico, 2014). The latter showed that both TM segments are required to maintain the characteristic modulatory function of the $\beta 1$ subunit (Kuntamallappanavar et al., 2014).

On the BK α subunit, by using chimeric BK α channels from different species, it was found that the unique S0 TM segment, as well as the extracellular N-terminus of BK α , was

important for BK channel modulation by $\beta 1$ and $\beta 2$ subunits (Lee et al., 2010; Morrow et al., 2006; Wallner, Meera, & Toro, 1996). The $\beta 1$ and $\beta 2$ subunits seem to be different in regulating the various functional domains of the BK α , although both affect the apparent voltage and Ca^{2+} sensitivity. Mutations on BK α that affected voltage-sensor activation altered the apparent changes in Ca^{2+} sensitivity induced by the $\beta 1$ subunit (Yang et al., 2008). In contrast, $\beta 2$ mainly affected Ca^{2+} sensitivity, presumably by altering the allosteric coupling between the Ca^{2+} sensors and the pore gate. Mutations in the BK α voltage sensor were found to have little effect on $\beta 2$'s modulatory effect on Ca^{2+} sensitivity (Yang et al., 2008). Instead, mutations on the N-terminus of RCK1 and on the linker region between S6 TM segment and RCK1 had a large impact on $\beta 2$'s but not $\beta 1$'s modulation on the BK channel's Ca^{2+} sensitivity (Lee et al., 2010). However, experiments with voltage-clamp fluorometry correlated the $\beta 2$ subunit with changes in voltage sensors (Savalli et al., 2007). The $\beta 1$ and $\beta 2$ subunits were also reported to alter the interactions between bound Mg^{2+} and R213 in the voltage sensor and to disrupt the engineered disulfide-bond formation between the voltage sensor and C-terminal intracellular domain (Sun et al., 2013).

5. MODULATION OF THE BK CHANNEL'S BIOPHYSICAL PROPERTIES BY γ SUBUNITS

Unlike the complex effects and mechanisms of different β subunits on many aspects of BK channel gating, the actions of the γ subunits appear to be remarkable in mechanistic simplicity and modulatory magnitudes (Yan & Aldrich, 2010; Zhang & Yan, 2014). The four γ subunits have distinct capabilities in shifting the voltage dependence of BK channel activation toward hyperpolarizing voltages by approximately 140 mV ($\gamma 1$), 100 mV ($\gamma 2$), 50 mV ($\gamma 3$), and 20 mV ($\gamma 4$) in the absence of Ca^{2+} (Fig. 6) (Yan & Aldrich, 2010, 2012). The gating shift produced by the $\gamma 1$ subunit is equivalent to the effect of $\sim 10 \mu\text{M}$ $[\text{Ca}^{2+}]_i$ on BK channels formed by BK α alone. However, Ca^{2+} and Mg^{2+} sensitivities were shown to be largely unaffected by the $\gamma 1$ subunit (Yan & Aldrich, 2010). In the presence of Ca^{2+} , the $\gamma 1$ –3 subunits all caused slight changes in the slope of the relationship between $Q_{\text{app}}V_{1/2}$ (Q_{app} , apparent gating charge obtained from a Boltzmann fit of the G – V curve for voltage dependence of channel activation) and $\log [\text{Ca}^{2+}]_i$ with some Ca^{2+} -dependent reduction in their $V_{1/2}$ shifting capabilities, suggesting some small but noticeable effects on the apparent calcium sensitivity of BK channel activation (Yan & Aldrich, 2010). It is unclear whether the observed Ca^{2+} effects on the function of γ subunits were mediated by the Ca^{2+} effects on the BK α or on the γ subunits.

The mechanistic actions of the $\gamma 1$ subunit were investigated and analyzed within the framework of the HA model in the absence of Ca^{2+} . By measuring the kinetics and open probabilities of the channels at very negative voltages to achieve good estimates of the Z_L and L_0 parameters, it was found that the pore's gating parameters L_0 and Z_L are largely unaffected by the $\gamma 1$ subunit (Yan & Aldrich, 2010). The lack of effect on L_0 also suggests that the $\gamma 1$ subunit is not a Ca^{2+} -like ligand that directly activates BK channels independently of voltage-sensor activation. By simulation with changes in other gating parameters, the $\gamma 1$ subunit's modulatory effect was found to be best explained by an approximate 20-fold increase in the allosteric coupling D factor (Yan & Aldrich, 2010) (Fig.

