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Review

Molecular mechanisms of BK channel activation

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Abstract. Large conductance, Ca^{2+}-activated potassium (BK) channels are widely expressed throughout the animal kingdom and play important roles in many physiological processes, such as muscle contraction, neural transmission and hearing. These physiological roles derive from the ability of BK channels to be synergistically activated by membrane voltage, intracellular Ca^{2+} and other ligands. Similar to voltage-gated K^+ channels, BK channels possess a pore-gate domain (S5–S6 transmembrane segments) and a voltage-sensor domain (S1–S4). In addition, BK channels contain a large cytoplasmic C-terminal domain that serves as the primary ligand sensor. The voltage sensor and the ligand sensor allosterically control K^+ flux through the pore-gate domain in response to various stimuli, thereby linking cellular metabolism and membrane excitability. This review summarizes the current understanding of these structural domains and their mutual interactions in voltage-, Ca^{2+}- and Mg^{2+}-dependent activation of the channel.

Keywords. Channel gating, MaxiK, calcium activation, voltage activation, metal binding, allosteric.

Introduction

BK channels have a large single-channel conductance (~100–300 pS), which is the basis for their designation as Big K^+ (or MaxiK) channels [1–3]. BK channels are found in neurons [4–8], chromaffin cells [9–13], inner hair cells of cochlea [14–18], and skeletal [19–21] and smooth muscles [22–29]. These channels are activated by membrane depolarization and elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (Fig. 1A). Due to these properties, the activation of BK channels results in repolarization of the membrane and closing of voltage-dependent Ca^{2+} channels to reduce Ca^{2+} entering the cell. Therefore, BK channels primarily serve as negative feedback regulators of membrane potential and $[Ca^{2+}]_i$, which are important in a number of physiological processes. These include controlling the interspike interval and spike frequency adaptation [5, 6, 30–34], modulating neurotransmitter release [17, 32, 35–38] and endocrine secretion [39–41], tuning hair cell firing frequencies in the auditory system [15, 16, 42–47], and regulating vascular [48–52], urinary bladder [53–56] and respiratory tone [26, 57–59]. Consistent with their physiological importance, malfunction of BK channels can lead to epilepsy [60, 61], motor impairment [62], noise-induced hearing loss [63], hypertension [49, 64–70], urinary incontinence [25], overactive urinary bladder [25, 71] and asthma [72].

In certain cells, BK channels are in close proximity to Ca^{2+} sources. For example, in neurons, BK channels are colocalized or physically associated with voltage-dependent Ca^{2+} channels (VDCCs) [73–77], NMDA

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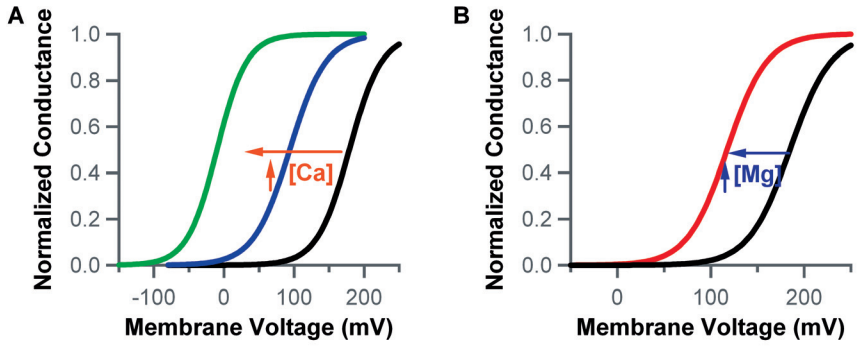

Figure 1. BK channel activation by voltage, Ca^{2+} and Mg^{2+}. (*A*) The conductance-voltage (G–V) relationship of mSlo1 BK channels in 0 (black), 2 (blue) and 100 μM Ca^{2+} (green). (*B*) The G–V relationship of BK channels in 0 (black) and 10 mM Mg^{2+} (red) With an increasing $[Ca^{2+}]_i$ (*A*) or $[Mg^{2+}]_i$ (*B*), the BK channels open at less-positive voltages.

receptors [78] and ryanodine receptors (RyRs) [74]. Normally, the global $[Ca^{2+}]_i$ is carefully regulated so that small changes in the global $[Ca^{2+}]_i$ will only open a few BK channels even at the peak of action potential. However, the $[Ca^{2+}]_i$ near the Ca^{2+} source, termed the microdomain $[Ca^{2+}]_i$, can be much higher [79] to ensure the opening of additional BK channels. The large K^+ efflux through BK channels efficiently repolarizes the membrane and halts Ca^{2+} entry.

Besides voltage and Ca^{2+}, intracellular Mg^{2+} also activates BK channels [80, 81]. Activation of the BK channel by multiple stimuli is an important property that allows the channel to integrate various cellular signals in modulating membrane excitability and Ca^{2+} homeostasis. This property also makes the BK channel a fascinating molecule in studying the mechanism of ion channel gating. The purpose of this review is to summarize the current understanding of the molecular gating mechanisms of BK channels in response to voltage, Ca^{2+} and Mg^{2+}, as well as the major historic developments leading to this understanding. We will first sketch the structure of BK channels, which reveals their homology to both voltage-gated and ligand-gated channels. Such a homology and functional similarities to both voltage- and ligand-gated channels suggest a similarity in the underlying molecular gating mechanisms. We will then dissect the gating mechanism for each stimulus: voltage, Ca^{2+} and Mg^{2+}. Channel gating involves two major steps: sensing of the stimulus by the sensor domain and conveying the conformational change from the sensor domain to open the activation gate, known as coupling. Structural information and functional studies of ion channels have shown that distinct structural domains underlie the activation gate and sensors for stimuli [82]. Accordingly, the gating mechanism of each of the stimuli for BK channels is divided into two parts, the identification of the sensor and the mechanism of coupling. We will then describe the effect of each stimulus on the mechanism of other stimuli in activating BK channels. Finally, we will describe the activation gate itself. Readers may obtain further insights from other excellent reviews about the BK channel's role in physiology [2, 33, 51, 83, 84], gene family [85], modulation [86–89], auxiliary subunits [39, 64, 90], pharmacology [91–93] and gating mechanisms [94–101].

