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REVIEW

The BK channel: a vital link between cellular calcium and electrical signaling

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ABSTRACT

Large-conductance Ca^{2+}-activated K^+ channels (BK channels) constitute an key physiological link between cellular Ca^{2+} signaling and electrical signaling at the plasma membrane. Thus these channels are critical to the control of action potential firing and neurotransmitter release in several types of neurons, as well as the dynamic control of smooth muscle tone in resistance arteries, airway, and bladder. Recent advances in our understanding of K^+ channel structure and function have led to new insight toward the molecular mechanisms of opening and closing (gating) of these channels. Here we will focus on mechanisms of BK channel gating by Ca^{2+}, transmembrane voltage, and auxiliary subunit proteins.

KEYWORDS RCK domain, voltage sensor, blood pressure, leucine-rich repeat-containing (LRRC) protein.

INTRODUCTION

Large-conductance Ca^{2+}-activated K^+ channels (known as Maxi-K or BK channels) are found in many different tissues in the human body, including nerve, smooth and skeletal muscle, and endocrine cells in the salivary and pituitary glands and pancreas, as well as a wide range of organisms in the animal kingdom, including nematodes, mollusks, *Drosophila*, and vertebrates (Gorman and Thomas, 1980; Barrett et al., 1982; Morris et al., 1986; Singer and Walsh, 1986; Adelman et al., 1992; Butler et al., 1993; Ferrer et al., 1996; Wang et al., 2001). These plasma membrane channels are characterized by their synergistic activation by cytoplasmic Ca^{2+} and electrical depolarization of the membrane, to yield rapid effux of K^+ under physiological conditions. The rapid K^+ efflux can be detected electrically as a large outward current, and this consequently results in a rapid hyperpolarization of the membrane.

The ubiquitous nature of BK channel expression points to the potential fundamental importance of BK channels in cellular physiology, in linking cytoplasmic Ca^{2+} signaling events with electrical signaling at the plasma membrane in a wide variety of organisms and tissues. Consistent with this idea, dysfunction of BK channels through mutations in its channel-forming subunits and its associated modulatory subunits can lead to disease in model organisms and humans. These include epilepsy and neurological disease, high blood pressure and cardiac hypertrophy, asthma, urinary incontinence, and erectile dysfunction (Brenner et al., 2000b; Meredith et al., 2004; Brenner et al., 2005; Werner et al., 2005; Imlach et al., 2008; Seibold et al., 2008; Wang et al., 2009; Semenov et al., 2011). Thus an understanding of the molecular interactions and events that control gating of BK channels will be important in the development of therapeutic measures to improve human health.

BASIC ARCHITECTURE AND SUBUNIT COMPOSITION

The BK channel is a member of the voltage-gated K (Kv) channel superfamily (Adelman et al., 1992; Butler et al., 1993; Pallanck and Ganetzky, 1994; McCobb et al., 1995). The pore-forming component of the channel is made up of four identical alpha subunits (Shen et al., 1994); in turn each BK alpha subunit contains seven transmembrane segments (Fig. 1) (named S0–S6; (Wallner et al., 1996; Meera et al., 1997)). The S1–S6 segments are analogous to the S1–S6 of the other Kv channels; the S5–S6 segments line the pore, and the S1–S4 segments contribute to “sensing” the transmembrane voltage, by way of a series of charged residues in the S4 region. The additional and relatively unique S0 region gives the BK channel an extracellular N-terminus, and forms