5). According to this study, the $\gamma 1$ subunit may mainly affect the coupling between voltage sensors and the pore. The γ subunits may serve as good tools to study BK channel gating mechanisms because little is currently known about the molecular basis underlying allosteric coupling between voltage sensors and the pore in this voltage- and ligand-gated channel. This earlier analysis, which was performed in the absence of Ca^{2+} and assumed that only one gating parameter was affected in simulation, was not comprehensive enough to allow complete assessment of some other gating parameters, such as some slight changes in the voltage- sensor parameters, J_0 and Z_j . Additionally, a later study observed some effects of the intracellular Ca^{2+} on the modulatory function of the γ subunits (Yan & Aldrich, 2012).

To understand the molecular mechanisms of BK channel regulation by auxiliary γ subunits, it is critical to identify key structural elements underlying their channel modulatory functions. By swapping structural elements among γ subunits and by mutations, we recently found that the differences in the various γ subunit-induced shifts of the BK channel $V_{1/2}$ are primarily determined by their single TM segments during the approximate -100-mV shift in $V_{1/2}$, in which the $\gamma 1$ and $\gamma 2$ TMs produced low $V_{1/2}$ BK channels, while the $\gamma 3$ and $\gamma 4$ TM domains all resulted in high $V_{1/2}$ channels (Fig. 7) (Li, Fan, Kwak, & Yan, 2015). We also found that their intracellular C-tails, particularly the juxta-membrane positively charged cluster regions that contain multiple positively charged amino acids, further adjust the modulatory functions of the four γ subunits by conferring to the BK channels an additional approximate -40 to -50 mV shift in $V_{1/2}$ from the $\gamma 1$ and $\gamma 3$ C-tails (Li et al., 2015) (Fig. 7). In a more recent study, we investigated in detail the structure and function in BK channel modulation by the $\gamma 1$ subunit's peptide region (rv40 amino acids) encompassing the single TM segment and the adjacent poly-Arg cluster (Li, Guan, Yen, Zhang, & Yan, 2016). We demonstrated that this peptide region, independent of the N-terminal LRR domain and the rest of the C-terminal tail, was sufficient to fully modulate BK channels. We found that Phe273 and its neighboring residues in the middle of the TM segment play a key role in BK channel association and modulation and that a minimum of three Arg residues in the charged cluster are required for the $\gamma 1$ subunit's modulatory function. Allosteric coupling between the TM segment and the intracellular positively charged cluster was also observed. We concluded that the TM segment is a key molecular determinant for channel association and modulation, and the intracellular positively charged cluster is involved mainly in channel association likely through its TM anchoring effect (Li et al., 2016). These new findings provide insights into the structure–function relationship of the γ subunits for understanding their potent modulatory effects on BK channels.

6. MODULATION OF THE BK CHANNEL'S PHARMACOLOGICAL PROPERTIES BY β AND γ SUBUNITS

As a result of decades of exploration, numerous endogenous and synthetic BK channel modulators have been identified and developed, some of which show BK auxiliary subunit-dependent characteristics. Generally, the β subunits confer alteration in sensitivity of the BK channel to peptide toxins, in which the extracellular loop of β subunits plays a critical role (Chen et al., 2008; Hanner et al., 1998; Meera et al., 2000; Xia et al., 1999). The four residues (Leu⁹⁰, Tyr⁹¹, Thr⁹³, and Glu⁹⁴) of BK $\beta 1$ were found to be crucial for the enhanced