Structure of BK channels

BK channels are encoded by a single *slowpoke* gene termed *slo1* [102–104]. These channels were first identified in mutant *Drosophila melanogaster,* which exhibited a lethargic phenotype due to a lack of Ca^{2+}-dependent K^+ current [102, 103]. Soon after, the mammalian orthologue of this gene was cloned from mouse and human [104, 105]. The initial hydropathy analysis suggested that a Slo1 protein may contain 10 hydrophobic segments [104]. Subsequent electrophysiology characterization and immunocytochemistry experiments on epitope-tagged BK channels revealed that the Slo1 protein comprises a membrane-spanning domain with seven transmembrane segments (S0–S6) and a large C-terminal cytoplasmic domain [106, 107] (Fig. 2A).

The channel's primary sequence of the membrane-spanning domain showed similarities to voltage-dependent K^+ (K_V) channels, including the voltage-sensing (VSD, S1–S4) and pore-gate (PGD, S5–S6) domains (Fig. 2A). Similar to K_V channels, a functional BK channel is formed by tetramerization of Slo1 proteins [108, 109]. However, unique to the BK channel is an additional transmembrane segment, S0, that confers the N-terminus to the extracellular side and a large cytoplasmic domain containing ~800 amino acids. The sequence and predicted secondary structure of the cytoplasmic domain are homologous to a regulatory domain for K^+ conductance (RCK domain) (Fig. 2) that is found in a number of K^+ channels and transporters [97, 110–114]. Thus, a functional BK channel can be divided into three major structural domains: the VSD senses voltage, the C-terminal cytoplasmic domain senses various intra-

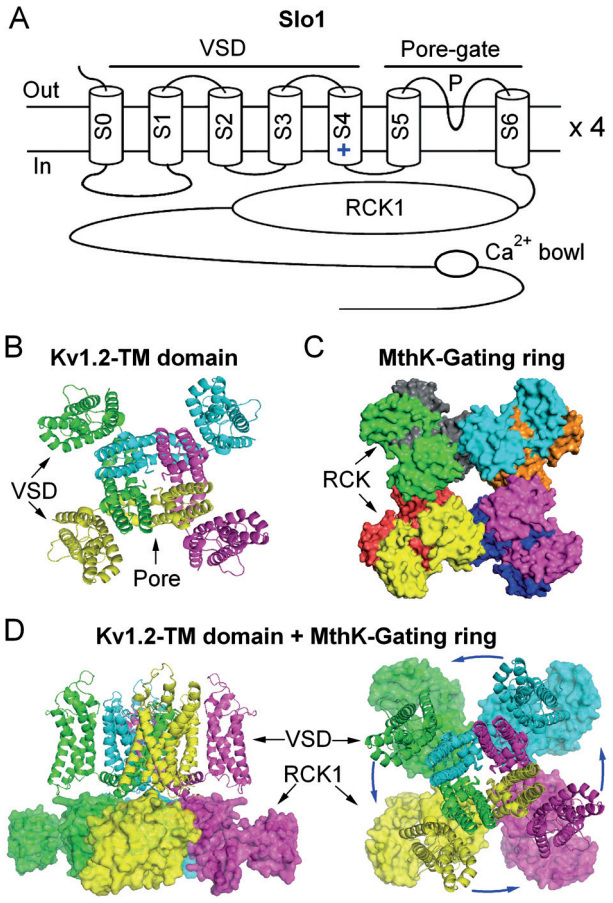

Figure 2. BK channel structure. (*A*) Membrane topology of the Slo1 subunit. VSD: voltage-sensor domain; P: pore loop. (*B*) $K_v1.2$ structure (PDB ID: 2A79) [115]. Four subunits are represented in different colors. Only the membrane-spanning domain of the structure is shown. (*C*) The gating ring structure of MthK (PDB ID: 1LNQ) [110]. Eight identical RCK domains are represented in different colors. (*D*) BK channel homology model based on the superposition of the $K_v1.2$ structure and the MthK structure by aligning their selectivity filter regions. Four different colors represent four subunits. Only the membrane-spanning domain of $K_v1.2$ (ribbons) and the four RCK domains of MthK close to the membrane (surface) are shown. Left: Side view. Right: Top view from the membrane. Blue arrows indicate that the relative arrangement of the VSD and the cytoplasmic domain of BK channels might be different from the homology model [118].

cellular ligands, and the PGD controls ion permeation in response to different stimuli.

The three-dimensional (3D) structure of the BK channel has not been solved. However, the X-ray crystallographic structure of $K_V1.2$ (Fig. 2B) [115] has been used as a model for the membrane-spanning domain of BK channels due to their sequence homology and functional similarity [116–119]. This model has not been systematically tested by experiments, but it is almost certain that the details of the BK channel structure would differ from that of $K_V1.2$, primarily because of the additional S0 transmembrane segment [117, 120] and the long linker between S0 and S1 (~70 amino acids). By performing a set of disulfide cross-linking experiments in which double residues, one flanking S0 and the other flanking S1, S2, S5, S6 transmembrane segments, or in the S3–S4 loop, were mutated to cysteine, Liu et al. showed that the extracellular end of S0 is likely to reside close to S1, S2 and the S3–S4 loop, but not S5 or S6 [117]. In a model based on $K_V1.2$ structure, these results would place the extracellular end of S0 in the cleft among S1–S4 helices that insulate S0 from the pore domain. Koval et al. inspected the function of S0 by performing a tryptophan scan and found that the mutants F25W, L26W and S29W, which are on the same side of the predicted S0 helix embedded in the membrane, altered channel function [120]. This result suggests that the middle of S0 may make direct contact with the VSD (S1–S4). The presence of S0 and its close contacts with S1–S4 would affect the conformation of BK channels differently in the open and the closed states [117, 120].