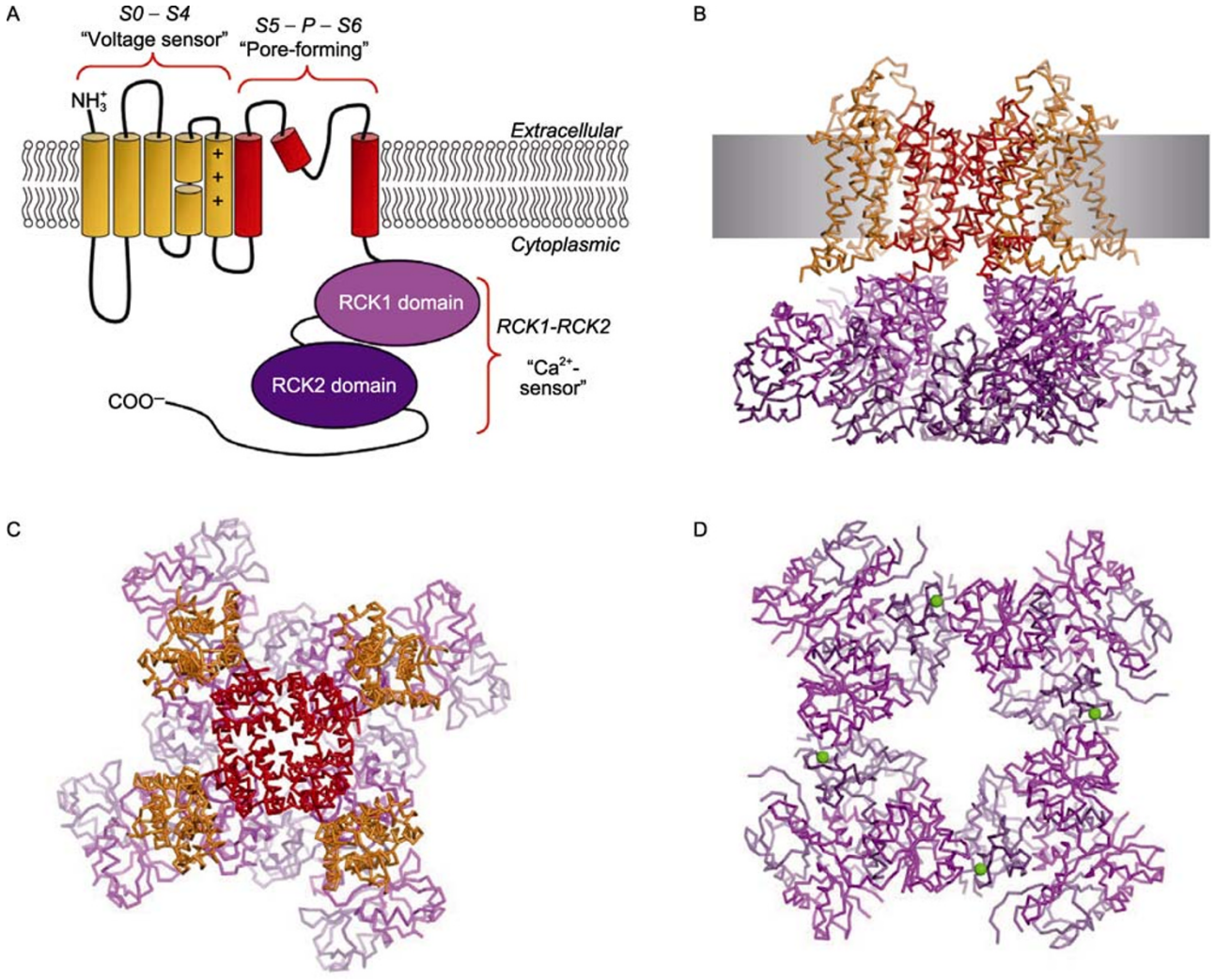

Figure 1. Molecular architecture of the BK channel. (A) Schematic diagram and topology of a BK channel subunit. Each subunit contains a transmembrane voltage-sensing domain (S0-S4 helices, orange) and pore domain (S5-P-S6 helices, red), and two tandem cytoplasmic RCK domains (RCK1, magenta; RCK2, purple). (B) Model of the human BK channel, based on alignment of the pore domain from the crystal structure of the Kv1.2-Kv2.1 chimera (PDB ID 2R9R) and MthK channel (PDB ID 3RBZ). The voltage-sensing domain, pore domain, and RCK domains have been colored according to the diagram in A, to illustrate the hypothetical three-dimensional arrangement of the domains. The approximate location of the plasma membrane is shaded gray. (C) BK channel model from part B viewed from above the extracellular side, illustrating the four-fold symmetrical arrangement of the domains about the central K^+ conduction pathway. (D) Crystal structure of the Ca^{2+}-bound "gating ring" of RCK domains (PDB ID 3U6N). Ca^{2+} ions are shown as green spheres.