affinity of ChTX, and the lysine-rich ring (K137, K141, K147, and K150) of subunit $\beta 2$ could reduce the ChTX sensitivity to BK channels. In addition, the $\beta 2$ and $\beta 3$ subunit were reported to reduce the degrees of blockade by ChTX (Xia et al., 1999, 2000; Zeng et al., 2008), together with a change in the magnitude and kinetics of the blocking reaction (Ding et al., 1998). However, the $\beta 4$ -complexed BK channel is extremely resistant to both iberiotoxin (IbTX) and ChTX (Bergeron & Bingham, 2012; Meera et al., 2000; Schneider, Rogowski, Krueger, & Blaustein, 1989). This kind of resistance is related with the so-called helmet structure constituted by BK α and three residues in extracellular loop (K120, R121, and K125) of the $\beta 4$ subunit, which can block the entry of ChTX by both electrostatic interaction and the limited space of the pore area (Gan et al., 2008). Moreover, compared with BK α alone, the BK α / $\beta 1$ channel, but not the BK α / $\beta 4$ channel, is more sensitive to the blockade by slotoxin (α KTx1.11). But the binding between slotoxin and the BK α / $\beta 4$ channel seemed to be irreversible in an unknown mechanism (Garcia-Valdes, Zamudio, Toro, & Possani, 2001). In contrast, martentoxin (Shi et al., 2008; Tao, Shi, Liu, & Ji, 2012) and conopeptide Vt3.1 (Li et al., 2014) were identified as the more selective blockers for the BK α / $\beta 4$ channel, but not BK α / $\beta 1$ channel, when compared with BK α channel alone. The β subunits were also reported to regulate BK channel modulation by ethanol. In heterologous expression system (HEK-293 cells), the $\beta 1$ subunit caused blockade of ethanol-induced potentiation of the BK channels (Feinberg-Zadek & Treistman, 2007; Martin et al., 2004). In the supraoptic nucleus neurons, the $\beta 4$ -complexed axonal terminal BK channels were highly potentiated by ethanol, but the $\beta 1$ -complexed somatic and dendritic channels were insensitive to ethanol (Dopico, Widmer, Wang, Lemos, & Treistman, 1999; Wynne, Puig, Martin, & Treistman, 2009). Similarly, the difference in sensitivity of BK channels to ethanol in the dendrites and cell bodies of nucleus accumbens medium spiny neurons can be explained by the differential expression of the $\beta 1$ subunit in these two compartments (Martin et al., 2004). In contrast to the nervous system, ethanol caused inhibition of BK currents in myocytes that led to cerebrovascular constriction, in which the $\beta 1$ subunit played a major role (Bukiya, Liu, et al., 2009). This group recently identified K361 on BK α as a putative binding site for ethanol (Bukiya, Kuntamallappanavar, et al., 2014). Further studies will be needed to clarify the different effects of different β subunits on the BK channel's responses to ethanol. A detailed review of BK channel modulation by ethanol can be found in Chapter "Modulation of BK Channels by Ethanol" by Dopico.

The earliest reported activator, dehydrosoyasaponin-I (DHS-I, the potent compounds extracted from *Desmodium adscendens*) activates BK channels only when coexpressed with the $\beta 1$ subunit in *Xenopus oocytes* (McManus et al., 1993, 1995), and such $\beta 1$ -dependent activation was also observed in smooth muscle membranes (Bukiya, Patil, Li, Miller, & Dopico, 2012). The xenoestrogen, tamoxifen, and the endogenous steroid hormone 17 β -estradiol were also found to activate the BK channel in a $\beta 1$ -dependent manner (De Wet et al., 2006; Dick, Rossow, Smirnov, Horowitz, & Sanders, 2001; Duncan, 2005; Valverde et al., 1999). Likewise, the $\beta 4$ -complexed BK channel was more sensitive to corticosterone for channel function potentiation by steroids, whereas the $\beta 2$ -complexed channel was more sensitive to dehydroepiandrosterone (King et al., 2006). The endogenous lithocholate was reported to selectively recognize the steroid-sensing site (T169, L172, and L173) in TM2 of the $\beta 1$ subunit to activate BK channels (Bukiya, McMillan, Parrill, & Dopico, 2008; Bukiya,