Homology models for the cytoplasmic domain of BK channels have been proposed based on the RCK domain structures of the *Escherichia coli* six-transmembrane K^+ channel and the Ca^{2+}-gated K^+ channel, MthK, from *Methanobacterium thermoautotrophicum* [94, 110, 111, 121–126]. These prokaryotic RCK domains adopt a Rossman fold topology at its core with β strands (βA–βJ) sandwiched by α helices (αA–αJ) [110, 111]. In the quaternary structure of the MthK channel, eight identical RCK domains assemble into an octameric ringlike shape that is known as the gating ring (Fig. 2C). Each RCK domain is associated with two neighboring RCK domains alternately with either a fixed (or assembly) or flexible interface. The cytoplasmic domain of Slo1 protein can be divided into two separate structures [127], both with sequence homology to the RCK domain [110], known as RCK1 and RCK2. Therefore, it was proposed that BK channels would also contain a gating ring formed by eight RCK domains from the four Slo1 subunits [94, 110, 124–126].

Several laboratories have published similar sequence alignments between the RCK1 of BK channels and the prokaryotic RCK domains (Table 1). The predicted RCK1 structure has been supported by a number of experimental results. First, based on the structure of the RCK domain in the *E. coli* K^+ channel, it was predicted that in the putative RCK1 domain, K448 at the C-terminus of αD and D481 in the loop between αE and βF would form a salt bridge [111]. The experimental data on the BK channel supported the existence of the salt bridge. Individual mutations, K448D and D481K, both shifted the G–V relation of BK channels to more positive voltages, but the double-mutation K448D:D481K restored the G–V

close to that of the wild-type channel [111]. Second, mutational studies suggested that E374 and E399 in RCK1 were part of the metal binding site for Mg^{2+}-dependent activation [118, 121, 122, 128, 129]. When mapped onto the prokaryotic RCK structures, these two residues are located at the N-terminus of the parallel βB and βC, respectively [118, 121], and are close to each other, supporting the mutational results that these two residues are part of the same metal binding site. Third, Hou et al. found that H365 and H394 in the RCK1 serve as the H^+ sensor for BK channel modulation [130]. In an RCK1 homology model based on the MthK structure, the two histidine residues were found close to D367, which serves as a Ca^{2+} sensor [122]. Hou et al. found that the mutations on H365 and H394 affected Ca^{2+} sensitivity, and reciprocally, the mutations of D367 altered H^+ sensitivity. These results are consistent with the model prediction that these residues affect both H^+ and Ca^{2+} sensitivities with an electrostatic interaction [130]. The above experimental results all demonstrated that residues far apart in the sequence are located close in the 3D structure, as predicted by the homology model of the RCK1 domain.

Unlike for RCK1, there has been little consensus in published sequence alignments for RCK2 (Table 1). Furthermore, based on a statistical calculation, Fodor and Aldrich suggested that the sequence homology between the BK channel and other RCK domains may not be sufficient to support the presence of the RCK-like structure in the putative RCK2 domain [112]. Nevertheless, using circular dichroism (CD) spectroscopy, Yusifov et al. measured the secondary structure composition of the C-terminus sequence of human Slo1 corresponding to 90% of the proposed RCK2 domain, and found that the α-helix and β-strand contents closely correlated with the predicted secondary structure based on their proposed structural alignments [124]. In another study, using the MthK gating ring as a guide, Kim et al. examined whether BK channel residues arising from different RCK domains participated in a putative fixed interface. Using a mutant cycle strategy, they measured G–V relations of mutant channels at a fixed $[Ca^{2+}]_i$ of 5 μM, and found that the coupling energy of the mutant cycles was not zero. These results led Kim et al. to argue that residues from RCK1 and RCK2 interact, thereby participating in an interface [126]. On the other hand, the proposed RCK2 structure was predicted to contain a salt bridge between K898 and D927, similar to that found in the RCK1 domain [111]. However, unlike the experimental results with the salt bridge in the RCK1 domain [111], mutations of K898 or D927 did not cause any significant functional change [126]. These mixed results make the homology model of RCK2 less certain than that of RCK1.

While the structures of $K_V1.2$ and MthK serve as a template for the homology model of the membrane spanning and cytoplasmic domains, no structural template is available to show how the two domains of the BK channel assemble. Recent studies show that residues from both the membrane-spanning VSD and the cytoplasmic RCK1 domain form the Mg^{2+} binding site [118], and the bound intracellular Mg^{2+} makes an electrostatic interaction with the voltage sensor [129]. These results indicate that the two domains are positioned close to each other and interact intimately. On the other hand, with a series of elegant experiments Zhang et al. showed that the BK channel has a central antechamber between the pore domain and the cytoplasmic domain that can accommodate the inactivation peptide from the accessory β2 subunit and protect it from intracellular trypsin digestion [131]. Together, these results suggest that at some positions the membrane-spanning and cytoplasmic domains come near each other, while at other positions they may be sufficiently separated to allow entry of bulky peptides. Besides the vertical distances between the membrane-spanning and cytoplasmic domains, another question concerning domain assembly is how the VSD and the cytoplasmic domain align laterally with each other in BK channels. Figure 2D shows the combined structures of $K_V1.2$ [115] and MthK [110] by aligning the selectivity filter of the two channels. This figure shows that, if the VSD and the RCK1 domain of BK channels pack against the PGD similarly to that of $K_V1.2$ and MthK, the VSD would be located just above the RCK1 domain from the same subunit. However, by studying the formation of the Mg^{2+} binding site, Yang et al. recently suggested that the VSD of one subunit is located on top of the RCK1 domain from the neighboring subunit (see "Mg^{2+}-dependent activation of BK channels" for more details) [118]. The contradiction between the experimental results and the homology model implies that the packing of VSD or the cytoplasmic domain relative to the PGD in BK channels may differ from that of $K_V1.2$ or MthK channels. Several unique features of BK channels may contribute to this discrepancy: 1) the VSD of BK channels contains an additional S0 segment and a long cytoplasmic loop connecting S0 and S1; 2) the interaction and packing between the RCK1 domain and the rest of the cytoplasmic domain of BK channels may differ from that of the RCK domains in the gating ring of the MthK channel; 3) other interactions may exist between the VSD and the cytoplasmic domain.

Table 1. Localization and identification of important structural features in BK channels. The residue numbers refer to the position on *mbr5* splice variant of mSlo1 [104]. The locations of the fixed or flexible interface in RCK1 or RCK2 are identified by comparing the sequence alignment to that of MthK [110, 111] without any experimental evidence, except for the residues denoted by * that were experimentally identified by Kim et al [125, 126].