a functionally important interaction with the β1 subunit (Wallner et al., 1996; Meera et al., 1997; Morrow et al., 2006; Liu et al., 2008). The S0 segment also forms an integral part of the BK channel voltage-sensor domain (Koval et al., 2007; Pantazis et al., 2010).

The remaining portion of each BK alpha subunit consists of a tandem pair of regulator of K^+ conductance (RCK) domains that form the cytoplasmic Ca^{2+} sensor of the channel (Schreiber and Salkoff, 1997; Schreiber et al., 1999; Bian et al., 2001; Jiang et al., 2001; Bao et al., 2002). The RCK domain is the key defining structural feature of the BK channel and its paralogs within the SLO K^+ channel subfamily, consisting of the BK channel (encoded by the KCNMA gene, also known as Kca1.1 or *slo*1), the Na^+-activated K^+ channel (KCNT, or *slo*2), and the voltage-dependent, H^+-inhibited K^+ channel known as *slo*3 (KCNU) (Wei et al., 1996; Schreiber et al., 1998; Yuan et al., 2000; Salkoff et al., 2006). Among these, BK and *slo*3 channels also appear to have the additional S0 transmembrane domain, defined by sequence homology, whereas *slo*2 channels do not (Yuan et al., 2000;

Koval et al., 2007).

Although BK channels are defined functionally by their relatively large unitary conductance and activation by both depolarization and cytoplasmic Ca^{2+}, BK channel currents measured in native tissues exhibit gating properties and Ca^{2+}-sensitivity that can vary across cell types. This diversity arises in part from mutiple alternative-splice sites that result in BK channels with different amino acid sequences, which can give rise to varied apparent sensitivities to depolarization and cytoplasmic Ca^{2+} in natively-expressed channels (Lagrutta et al., 1994; Johnson et al., 2011). In addition, BK channels in native tissues are known to co-assemble with at least two different classes of modulatory auxiliary subunits: the BK β subunits (β1–4), and a family of leucine-rich repeat containing proteins (LRRC proteins), now referred to as BK "γ" subunits (Knaus et al., 1994; Tanaka et al., 1997; Brenner et al., 2000a; Yan and Aldrich, 2010, 2012). The β subunits are each ~20 kDa per subunit, with two transmembrane segments and a large extracellular domain, whereas the γ subunits are each ~35 kDa per subunit, with a large, extracellular leucine-rich repeat domain consisting of six leucine-rich repeat units (LRR1–6), and a single transmembrane segment (Fig. 2). Because both of these classes of auxiliary subunits contain putative membrane-spanning segments and substantial extracellular domains, it seems likely that a component of their functional effects arises from interactions with the BK voltage-sensing domain (Morrow et al., 2006; Liu et al., 2008; Wu et al., 2009).