Singh, Parrill, & Dopico, 2011; Bukiya, Vaithianathan, Toro, & Dopico, 2009). T169 in TM2 of the $\beta 1$ subunit was also involved in the activation of BK α / $\beta 1$ channels by nonsteroidal lithocholic acid (Bukiya et al., 2013). Current research has shown that lipid agents can act through β subunits, although the underlying mechanisms are unclear. Omega-3 docosaheptaenoic acid could increase BK channel activity in a $\beta 1$ and $\beta 4$ subunit-dependent manner, while not in a $\beta 2$ or $\gamma 1$ subunit-dependent manner (Hoshi et al., 2013). In addition, phosphatidylinositol 4,5-bisphosphate (PIP2) was reported to inhibit macroscopic currents of BK α and BK α / $\gamma 1$ channels but increase macroscopic currents of BK α / $\beta 1$ and BK α / $\beta 4$ channels without altering those of BK α / $\beta 2$ channels (Tian et al., 2015). Endogenous leukotrienes (LTB4) can potentially activate BK channels when coexpressed with the $\beta 1$ subunit in *X. oocytes*, which makes LTB4 a good template for the future development of $\beta 1$ -specific BK channel activators (Bukiya, McMillan, et al., 2014). A recent study reported that bis-(1,3-dibutylbarbituric acid)trimethine oxonol [DiBAC4(3)] and *N*-arylbenzamide can activate the BK channel in $\beta 1$ subunit dependence, but DiBAC4(3) partially blocked the BK α / $\beta 2$ channel's currents (Kirby, Martelli, Calderone, McKay, & Lawson, 2013; Morimoto et al., 2007). Additionally, HBD2 (human β -defensin 2) could activate BK channels via interactions with Leu41 and Gln43 of the $\beta 1$ extracellular loop (Liu et al., 2013). A recent report showed that the newly identified BK channel opener GoSlo-SR-5-130, but not its analogue GoSlo-SR-5-6, required the presence of $\beta 1$ or $\beta 4$ subunit to achieve its the maximal modulatory effects on BK channels (Large et al., 2015).

Little has been done to investigate the interaction between the newly identified auxiliary γ subunits and the BK channel's modulators. Nevertheless, the BK channel in LNCaP cells was found to be still sensitive to NS1619, ChTX, IbTX, paxilline, and penitrem A (Gessner et al., 2005), suggesting that most known pharmacological properties of the BK channels are likely retained in the presence of the endogenous $\gamma 1$ subunit. Interestingly, the $\gamma 1$ subunit can inhibit the effect of some BK activator (Almassy & Begenisich, 2012). In native salivary gland parotid acinar cells, the endogenous $\gamma 1$ subunit blocked the activating effect of mallotoxin but not NS1619 (Almassy & Begenisich, 2012). A similar blocking effect of the $\gamma 1$ subunit on mallotoxin action was also observed in HEK-293 cells when the $\gamma 1$ subunit was heterologously coexpressed with BK α . It was proposed that mallotoxin may displace the $\gamma 1$ subunit instead of lacking accessibility to the binding site (Almassy & Begenisich, 2012). Further biophysical studies and biochemical binding assays will be needed to clarify the detailed mechanisms. NS1619 was recently shown to bind to the S6/RCK linker region (Gessner et al., 2012), but little is known about the mallotoxin binding site. Identification of the mallotoxin binding site may complement our understanding of the actions of γ subunits on BK channels.

7. STOICHIOMETRY

When the $\beta 1$ subunit was first identified from bovine tracheal and aortic smooth muscle with immunoprecipitation, the isolated BK channel complex was found to contain an octameric assembly of BK α and BK $\beta 1$ subunits in 1:1 stoichiometry (Knaus, Eberhart, et al., 1994; Knaus, Garcia-Calvo, et al., 1994). In a classic model of tetrameric ion channel complex, auxiliary subunits bind to the pore-forming α subunit with a fourfold symmetry so that the regulatory effect is incremental upon variation in the relative molecular ratio of the auxiliary

subunit to core subunit. Consistently, the β subunits regulated the voltage dependence of BK channel activation in the titration-dependent manner in that the G - V curves shifted in a parallel manner as a function of the injected ratio of β to α subunit mRNA in *X. oocytes* (Wang, Ding, Xia, & Lingle, 2002). Variation in stoichiometry of BK α and β subunits likely contributed to the heterogeneity in BK channel activity in rat chromaffin cells (Ding et al., 1998; Xia et al., 1999) and turtle auditory hair cells (Jones et al., 1999). As the newly identified BK channel auxiliary subunits, no biochemical or stoichiometric study has been reported on the assembly of BK α and γ subunits.