Ref. (structure used for alignment)	Domains		Interfaces				Ca^{2+} Binding Sites		
	RCK1	RCK2	Fixed		Flexible		D362	D367	C^{a2}+ bowl
			RCK1	RCK2	RCK1	RCK2			
Jiang et al., 2001 [111] (*E.coli*)	344–514 (βA–αG)		αD (440–448)		αF (489–497) αG (507–514)		middle–end of αA	between αA and βB	
Jiang et al., 2002 [110] (MthK)	344–611 (βA–αJ)		αD (437–450) αE (465–473)		αF (487–496) αG (502–512)		middle–end of αA	between αA and βB	
A.Pico's thesis, 2003 [177] (MthK)	344–612 (βA–αJ)	721–1055 (βA–βJ)	αD (437–450)	αD (820–833)	αF (487–498) αG (501–511)	αF (920–931) αG (934–944)	middle–end of αA	between αA and βB	between βE and αE
Roosild et al., 2004 [234] (MthK)	345–498 (βA–αF)	722–871 (βA–αF)	αD (435–447)	αD (818–830)	αF (487–498)	αF (860–871)	middle of αA	end of αA	after RCK2 domain
Kim et al., 2006 and 2008 [126, 125] (MthK)	344–619 (βA–αJ)	721–879 (βA–βF)	αD (437–456) αD (441)* αD (442)* αD (445)*	αD (820–830) αD (824)* αD (825)* αD (828)*	αF (487–496) αG (502–512) αF (490)*	αA (736)*	middle of αA	end of αA	after RCK2 domain
Latorre and Brauchi, 2006 [94] (*E.coli*)	344–512 (βA–αD: normal β–α–β structure; after that all α helices: αX, αE, αF and αG)		αD (437–450)		αF (482–496) αG (504–512)		middle of αA	between αA and βB	
Yusifov et al., 2008 [124] (MthK)	344–612 (βA–αJ)	721–987 (βA–αJ)	αD (437–450)	αD (820–833)	αF (486–494) αG (503–512)	αF (865–873) αG (882–891)	end of αA	between αA and βB	between αG and βG (891–902)

Voltage-, Ca^{2+}- and Mg^{2+}-dependent activation of BK channels

Voltage-dependent activation of BK channels

Voltage sensors. The voltage dependence of BK channels changes in different $[Ca^{2+}]_i$ (Fig. 1A). In the early days, there were two different views on the origin of voltage dependence. One suggested that voltage-dependent activation derives from the voltage-dependent binding of Ca^{2+}; the other proposed that voltage dependence is one of the intrinsic properties of BK channels, independent of Ca^{2+}-dependent activation. The latter view gained more support after cloning of the BK channel, which predicted the presence of a VSD. Specifically, the S4 transmembrane segment contains multiple regularly spaced Arg residues, a hallmark of VSD in K_V channels [102–104]. Macroscopic ionic current measurements in the absence of Ca^{2+} [132, 133] and gating current recordings [134, 135] support the notion that BK channels, similar to K_V channels, contain an intrinsic VSD. Mutation of the Arg residues in S4 to neutral amino acids reduced the steepness of the G–V relation [136, 137], further suggesting that the S4 segment in BK channels also serves as a voltage sensor.

To identify the charged residues in the VSD (S1–S4) that contribute to voltage sensing, Ma et al. mutated each of these residues and measured the reduction in the effective gating charge [116]. The number of gating charges in the WT and mutant BK channels in their study was estimated by measuring the probability of channel opening (P_O: 10^{-6} ~ 10^{-1}) over a broad voltage range and fitting the data to the allosteric HA model (Fig. 3A, see below). Four residues were thus identified and referred to as the voltage-sensing residues: D153 and R167 in S2, D186 in S3 and R213 in S4 (Fig. 2A). Neutralization of each of these residues in four mSlo1 subunits reduced effective gating charge by 0.92, 0.48, 0.88 and 1.20, respectively, from 2.32 of the WT channel [116, 135]. In an earlier study, Diaz et al. reported that, besides R213, R210 in S4 also contributed to gating charge based on limiting slope measurements [136]. However, Ma et al. found that with more stringent conditions (at $P_O \leq 10^{-6}$ vs. at $P_O \geq 10^{-3}$ in [136]), limiting slope measurements indicate that R210 is not a voltage-sensing residue [116]. Compared with K_V channels such as Shaker, which has ~12–13 effective gating charges [138, 139], the BK channel is less sensitive to voltage. Besides this difference, two unique features of the voltage sensor of BK channels are noteworthy: 1) Out of three Arg residues in S4, only R213 is a voltage-sensing residue. Mutations of the other Arg residues (R207 and R210) do not affect the amount of gating charge [116].