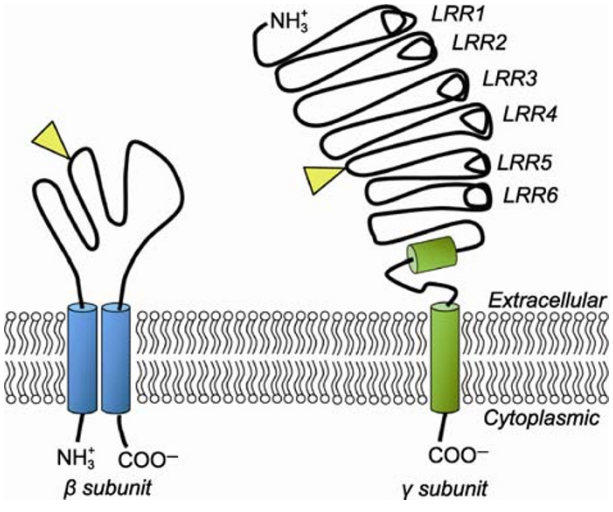

Figure 2. Schematic diagrams and topology of a BK channel β and γ auxiliary subunits. Each β subunit family member (left) contains two transmembrane segments connected by an extracellular domain, which contains a conserved N-linked glycosylation site (indicated by the yellow triangle). Each γ subunit family member (right) contains an extracellular leucine-rich repeat domain comprised of six leucine-rich repeat (LRR) units (LRR1–LRR6), followed by a single transmembrane segment. These subunits likely effect BK channel gating in part through interactions with the BK channel voltage-sensing domains.

Both β and γ subunits exhibit tissue-specific expression, and impose an array functional effects on BK channel activation properties, which can be inhibitory (β2 and β3), facilitatory (β1, γ1–4), or mixed (β4) (summarized in Table 1) (Nimigean and Magleby, 1999; Brenner et al., 2000a; Cox and Aldrich, 2000; Nimigean and Magleby, 2000; Bao and Cox, 2005; Wang et al., 2006; Yan and Aldrich, 2012). The inhibitory effect of the β2 subunit arises from an apparent "ball and chain" mechanism, with the inactivation "ball" encoded at the cytoplasmic carboxy-terminal end of the β subunit, binding at the cytoplasmic side of the BK channel pore and blocking current flow (Brenner et al., 2000a; Xia et al., 2003; Benzinger et al., 2006; Savalli et al., 2007; Gonzalez-Perez et al., 2012). In contrast, the inhibitory effect of the β3 subunit (specifically the β3b subtype) arises from inactivation imparted by the cytoplasmic amino-terminal end of the subunit, as well as effects on voltage-dependent gating independent of the amino-terminus (Zeng et al., 2001).

The functional effects of mixtures of different β and γ subunits on BK channel gating are not yet known. Because these different classes of auxiliary subunits can exhibit overlapping expression patterns (e.g., β4 with γ3 and γ4, and β1 with γ1), it will be important learn how combinations of β and γ subunits impact channel function and cellular physiology.

PHYSIOLOGY AND FUNCTION

As mentioned above, BK channels are opened by both membrane depolarization and cytoplasmic Ca^{2+} (Barrett et al., 1982; Moczydlowski and Latorre, 1983; Rothberg and Magleby, 2000; Horrigan and Aldrich, 2002). In biophysical studies of BK channel activation and gating using either native BK channels expressed in skeletal muscle cells or mouse or human BK channels heterologously-expressed in mammalian cell lines or *Xenopus* oocytes, it was found that BK channels required micromolar levels of Ca^{2+} to be completely activated with physiological levels of membrane depolarization (Barrett et al., 1982; Magleby and Pallotta, 1983; McManus and Magleby, 1991; Cox et al., 1997a; Cui et al., 1997; Rothberg and Magleby, 1999, 2000; Horrigan and Aldrich, 2002). This seemed to be at odds with the levels of global [Ca^{2+}] typically estimated during Ca^{2+} signalling events, which are thought to be <1 μmol/L.

It is now clear that BK channel opening in vascular smooth muscle is linked to Ca^{2+} release from the endplasmic reticulum (ER), which in turn leads to repolarization of the smooth muscle cell membrane (Brayden and Nelson, 1992; Jaggar et al., 1998; Knot et al., 1998; Perez et al., 1999). Although it was initially unclear whether ER Ca^{2+} release was sufficient to activate BK channels, it was discovered that highly localized Ca^{2+} release events, known as "Ca^{2+} sparks", are responsible. A Ca^{2+} spark results from concerted opening of

Table 1 Summary of tissue localization and functional effects of BK channel auxiliary subunits