A recent study indicated that the regulatory mechanism of the $\gamma 1$ subunit may be fundamentally different from that of the β subunit (Gonzalez-Perez, Xia, & Lingle, 2014). In contrast to β subunits (Gonzalez-Perez et al., 2014), the $\gamma 1$ subunit caused the voltage dependence of channel activation to be either fully shifted or unchanged, independent of the molar ratio of the injected BK α : $\gamma 1$ RNA to *X. oocytes*, although the ratio of these two populations of channels varied (Gonzalez-Perez et al., 2014). It is unknown whether one $\gamma 1$ subunit per channel complex is sufficient to fully modulate BK channels. Alternatively, the $\gamma 1$ subunit may preferably exist in a tetrameric form when forming a complex with BK α . Additional studies will be required to determine the detailed mechanisms, particularly the stoichiometry and the interaction sites between the BK α and γ subunits in the tetrameric channel complex.

8. PHYSIOLOGICAL AND PATHOLOGICAL RELEVANCE OR ROLES

The BK channel β and γ auxiliary subunits have been reported to be involved in tissue-specific functions in health or disease (Table 1). An early study of epigenetic epidemiology showed that the individuals carrying E65K polymorphism in $\beta 1$ subunit experienced low prevalence of diastolic hypertension and cardiovascular disease compared to those with normal $\beta 1$ subunit (Fernandez-Fernandez et al., 2004). The mRNA level of $\beta 1$ subunit was markedly decreased in the vascular smooth muscle of patients with acquired hypertension (Amberg et al., 2003). Studies with knockout mice showed that the presence of the $\beta 1$ subunit was critical to normal BK channel function in vascular smooth muscle cells (Werner et al., 2007, 2008). One recent report showed that $\beta 1$ deficiency exacerbated vascular fibrosis and remodeling (Xu et al., 2015). The knockout mice of $\beta 4$ subunit showed signs of an epileptic phenotype (Brenner et al., 2005), and the $\beta 2$ knockout mice had an increased tendency toward spontaneous burst firing in the adrenal medullary chromaffin cells (Martinez-Espinosa et al., 2014). A $\beta 3$ (delA750) mutant, which led to truncation of the C-terminus, was reported to be associated with a form of generalized epilepsy (Lorenz et al., 2007). The results obtained from the β subunit KO mice so far have used method of global ablation of the β subunit in the whole animal body which could complicate the results and interpretations because of functional communications between different tissues and organs. Studies with tissue- or cell- specific KO mice will be needed to further clarify the physiological functions of different β subunits in different cells or tissues.

The tissue-specific distribution patterns of the four γ subunits at the mRNA level had been investigated with use of TaqMan quantitative PCR in various human tissues (Yan & Aldrich, 2012). The $\gamma 1$ subunit was highly expressed in the salivary glands, prostate, and trachea,

whereas $\gamma 2$ (LRRC52) was found predominantly in the testes, and $\gamma 3$ (LRRC55) was found primarily in the nervous system. The $\gamma 4$ (LRRC38) subunit was observed mainly in skeletal muscle, adrenal glands, and the thymus. These results suggest that like β subunits, γ subunits have different tissue- specific distributions to fit the diverse functional requirements of various tissues and cell types (Yan & Aldrich, 2012). The $\gamma 1$ subunit's endogenous functional regulation of BK channels has been confirmed in prostate and salivary gland cells (Almassy & Begenisich, 2012; Yan & Aldrich, 2010). The physiological roles of the $\gamma 1$ subunit in prostate and salivary glands remain to be determined. Conceivably, constitutive activation of BK channels might be required for K^+ flow-mediated fluid secretion in these nonexcitable tissues. A very recent study suggested that the $\gamma 1$ subunit in airway epithelial cells may participate in BK channel-mediated airway hydration for effective mucociliary clearance (Manzanares et al., 2014). The K^+ flow through the apically expressed BK channels in airway epithelial cells provides an electro- chemical driving gradient for Cl^- secretion and thus plays a role in airway hydration. It was found that both the mRNA level of the $\gamma 1$ subunit and the sensitivity of BK channels to mallotoxin were decreased after IFN- γ treatment, suggesting that the $\gamma 1$ subunit might be involved in IFN- γ -mediated reduction in BK channel activity and the resulting mucociliary dysfunction (Manzanares et al., 2014). The $\gamma 1$ subunit under a different name (CAPC) was reported to be able to suppress tumor growth and metastasis, which may likely involve ion channel-independent function (Liu et al., 2012). The enhanced K^+ channel activity generally promoted cancer cell proliferation (Pardo & Stuhmer, 2014). It will be necessary to determine whether the association of the $\gamma 1$ subunit with BK channels will affect tumor growth because of change in BK channel activity.