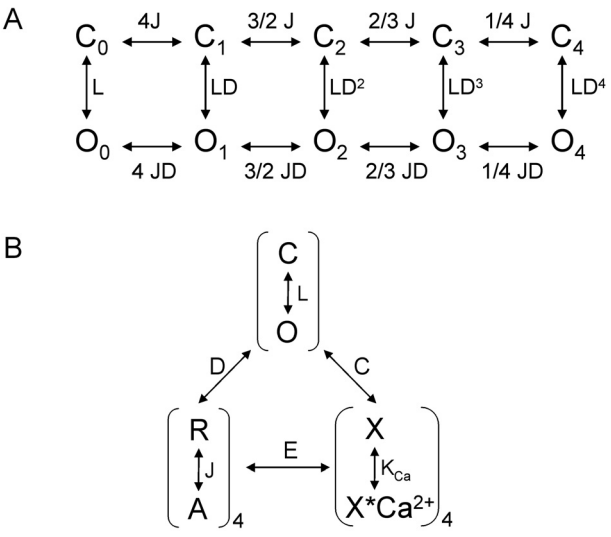

Figure 3. Allosteric gating mechanisms of BK channels. (*A*) Mechanism of voltage-dependent gating [143]. Channels can open when 0–4 voltage sensors activate in the four subunits, resulting in five closed (C_0–C_4) and five open (O_0–O_4) states. J represents the equilibrium constant for voltage-sensor activation in each subunit. $J = exp[(V-V_{hC})(z_J e/kT)]$, where V is voltage, $V_{hC} = +150$ mV is the half-activation voltage at the closed state, $z_J = 0.58$ is the amount of charge movement during voltage-sensor activation, e is the charge of an electron, k is Boltzmann's constant and T is absolute temperature. L is the intrinsic equilibrium constant for channel opening when all voltage sensors are in the resting state. $L = L_0\ exp[(V)(z_L e/kT)]$, where $L_0 = 9.8 \times 10^{-7}$ is the equilibrium constant at 0 mV and $z_L = 0.3$ is the number of equivalent charge movement during gate opening. $D = 25$ is the allosteric factor coupling voltage-sensor activation to channel opening. (*B*) A general allosteric mechanism, including voltage and Ca^{2+}-dependent activation, and the interaction between Ca^{2+} binding and voltage-sensor activation [179]. The allosteric coupling (with allosteric factor D) between voltage-sensor activation $[R-A]_4$ and channel opening [C–O] can be expanded as the 10-state scheme in *A*. Likewise, the allosteric coupling (with allosteric factor C) between Ca^{2+} binding $[X-XCa^{2+}]_4$ and channel opening [C–O] can be expanded into a similar 10-state scheme. Here it is assumed that the channel contains four Ca^{2+} binding sites although the model can be easily expanded to contain eight Ca^{2+} binding sites. Channels can open when 0–4 Ca^{2+} are bound, resulting in five closed (C_0–C_4) and five open (O_0–O_4) states. K_{Ca} is the equilibrium constant for Ca^{2+} binding. $K_{Ca} = [Ca^{2+}]_i/K_{dC}$ at closed states and $[Ca^{2+}]_i/K_{dO}$ at open states, where $K_{dC} = 11$ μM and $K_{dO} = 1.4$ μM are the elementary Ca^{2+} dissociation constants. $C = K_{dC}/K_{dO} = 8$. The allosteric factor E describes the interaction between Ca^{2+} binding and voltage-sensor activation. At saturating Ca^{2+} binding, J increases by the factor $E = 2.4$.

According to the alignment of Shaker and BK channels [116, 140], R213 in S4 of BK channels corresponds to the fourth Arg in S4 of K_V channels. Each of the first four Arg residues (R1–R4) in S4 of K_V channels moves across partial or the entire transmembrane electric field, and accounts for either a half or a full gating charge [139]. However, in S4 of BK channels, only R213 accounts for a mere 0.3 gating charge [116]. This result suggests that either S4 in BK channels does not move a distance along the voltage gradient as large as that in K_V channels during

activation or the voltage drop along the path of S4 movement is small. 2) Besides R213 in S4, other voltage-sensing residues, D153, R167 and D186, are located in either S2 or S3. These residues are also conserved in K_V channels; none of them, however, serves as gating charge. Interestingly, in Shaker K^+ channels, the residues corresponding to D153 and D186 have been shown to form a network of electrostatic interaction with Arg residues in S4 at either the resting or active state [115, 141, 142]. If such a network of electrostatic interaction exists among the voltage-sensing residues in the BK channel, it is possible that a mutation to any one of these residues could alter the movement of others, resulting in an indirect effect on the number of effective gating charges. This or other unspecified indirect effects must exist, because the summed decreases in the amount of effective gating charge produced by neutralizing these residues (3.48) exceeds the total charge of the WT channel (2.32) [116]. At present, it is not clear which of the four voltage-sensing residues are affected by such indirect effects and how much of their reported contribution to the gating charge is due to indirect effects. The contribution of specific amino acid residues to the effective gating charge in K_V channels has been estimated by a number of different approaches [138, 139], and the results, in general, agree with one another. For BK channels, more studies using different approaches would help to more firmly establish the identity and contribution of individual amino acid residues as voltage sensors.

Coupling between voltage sensors and the activation gate. In response to depolarization, the voltage sensor in BK channels moves from a resting state to an active state, resulting in transient gating currents (I_g) [134, 135]. While it enhances P_O, voltage-sensor activation is not obligatory for channel opening. At negative voltages ($\leq$ –20 mV), where the voltage sensor of the BK channel is mostly at the resting state and the probability of channel opening due to voltage-sensor movement is small ($P_O \leq 10^{-4}$), residual channel opening can be detected [143]. Channel opening at this voltage range has a much weaker voltage dependence than in positive voltages (30–80 mV), indicating that the activation gate can open with a weak voltage dependence while voltage sensors are in the resting state.

Analysis of the kinetics and steady-state properties of BK currents suggests that, at positive voltages, the voltage sensor in each of the four subunits moves from a resting state to an active state independently, and the channel can open when 0, 1, 2, 3 or 4 voltage sensors are activated. Horrigan et al. proposed an allosteric model (the HCA model) to describe the coupling between voltage sensor and the activation gate (Fig. 3A) [143]. In this model, voltage sensors can move from the resting state to the active state at either closed or open conformation, and voltage sensor activation promotes channel opening (with an equilibrium constant L) by selectively destabilizing the closed conformation with an allosteric factor D. Similarly, the equilibrium constant for voltage-sensor activation (J) increases D-fold in favor of the active state, when the channel opens (Fig. 3A). The model illustrates the idea that the BK channel undergoes two types of conformational change: voltage-sensor activation and channel opening; neither type is obligatory for the other to occur, but they influence each other via an allosteric mechanism. This two-tiered allosteric model is consistent with the kinetic properties of the macroscopic K^+ current in response to voltage pulses; the activation and deactivation time courses of the current are exponential after a brief delay [133, 143]. Single-channel analyses have also revealed multiple open and closed states in 0 Ca^{2+}, supporting the two-tiered allosteric model for voltage-dependent activation [144–146]. The voltage dependence of channel gating derives primarily from the voltage dependence of transitions among closed (C–C) or open states (O–O) [146], compatible with the idea that the voltage sensor activates at either closed or open states, while channel opening has a weak voltage dependence [143].