Subunit	Tissue(s)	Functional effect	References
β1	Smooth muscle	Facilitates voltage-sensor activation	Nimigean and Magleby, 1999; Brenner et al., 2000a, 2000b; Cox and Aldrich, 2000; Nimigean and Magleby, 2000; Patterson et al., 2002; Qian et al., 2002; Bao and Cox, 2005; Wang and Brenner, 2006
β2	Chromaffin cells; ovary	Inactivation	Xia et al., 2003; Benzinger et al., 2006; Gonzalez-Perez et al., 2012
β3	Testis	Inactivation	Lingle et al., 2001; Zeng et al., 2001, 2007
β4	Brain	Facilitates voltage-sensor activation	Brenner et al., 2000a, 2005; Wang et al., 2006
γ1 (LRRC26)	Aorta; intestinal epithelium; trachea; prostate; thyroid; thymus; salivary gland	Facilitates voltage-sensor activation	Yan and Aldrich, 2010, 2012
γ2 (LRRC52)	Testis; skeletal muscle	Facilitates voltage-sensor activation	Yan and Aldrich, 2012
γ3 (LRRC55)	Brain	Facilitates voltage-sensor activation	Yan and Aldrich, 2012
γ4 (LRRC38)	Brain; testis; skeletal muscle; thymus; adrenal gland	Facilitates voltage-sensor activation	Yan and Aldrich, 2012

several ryanodine receptor (RYR) channels, which form apparent microdomains where the ER membrane in close proximity to the plasma membrane, localized near BK channels (ZhuGe et al., 1998, 2002). Within these microdomains, local [Ca^{2+}] can reach 10 μmol/L, which is sufficient for activation of BK channels leading to membrane repolarization (ZhuGe et al., 2002). Through this repolarization effect, BK channel activity deactivates voltage-dependent Ca^{2+} channels at the plasma membrane, limiting both Ca^{2+} influx and subsequent smooth muscle contraction (Fig. 3) (Filosa et al., 2006; Ledoux et al., 2006; Girouard et al., 2010).

In addition, BK channels in smooth muscle are co-expressed with the β1 subunit that contributes to enhancement of apparent Ca^{2+} sensitivity compared with BK channels expressed in the absence of the β1 subunit

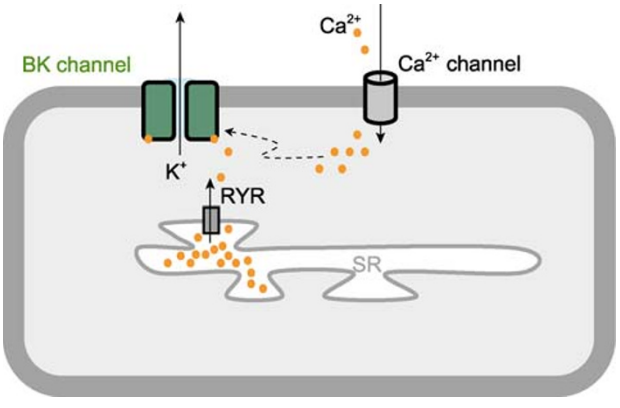

Figure 3. Representative pathway depicting modulation of BK channel activity through Ca^{2+} influx via Ca^{2+} channels at the plasma membrane (gray cylinder), or localized Ca^{2+} release events ("Ca^{2+} sparks") from intracellular stores such as sarcoplasmic reticulum (SR) via ryanodine receptors (RYR).

(Nimigean and Magleby, 1999; Brenner et al., 2000b; Cox and Aldrich, 2000; Nimigean and Magleby, 2000; Patterson et al., 2002; Qian et al., 2002; Zhu et al., 2002; Bao and Cox, 2005). Consistent with the presumed physiological role of these channels in regulating smooth muscle contraction, mice in which the BK β1 subunit has been knocked-out display chronic high blood pressure, coupled with cardiac hypertrophy that likely results from the chronically increased load on the heart muscle from pumping against a higher resistance (Brenner et al., 2000b). It is likely that BK channels in these tissues are also coexpressed with the γ1 subunit (LRRC26), which would be expected to further enhance opening of the channels arising from physiological Ca^{2+} signaling events (Yan and Aldrich, 2012). The physiological effects of targeted LRRC26 deletion are not yet known.