Because of the drastic activating effect caused by the $\gamma 1$ subunit, expression of this protein even at low levels might exert significant effect on BK channel currents. For example, a low level of mRNA expression of the $\gamma 1$ subunit has been detected in aorta cells (Yan & Aldrich, 2012). A very recent study reported that knockdown of $\gamma 1$ subunit expression in rat cerebral artery myocytes led to reductions in the apparent voltage/ Ca^{2+} sensitivity, in current frequency and amplitude of the BK channels, and in the extents of BK channel-specific inhibitor-induced vasoconstriction and activator-induced vasodilation (Evanson et al., 2014). This study suggested that the $\gamma 1$ subunit may play broad physiological roles that are not limited to nonexcitable cells. In excitable cells, the voltage and Ca^{2+} sensitivities of the BK channels are more finely tuned to be properly responsive to different levels of voltage and Ca^{2+} in different cell types; therefore, even a low expression of this potent BK channel modulator might exert a significant physiological effect. It is worth noting that the $\gamma 1$ subunit is also expressed in fetal brain tissue (Yan & Aldrich, 2012) and that the $\gamma 1$ subunit might participate in maintaining proper neuronal excitability in the fetal nervous system during early development. The recent finding of independent association and function of the β ($\beta 2$) and γ ($\gamma 1$) subunits in the same functional BK channel complex (Gonzalez-Perez, Xia, & Lingle, 2015) suggests that the BK channel function can be extremely diversified across different tissues or cells types when multiple auxiliary subunits (β and γ) are coexpressed.

9. PERSPECTIVES

The four β subunits have been found to confer the BK channel a high diversity in the channel's biophysical properties. Over the past two decades, BK channel modulation by β subunits has been extensively investigated. Studies in both humans and knockout mice have led to an appreciation of the physiological and pathological significance of the BK channel β subunits. With research efforts using heterologous expression systems *in vitro*, we have overall achieved a good understanding of the mechanisms underlying the β subunits' modulatory functions conferring the BK channel inactivation and alterations in ion permeation and toxin sensitivity, which involve more specific regions of interactions between the β and BK α subunits. Significant progress has been made in understanding the mechanisms involved in the β subunits' effect on BK channel voltage and Ca^{2+} dependence of channel activation, including the identification of altered gating processes and the important regions and residues involved on the β subunits. However, it remains poorly understood how the β subunit interacts with the BK channel, particularly the interacting amino acid residues on the BK α , to modulate the voltage and/or Ca^{2+} sensitivity. This is in parallel with our very limited understanding of the detailed molecular mechanisms underlying BK channel activation by voltage and Ca^{2+} , which is partly due to the lack of atomic structure, particularly the structure that includes the channel's TM domain.

Our understanding of BK channel modulation by γ subunits is still in its very early stage. In particular, very little is known about the physiologic functions and the structural basis underlying the regulatory mechanisms of γ subunits. The few published studies examining the modulatory mechanisms and physiological functions of BK channel γ subunits have mainly focused on the $\gamma 1$ subunit. The regulation of BK channels by $\gamma 2-4$ subunits has so far been demonstrated only in the heterologous expression system. It will be important to determine whether $\gamma 2-4$ subunits also play any functional or physiological role in BK channel modulation *in vivo*. The mouse $\gamma 2$ subunit has been found to function as an accessory subunit of the sperm-specific mouse Slo3 channels (Yang et al., 2011), and its ablation in mice was recently found to cause a severe fertility impairment (Zeng et al., 2015). It will be intriguing to determine whether in any stage of germ cells the $\gamma 2$ subunit also modulates BK channels and whether Slo3 and BK channels can form functional heterotetrameric channels. When the BK and Slo3 channels were coexpressed in a heterologous expression system (X. oocytes), only two distinct BK-like and Slo3-like channels were observed, arguing against the presence of functional heterotetrameric channels (Yang, Zeng, Xia, & Lingle, 2009). BK channels and Slo3 belong to the Slo channel family, which also includes two more distantly related Na^{+} -activated channels, Slo2.1 (slick) and Slo2.2 (slack). It remains an open question whether the γ subunits may broadly function as auxiliary proteins of the Slo channel family.