The properties of gating currents (I_g) are also consistent with key features of the HCA model. First, the kinetics of gating current relaxation is rapid [134, 143]. The time course of the fast on-gating current accounts for the initial delay in channel opening after a depolarizing voltage pulse, and its relaxation is almost completed before channels begin to open [143]. This result reveals voltage-sensor activation and channel opening as two types of conformational change, and the movement of voltage sensors promotes channel opening. Second, gating currents contain kinetically distinctive components, which can be assigned to voltage-sensor movements during C–C, O–O and C–O transitions [143]. These results clearly demonstrate that voltage sensors can move in both the closed and open conformations. Third, voltage-sensor activation contains a slow component that increases with a time course in parallel with channel opening [143]. This result is consistent with the idea that voltage-sensor activation is facilitated at the open conformation; as more channels open, the easier it is for the gating charges to move to the active state. In a study in which residues in the S3–S4 linker of hSlo1 were tagged with fluorescent molecules, Savalli et al. showed that the change of fluorescence had time constants similar to that of ionic currents (I_K), but voltage dependences

similar to that of I_g, consistent with the view that the VSD moves as the BK channels open [147].

In contrast to the allosteric model for BK channel gating, so-called obligatory models are usually used to describe K_V channel gating. The simplest obligatory model is the Hodgkin–Huxley type sequential model, C–C–C–C–O, in which four identical voltage sensors are activated independently by depolarization, and the channel remains closed (C) until all four sensors are activated, which opens the channel (O) [82]. This model assumes an obligatory coupling between voltage-sensor activation and channel opening: the voltage sensor must move to an active state in order for the channel to open, and channel opening is obligatory once all four sensors are activated. However, one prevalent model proposed for Shaker K^+ channels includes a C–O transition after all four voltage sensors are activated [148, 149]. This model shares similarity with the allosteric model for BK channels based on the idea that the channels undergo two types of conformational changes: voltage-sensor activation and channel opening. In this model, although voltage-sensor activation is obligatory for the channel to open, channel opening is not obligatory after voltage sensors are activated. This concept was best illustrated by the ILT mutation of Shaker K^+ channels [149], which includes three conservative amino acid substitutions in S4, V369I, I372L and S376T. The ILT mutation alters the rate constants of channel opening to make this conformational change rate-limiting, resulting in a separation of voltage ranges for channel opening and for voltage-sensor activation. Therefore, activation of voltage sensors saturates at a voltage ~60 mV below the threshold of activation of ionic currents [149]. In other words, within the 60 mV voltage interval, the activation gate does not open even though voltage sensors have been activated. In Shaker channels, the voltage dependence of P_O does not change even when P_O is as low as 10^{-7} at negative voltages [148], which differs from the results with BK channels [143]. This result is consistent with the idea that voltage-sensor activation is obligatory for the K_V channel to open [138]. However, in the case of BK channels, it was shown that, if the parameters of the HCA model are altered in certain ways, channel opening can also be difficult to observe when voltage sensors are at the resting state [135, 143]. In other words, the possibility remains that the coupling between voltage-sensor activation and channel opening is actually allosteric even in Shaker channels, but the properties of the two types of conformational changes happen to be such that they would not allow observation of channel opening when voltage sensors are at the resting state. Consistent with this view, some mutations that disrupt physical connections between voltage sensors and the pore in Shaker channels make the channels open at negative voltages [150]. In fact, an allosteric model of Shaker channel gating was proposed to account for the block of 4-aminopyridine (4-AP) on both the ionic and gating currents [151]. Taken together, although different models have been developed to describe voltage-dependent gating of BK and K_V channels, the coupling between voltage-sensor activation and channel opening in these channels may be fundamentally similar. The structural basis of the coupling between voltage sensors and the activation gate has not been explored in BK channels. In K_V channels, interactions between the S4–S5 linker and the C-terminus of S6 are important for the coupling between voltage-sensor activation and channel opening [152]. Experimental evidence suggests that a direct interaction between S4 and S5 also contributes to the VSD-PGD coupling [149, 153]. Based on above discussions and the structural homology between BK and K_V channels, it is likely that similar molecular mechanisms apply to BK channels. However, the unique structural features of BK channels may alter the molecular mechanism of the coupling between voltage sensors and the activation gate: 1) The additional S0 transmembrane segment and the long intracellular S0–S1 linker increase the molecular mass of the VSD and may interact with parts of the VSD, thereby potentially altering the interactions of the VSD with the pore domain [118]. 2) The residues in the large cytoplasmic domain interact with those in the VSD to affect voltage-sensor activation [118, 129]. On the other hand, the cytoplasmic domain may affect channel opening by pulling S6 via the peptide linker [154]. Thus, the cytoplasmic domain of the BK channel may affect the coupling between voltage sensor and the activation gate by interactions with both domains.

Ca^{2+}-dependent activation of BK channels

Ca^{2+} binding sites. Intracellular Ca^{2+} binds to the cytoplasmic domain of BK channels to promote channel opening [127]. Each Slo1 subunit of BK channels contains two high-affinity Ca^{2+} binding sites (K_d: 0.8 ~ 11 μM) [85, 97, 122, 155–157]. One site is located in a motif in the C-terminus of the cytoplasmic domain that contains a series of Asp residues known as the 'Ca^{2+} bowl' [155], and the other is in the RCK1 domain [122].

The Ca^{2+} bowl was first proposed as a Ca^{2+} binding site because it may structurally resemble the Ca^{2+} binding loop of serine proteinases [158, 159] and it contains a high density of acidic residues [155]. Mutations to the Asp residues in the Ca^{2+} bowl reduced Ca^{2+} sensitivity of channel activation [122, 155, 156, 160, 161]. By eliminating 1, 2, 3 and 4 functional Ca^{2+} bowls from