Aside from their well-established role in controlling Ca^{2+} entry in smooth muscle cells, BK channels also modulate the shapes of neuronal action potentials, which consequently controls Ca^{2+} entry in neurons (Brenner et al., 2005; Meredith et al., 2006; Wang et al., 2006; Jaffe et al., 2011). Knockout mice lacking the neuron-specific BK β4 subunit are prone to non-convulsive temporal lobe seizures, which arise in part from hyperexcitability of dentate gyrus neurons. This hyperexcitability, in turn, arises from rapid activation of BK channels lacking the β4 subunit. Normally, BK channels activate slowly relative to the action potential; this allows Ca^{2+} entry (which occurs during the action potential) to activate SK channels, thus inhibiting subsequent repetitive firing. In the β4 knockout mouse, BK channels are activated much more rapidly, thus terminating the action potential before Ca^{2+} entry is sufficient to allow opening of SK channels. Similarly, mice lacking the pore-forming BK alpha subunit exhibit altered neuronal function and poorly-regulated circadian rhythms, a phenotype that arises from hyperexcitability of circadian pacemaker neurons in the

suprachiasmatic nucleus (Meredith et al., 2006).

CURRENT WORKING HYPOTHESIS OF BK CHANNEL GATING

The key role of BK channels in regulating the cell membrane potential in response to cytoplasmic Ca^{2+} signals has stimulated investigation into the molecular mechanisms of their function. Because of their large ionic currents and conspicuous lack of inactivation, the voltage- and Ca^{2+}-dependent activation mechanisms of BK channels have been studied by elecrophysiological analysis with great quantitative rigor, leading to a remarkably detailed understanding of the energetics coupling between the voltage-sensor, the Ca^{2+}-sensor, and the molecular gate of the channel (Fig. 4) (Magleby and Pallotta, 1983; McManus and Magleby, 1991; Rothberg et al., 1996; Cox et al., 1997b, a; Cui et al., 1997; Rothberg and Magleby, 1998; Horrigan and Aldrich, 1999; Horrigan et al., 1999; Rothberg and Magleby, 1999; Cui and Aldrich, 2000; Rothberg and Magleby, 2000; Magleby, 2001; Horrigan and Aldrich, 2002; Magleby, 2003; Li and Aldrich, 2004; Rothberg, 2004; Piskorowski and Aldrich, 2006; Shelley et al., 2010).

We now understand the gating energetics of BK channels comprised of alpha subunits, as well as alpha + β1 and alpha + β4 subunits in terms of well-defined allosteric models, in which channel gating is parameterized as being modulated by the movement of four voltage sensors and four "Ca^{2+} sensors", where each Ca^{2+} sensor is governed by the binding of 1 Ca^{2+} ion. For BK channels comprised only of alpha subunits, for example, the channels are rarely observed to open under conditions in which the membrane is hyperpolarized and the cytoplasmic [Ca^{2+}] is less than 0.5 μmol/L; thus under these conditions, the closed state of the pore is energetically much more stable than the open state, by a factor of ~10^7. However, the equilibrium between the open and closed states is driven toward the open state by a factor of ~25 with the activation of each voltage sensor, and by a factor of ~8 with the activation of each Ca^{2+} sensor. Thus activation of all four voltage sensors and all four Ca^{2+} sensors will shift the gating equilibrium toward the open state by a factor of $(25)^4(8)^4$, or 1.6×10^9.

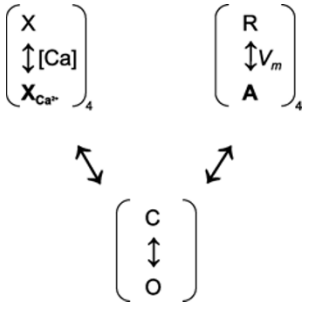

Figure 4. Abbreviated kinetic scheme to describe gating of BK channels by voltage and Ca^{2+} (adapted from Horrigan and Aldrich, 2002). BK channels consist of a four Ca^{2+} sensors that can be empty (X) or bound (X_{Ca}), four voltage sensors that can exist in Resting (R) or Activated (A) states, and a pore that can be Closed (C) or Open (O). Binding of Ca^{2+} or activation of voltage sensors drives the pore from the closed to the open state.