Effective BK channel openers have been sought or explored to treat a variety of diseases such as stroke, epilepsy, psychoses, bladder overactivity, erectile dysfunction, asthma, arterial hypertension, ischemic heart disease, and gastric hypermotility (Nardi & Olesen, 2008; Chapter "Developing the Molecular Pharmacology of BK Channels for Therapeutic Benefit" by Kaczorowski and Garcia). Although the widely used BK channel opener NS1619 can give an approximate -40-mV shift in $V_{1/2}$ at a high concentration (30 μM)

(Gessner et al., 2012), its specificity was recently questioned because of its direct inhibiting effect on the sarcoplasmic/endoplasmic reticulum Ca^{2+} -ATPase (SERCA) (Wrzosek, 2014) in addition to a previous report on its stimulatory effect on Ca^{2+} release from caffeine/ryanodine-sensitive intracellular store (Yamamura, Ohi, Muraki, Watanabe, & Imaizumi, 2001). Currently, no BK channel-targeted drug has been approved for clinical use, in spite of extensive academic and pharmaceutical efforts over the past two decades. Drug development specifically targeting auxiliary β or γ subunits of BK channels is likely to be more effective in modulating BK channel function, while minimizing the global adverse effects originated from the ubiquitously expressed $\text{BK}\alpha$ subunit. Deciphering the biochemical mechanisms underlying BK channel activation by auxiliary subunits will be useful for the development of new BK channel-targeted drugs.

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Fig. 1.

Schematic structure and membrane topology of BK channel α (A), β (B), and γ (C) subunits and their models of assembly. *VSD*, voltage-sensor domain; *PGD*, pore-gate domain; *RCK*, regulator of K⁺ conductance; *NH₂*, aminotermminus; *COO⁻*, carboxyterminus; *LRRD*, leucine-rich repeat domain; *S* or *TM*, transmembrane segment.

Fig. 2. The protein sequence alignment of human BK channel β (A) and γ (B) subunits. The cysteine pairs for potential disulfide formation are based on previous publications (Brenner, Jegla, et al., 2000; Yan & Aldrich, 2012).

Fig. 3. Structural models. (A) Predicted leucine-rich repeat domain structure and membrane topology of the γ subunit (Yan & Aldrich, 2012). (B) Docking model of BK β 1 transmembrane segments (TMs) located in BK α subunit (Liu, Zakharov, Yao, Marx, & Karlin, 2015). For panel B, each BK α subunit is shown in a different *color* (*gray shades* in the print version). The β 1 TM1 and TM2 are in black.

Fig. 4.

The voltage- and Ca^{2+} -dependent activities of human $\text{BK}\alpha$ alone (A) or together with $\beta 1$ (B), $\beta 2$ (C), $\beta 3$ (D), or $\beta 4$ (E). The current traces were recorded in an inside-out patch from HEK-293 cells at $10\ \mu\text{M}$ (upper) or $1\ \mu\text{M}$ $[\text{Ca}^{2+}]_i$ (lower). The normalized conductance–voltage relations (G – V) for the activation of BK channels were plotted at $10\ \mu\text{M}$ $[\text{Ca}^{2+}]_i$ (●) and $1\ \mu\text{M}$ $[\text{Ca}^{2+}]_i$ (○). *Inactivation was removed by exposing the patch to $1\ \text{mg/mL}$ trypsin for $60\ \text{s}$. Modified from Pongs, O., & Schwarz, J. R. (2010). Ancillary subunits associated with voltage-dependent K^+ channels. *Physiological Reviews*, 90, 755–796.

Fig. 5.

The allosteric HA model of BK channels and the possible effects of the β and γ subunits. The three processes of channel opening (C-O), voltage-sensor activation (R-A), and Ca²⁺-binding transition (X-XCa²⁺) are linked, respectively, by the allosteric coupling factors C, D, and E. The possible gating processes or parameters affected by the β and γ subunits are indicated by *gray arrows*, which are based on previous reports (Contreras et al., 2012; Ma, Lou, & Horrigan, 2006; Orio & Latorre, 2005; Wang & Brenner, 2006; Yan & Aldrich, 2012; Yang et al., 2008). The HA model was taken from Horrigan, F. T., & Aldrich, R. W. (2002). Coupling between voltage sensor activation, Ca²⁺ binding and channel opening in large conductance (BK) potassium channels. *The Journal of General Physiology*, 120, 267–305.