four subunits, the apparent Ca^{2+} binding affinity and the Hill coefficient, which represents the allosteric interaction among multiple Ca^{2+} binding sites, diminish in a stepwise fashion [162]. By studying channels containing C-terminus chimeras between the Ca^{2+}-sensitive mSlo1 and the Ca^{2+}-insensitive mSlo3, Schreiber et al. revealed that the Ca^{2+} bowl region of mSlo1 is required for part of Ca^{2+} sensitivity [163]. In most studies, Ca^{2+} sensitivity of the channel is measured by the G–V shift caused by varying $[Ca^{2+}]_i$ (Fig. 1). The method to directly and quantitatively measure Ca^{2+} binding to BK channels is not available. However, employing a $^{45}Ca^{2+}$-overlay technique, Bian et al. and Braun and Sy showed that purified C-terminal peptides of Slo1 containing the Ca^{2+} bowl region binds Ca^{2+} [160, 161]. Furthermore, mutating Asp residues in the Ca^{2+} bowl significantly reduced Ca^{2+} binding [160]. More recently, using CD spectroscopy, Yusifov et al. found that the secondary structure composition of an hSlo1 C-terminal peptide that includes the Ca^{2+} bowl changed with Ca^{2+} concentration in solution, and the mutation of five sequential Asp to Asn (5D5N) residues in the Ca^{2+} bowl significantly reduced the Ca^{2+} effect [124]. These experiments provide strong support for Ca^{2+} binding to the Ca^{2+} bowl. In a comprehensive study of mSlo1, it was shown that the acidic residues were not equal in binding to Ca^{2+} [156]. Mutation to individual Asp residues showed various degrees of reduced Ca^{2+} sensitivity, as measured by G–V shifts, and Ca^{2+} binding, as measured by $^{45}Ca^{2+}$ overlay [156]. Among these mutations, D898A and D900A eliminated the Ca^{2+} sensitivity contributed by the Ca^{2+} bowl and caused the largest reduction in Ca^{2+} binding. Therefore, it was proposed that D898 and D900 serve as ligands for Ca^{2+} coordination. In the same study, due to discrepancies between the effects of mutations in the intact subunit on Ca^{2+}dependent gating and in the isolated peptides on Ca^{2+} binding, Bao and Cox suggested that in the biochemistry studies the purified peptides may have a somewhat distorted structure [156]. In addition, in the studies using a $^{45}Ca^{2+}$-overlay technique or CD spectroscopy, mutations in the Ca^{2+} bowl could not abolish Ca^{2+} effects entirely, suggesting that Ca^{2+} binding to the purified peptides may not be specific to the Ca^{2+} bowl [124, 160, 161].

Mutations in the Ca^{2+} bowl reduce, but do not eliminate the Ca^{2+} sensitivity of BK channels [155, 163]. Additionally, besides Ca^{2+}, Cd^{2+} activates BK channels, but Cd^{2+} activation is not modified by the mutations in the Ca^{2+} bowl [155]. Therefore, it was proposed that the channel contained a second high-affinity Ca^{2+} binding site [155, 163]. In 2002, mutations of two acidic residues (D362A/D367A) in the RCK1 domain were found to eliminate the rest of the Ca^{2+} sensitivity [122]. Subsequent characterization of this mutation indicated that it also abolished Cd^{2+}-dependent activation [164], further supporting D362/D367 as another putative Ca^{2+} binding site. Interestingly, the human hereditary disease of generalized epilepsy and paroxysmal dyskinesia (GEPD) was found to be associated with a missense mutation of an Asp residue two amino acids downstream of D367 [60]. The Asp-to-Gly mutation increased BK channels' Ca^{2+} sensitivity. The identification of this mutation further highlights the importance of the Ca^{2+} binding site in RCK1 for Ca^{2+} sensing of BK channels. Consistent with this idea, recent studies on carbon monoxide (CO) and intracellular pH modulation of BK channel function [130, 165] indicate that these ligands may bind in the vicinity of the D362/D367 site, exerting direct or indirect effects on Ca^{2+} binding to this site.

Many molecular details about the putative Ca^{2+} binding site in RCK1 are still unclear. D362 seems to be less important than D367 in Ca^{2+} binding to the RCK1 site [121, 122, 166]. While D367A resembles the effect of D362A/D367A, D362 only causes a small reduction of Ca^{2+} sensitivity. More intriguingly, another mutation in the RCK1 domain, M513I, significantly reduces, but does not abolish, the Ca^{2+} sensitivity derived from the RCK1 domain [157]. Ca^{2+} prefers to bind to 'hard' oxygen-containing ligands, such as carboxylates, carbonyls, water and hydroxyl oxygen atoms. The 'soft' sulfur in the side chain of Met residues usually is not considered to be a Ca^{2+} ligand [167, 168]. Nevertheless, since the D362/D367 site can also bind to Cd^{2+} [164], which prefers 'soft' ligands such as sulfur and nitrogen, the possibility that M513 also contributes to Ca^{2+} binding to the RCK1 site cannot be completely ruled out [169]. Because the loop containing D367 is not conserved in the MthK RCK1 domain, it is not known whether D362, D367 and M513 are indeed close enough to each other to participate in the same Ca^{2+} binding site. Further studies are needed to clarify their individual contribution to Ca^{2+} binding to the RCK1 site.

The RCK1-related Ca^{2+} binding site has somewhat of a lower affinity for Ca^{2+} than the Ca^{2+} bowl, but is responsible for a larger portion of Ca^{2+} sensitivity, accounting for ~60 % of total G–V shift due to Ca^{2+} binding [95, 122, 157]. Consistent with this, the Ca^{2+} bowl accelerated activation kinetics mainly at $[Ca^{2+}]_i$'s below 10 μM, while the RCK1-related site altered both the activation and deactivation rate at $[Ca^{2+}]_i$'s between 10 and 300 μM [164]. The contribution to the Ca^{2+} sensitivity by each of the two sites was studied by eliminating the function of the other site. The results showed a roughly additive Ca^{2+} sensitivity, indicating that each site contributes to the channel gating

independently [122, 157]. However, by studying a combination of mixed mSlo1 WT and mutant subunits, in which the function of either the Ca^{2+} bowl or the RCK1-related site was eliminated, Qian et al. found that the two sites in the same Slo1 subunit cooperate positively; the two intact sensors on the same subunit are more effective in increasing channel opening than when they are on different subunits [170]. It is not clear how the cooperativity observed by Qian et al. affects the overall Ca^{2+} sensitivity quantitatively.