Electrophysiological analysis of heterologously-expressed BK channels comprised of alpha + β1 subunits demonstrated that the functional effects of the β1 subunit could be explained primarily by a stabilization of the BK channel voltage sensor in the activated state (Nimigean and Magleby, 1999; Cox and Aldrich, 2000; Nimigean and Magleby, 2000; Bao and Cox, 2005). Direct structural interaction between the β1 subunit and the BK voltage-sensing domain is supported by functional and biochemical analysis, which has demonstrated a key role for the S0 transmembrane helix and suggested that there are direct molecular contacts between S0 and the second transmembrane segment (TM2) of the β1 subunit, and between S1 and S2 and the first transmembrane segment (TM1) (Wallner et al., 1996; Morrow et al., 2006; Liu et al., 2008). Similarly, gating effects of the neuronal β4 subunit could be explained by stabilization of the BK channel voltage sensor in the activated state, in combination with relative stabilization of the pore in the closed state in the absence of voltage-sensor activation, giving rise to a gating phenotype in which the voltage-dependent activation is shift toward more positive voltages at low [Ca^{2+}] and more negative voltages at higher [Ca^{2+}] in channels comprised of alpha + β4 subunits (Wang et al., 2006; Wu et al., 2009).

It can be seen from the studies described above that conclusions drawn from kinetic modeling of BK channel gating have been remarkably informative in terms of functional and structural mechanisms of gating and association with auxiliary subunits. It is likely that functional and quantitative kinetic strategies will continue to be useful in continued analysis of the gating mechanism.

THE CYTOPLASMIC Ca^{2+}-SENSING DOMAIN

In structural terms, one of the most intriguing and unique aspects of the BK channel is its large cytoplasmic domain, which accounts for approximately 2/3 of the mass of its pore-forming alpha subunit (Butler et al., 1993). Initially, a cluster of five aspartate residues (D897–D901) within the sequence of cytoplasmic domain was proposed to form a possible Ca^{2+} binding site; consistent with this idea, charge-neutralization of these residues resulted in reduction of the channel's Ca^{2+} sensitivity (Schreiber and Salkoff, 1997; Schreiber et al., 1999). However, mutations at this site, dubbed the "Ca^{2+} bowl", could not on their own abolish Ca^{2+} sensitivity of the channel. It was soon found that additional charge-neutralizing mutations had to be introduced to eliminate Ca^{2+} sensitivity, suggesting that the cytoplasmic domain contains at least three different sites that underlie activation