Fig. 6.

Modulatory effects of the γ subunits on BK channels heterologously expressed in HEK-293 cells. (A and B) Voltage dependence of the BK channel activation for the BK α alone or together with γ 1, γ 2, γ 3, or γ 4 in the virtual absence of $[\text{Ca}^{2+}]_i$ (A) or in the presence of 1.9 μM $[\text{Ca}^{2+}]_i$ (B). (C) Plot of $V_{1/2}$ vs $[\text{Ca}^{2+}]_i$ (log scale after break). (D) Plot of $Q_{\text{app}} V_{1/2}$ vs $[\text{Ca}^{2+}]_i$ normalized at 0 $[\text{Ca}^{2+}]_i$. This figure is taken from Yan, J., & Aldrich, R. W. (2012). BK potassium channel modulation by leucine-rich repeat-containing proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 7917–7922.

Fig. 7.

Schematic of the structure, membrane topology, and generalized relative functional contributions of individual structural elements in the BK channel γ subunits. The different colors (*gray shades* in the print version) indicate the structural elements' relative contributions (in millivolts, mV) to the γ subunit-induced shifts of voltage dependence of BK channel activation. This figure is taken from Li, Q., Fan, F., Kwak, H. R., & Yan, J. (2015). Molecular basis for differential modulation of BK channel voltage-dependent gating by auxiliary gamma subunits. *The Journal of General Physiology*, 145, 543–554.

Characteristics of BK Channel Auxiliary Subunits

Table 1

Effect on BK Channels Function						
Subunit	Gene Symbol (Human)	Ca ²⁺ Sensitivity	Voltage Sensitivity	Inactivation	Deactivation	References
BKβ1	KCNMB1	Increase	Decrease	No inactivation	Slow	Walner et al. (1995), Tseng-Crank et al. (1996), Cox and Aldrich (2000), and Orto and Latorre (2005)
BKβ2	KCNMB2	Increase	No significant decrease	Fast and complete	Slow	Orto and Latorre (2005), Lee, Shi, and Cui (2010), Walner et al. (1999), Xia et al. (1999, 2000), and Bentrop, Beyermann, Wissmann, and Fakler (2001)
BKβ3	KCNMB3	β3a No effect	Increase	Incomplete, comparing to hβ3a, mβ3a: more rapid, more complete	No effect	Uebele et al. (2000), Brenner, Jegla, et al. (2000), Orto and Latorre (2005), Zeng, Benzinger, Xia, and Lingle (2007), and Zeng et al. (2008)
β3b		No effect	Decrease, while mβ3b: gating shift more to negative potentials at a given Ca ²⁺ concentration	Inactivation at a given Ca ²⁺ concentration, hβ3b: rapid inactivation; mβ3b: no inactivation	Slow	Uebele et al. (2000), Xia et al. (2000), Zeng et al. (2008), and Lingle, Zeng, Dng, and Xia (2001)
β3c		No effect	No effect	Incomplete	No effect	Uebele et al. (2000)
						Spleen, pancreas, kidney, heart, testes, brain, placenta, lung, and liver (Brenner, Jegla, et al., 2000; Uebele et al., 2000; Xia et al., 2000)
						Spleen, pancreas, kidney, heart, testes, brain, placenta, lung, and liver (Brenner, Jegla, et al., 2000; Uebele et al., 2000; Xia et al., 2000)
						Spleen, pancreas, liver, kidney, prostate, placenta,
						Blood pressure, vascular smooth muscle function, bladder function, alcohol dependence, and tolerance (Amberg, Bonev, Rossow, Nelson, & Santana, 2003; Bukiya, Liu, & Dopico, 2009; Kreifeldt, Cates-Gatto, Roberts, & Coniet, 2015; Kreifeldt, Le, Treistman, Koob, & Coniet, 2013; Werner, Knorn, Meredith, Aldrich, & Nelson, 2007; Werner, Meredith, Aldrich, & Nelson, 2008)
						The adrenal medullary chromaffin cells (CCs) function (Martinez-Espinosa, Yang, Gonzalez-Perez, Xia, & Lingle, 2014)
						Generalized epilepsy (Lorenz, Heils, Kasper, & Sander, 2007)

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