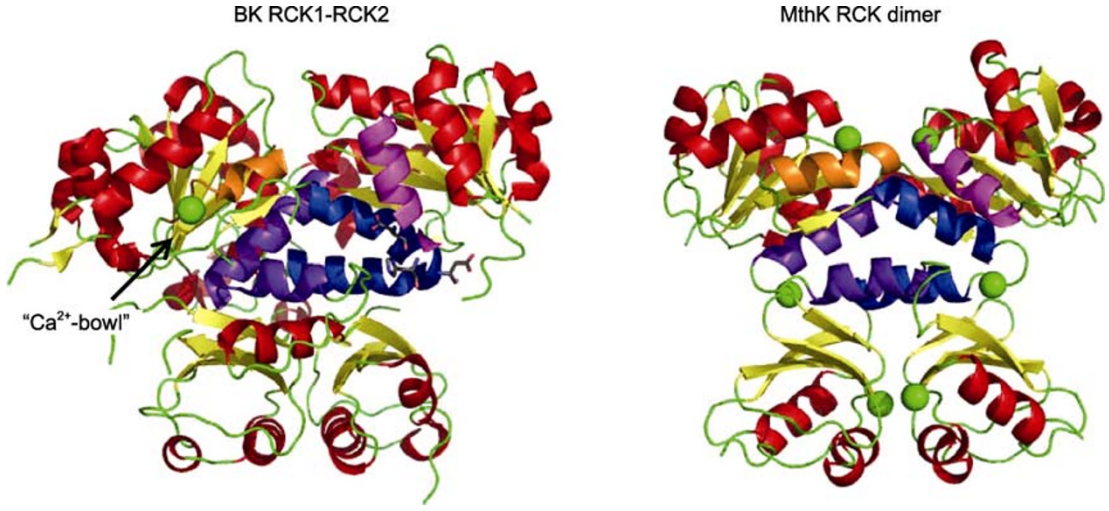

Figure 5. Comparison of BK and MthK RCK domains. The BK channel tandem RCK domains (RCK1-RCK2, left, PDB ID 3MT5; Yuan et al., 2010), shown next to a MthK channel RCK dimer (right, PDB ID 3RBZ; Pau et al., 2011). Selected helices have been colored to illustrate apparent homologous regions common to BK and MthK. Other secondary structural elements are colored according to: alpha = red, beta = yellow, coil = green. Ca^{2+} ions identified in the crystal structures are shown as green spheres.

by divalent cations (Zhang et al., 2001; Bao et al., 2002; Shi et al., 2002; Xia et al., 2002; Bao et al., 2004; Cox, 2005; Zeng et al., 2005; Hu et al., 2006; Zhou et al., 2012).

Initial insight toward the molecular architecture of the cytoplasmic domain came from the discovery of a class of prokaryotic orthologues of the BK channel. This class of channels was defined by the presence of the conserved RCK domain, which was found to be similar to the BK cytoplasmic domain. One of these prokaryotic K^+ channels, MthK (from *Methanobacterium thermoautotrophicum*) was crystallized, revealing a structure in which a four-fold symmetrical ring of eight RCK domains, called the "gating ring", undergoes conformational changes with the binding of multiple Ca^{2+} ions (Jiang et al., 2002; Dong et al., 2005; Ye et al., 2006; Pau et al., 2011). The discovery of this prokaryotic structure provided a great deal of insight, allowing for structural interpretation of functional studies performed on BK channels. For example, it was this work that led to the idea that Ca^{2+}-dependent conformational changes in the RCK domains acted on the pore-lining helices of the channel through an apparently mechanically-passive linker sequence (Niu et al., 2004).

Recently, the BK channel cytoplasmic domain was crystallized, revealing a remarkable degree of conservation in the domain structure (Fig. 5), and formation of an RCK gating ring arrangement that is similar to that observed in MthK (Fig. 1D) (Wu et al., 2010; Yuan et al., 2010, 2011). Along with this groundbreaking achievement, however, further intriguing questions are raised. For example, structures of the BK cytoplasmic domain in the presence of Ca^{2+} revealed a Ca^{2+} ion bound in the predicted Ca^{2+} bowl, showing a structural motif that was remarkably well-predicted by previous functional studies (Bao et al., 2004). However, no Ca^{2+} ions are observed at the other Ca^{2+} binding sites predicted by mutagenesis and functional experiments. Because the known crystal structures consist only of the cytoplasmic domain, one could ask whether Ca^{2+} binding at the other sites requires crystallization of the entire channel complex? Additional experiments are likely to hold the answer.

CONCLUSIONS

BK channels are physiologically important molecules involved in signaling pathways in neurons and smooth muscle, tissues that are critical to human health and are thus vulnerable to injury and disease. A great deal of effort over the past 30 years has led to elucidation of auxiliary proteins involved in the tissue-specific functions of BK channels, as well as the structural and molecular basis of BK channel gating. However, as structural studies reveal the atomic basis of Ca^{2+} coordination by the channel, new questions arise concerning the molecular basis of BK channel gating (Wilkens and Aldrich, 2006; Chen and Aldrich, 2011), suggesting that new mechanisms have yet to be revealed.

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