Piskorowski and Aldrich [171] reported that a truncated BK channel that lacks the entire cytoplasmic C-terminus, made by deleting the amino acids right after the transmembrane segment S6 (after residue 323 of mSlo1), still has a Ca^{2+} sensitivity similar to that of the wild-type channels. Since neither the Ca^{2+} bowl nor the RCK1 is present in the truncated channel, this result suggested that the observed Ca^{2+}-induced activation was either due to a Ca^{2+} binding site within the membrane-spanning domain or derived from a Ca^{2+} -sensing molecule physically associated with the truncated channel. The truncation reduced the expression of the channel on the cell membrane of *Xenopus laevis* oocytes and mammalian cell lines [171–173] so that only single-channel activities of the truncated channel could be recorded. However, the surface expression level of the truncated channel in *Xenopus* oocytes was apparently higher than that of the endogenous BK channels [171, 174]. Piskorowski and Aldrich were careful to make sure that they were indeed studying the truncated channels by including a secondary mutation, T294V, which reduces the channel blockade by TEA [109] and thus can be distinguished from endogenous or contaminating WT BK channels. Consistent with these results, Qian and Magleby showed that the coexpression of the accessory β1 subunit of BK channels with a triple mutant mSlo1, which eliminates the function of the Ca^{2+} bowl (5D5N), the RCK1-related Ca^{2+} binding site (D362A/D367A) and the Mg^{2+} binding site (E399A), partially restored Ca^{2+} sensitivity [175]. Since the β1 subunit itself does not contain any Ca^{2+} binding site, the restored Ca^{2+} sensitivity should derive from a Ca^{2+} binding site other than the Ca^{2+} bowl or the RCK1-related site. On the other hand, there are controversies in regard to the results of Piskorowski and Aldrich [171] and how to interpret these results in relation to the large body of studies on the Ca^{2+} bowl and the RCK1-related Ca^{2+} binding sites discussed above. In a direct comparison, Schmalhofer et al. found that the identically truncated Slo1 channels were trapped inside the TsA-201 cells and no single channel activities could be detected on the cell membrane under various recording conditions [173]. Using an iberiotoxin binding assay, they showed that the channels trapped in the intracellular membranes either were not tetramerized or had an altered architecture in the outer vestibule of the channel. Even in *Xenopus* oocytes, the same truncated channel failed to express in different laboratories [176, 177]. To examine whether the Ca^{2+} sensor resides in the membrane-spanning domain or in the cytoplasmic domain, Xia et al. studied chimera channels between the Ca^{2+}-sensitive mSlo1 and the pH-sensitive mSlo3 channels [176]. The results showed that the cytoplasmic domain of mSlo1 conferred Ca^{2+} sensitivity when connected to either the mSlo1 or mSlo3 membrane-spanning domain, while the cytoplasmic domain of mSlo3 conferred pH sensitivity. Therefore, the specific ligand sensors of the two channels are defined by the cytoplasmic domain. Another important unsettling aspect in regard to the results of Piskorowski and Aldrich is that no studies have identified any Ca^{2+} binding site in the membrane-spanning domain, while a large body of data identified the Ca^{2+} bowl and the RCK1-related site in the cytoplasmic domain. Braun and Sy proposed an EF handlike Ca^{2+} binding site in the S0–S1 linker, but mutations in this putative site only reduced Ca^{2+} sensitivity by less than 20 % [161]. In a recent study, each of the oxygen-containing residues in the intracellular side of transmembrane segments and loops between them were mutated to Ala. The results showed that none of these mutations reduced Ca^{2+} sensitivity in 0–100 μM Ca^{2+} [178]. Since oxygen is the preferred ligand for Ca^{2+} coordination [168] and these residues are possibly exposed to the cytosol, this result would normally indicate that there is no Ca^{2+} binding site in the membrane-spanning domain. However, both studies [176, 178] were based on the entire mSlo channels containing both the membrane-spanning and the cytoplasmic domains, which may use different Ca^{2+} binding sites for channel activation as compared to the truncated channel lacking the cytoplasmic domain. Therefore, taken together, the results from both studies suggest that if the membrane-spanning domain contains a Ca^{2+} binding site, it does not contribute to Ca^{2+} sensing in the intact BK channel. Such a Ca^{2+} binding site may be exposed only after the removal of the cytoplasmic domain to give rise to Ca^{2+} sensitivity in the truncated channel. Alternatively, the truncated channel may associate with intracellular factors that provide a Ca^{2+} sensor.

Coupling between Ca^{2+} binding and channel activation. In the absence of Ca^{2+}, BK channel gating involves two types of conformational changes, the activation of voltage sensors and the opening of the activation gate. Ca^{2+} binding primarily promotes gate opening, with small facilitating effects on voltage-

sensor activation. The effects of Ca^{2+} on gate opening can be measured at negative voltages where voltage sensors are at the resting state; under such conditions, the P_O increased $\geq$2000 times between 0 and 100 μM $[Ca^{2+}]i$ [137, 179]. Thus, voltage and Ca^{2+} activate BK channels using two parallel mechanisms, with the voltage sensors and the Ca^{2+} binding sites coupling to the activation gate independently, except for a weak interaction between the two mechanisms. Here we will review the coupling between Ca^{2+} binding and gate opening. The effect of Ca^{2+} binding on voltage-sensor activation will be discussed in the section titled 'Relationship between activation pathways'.

The relationship between BK channel open probability and Ca^{2+} concentration can be fitted by the Hill equation, with the Hill coefficient ranging from 1.5 to 6 [133, 160, 162, 180–183]. The Hill equation is a semi-empirical relationship that describes cooperative association of ligands by multiple binding sites. The values of the Hill coefficient for Ca^{2+}-dependent activation indicate that there are at least 2–6 Ca^{2+} binding sites in a BK channel molecule, which is consistent with our current knowledge that each Slo1 subunit contains two Ca^{2+} binding sites (see above), and that, perhaps more important, there is a positive cooperativity among these Ca^{2+} binding sites. Such a cooperative interaction among Ca^{2+} binding sites in BK channels can be explained by the MWC model that was originally proposed by Monod, Wyman and Changeux [184] to account for the cooperative binding of oxygen to hemoglobin (Fig. 3B) [133, 179, 185]. The central idea of the MWC model is that ligand binding to any of the multiple binding sites will promote a conformational change that alters all binding sites and thus the affinity of ligand binding. In the MWC model for BK channels, the conformational change of the activation gate in channel opening (C–O) is accompanied by a conformational change of the Ca^{2+} binding sites that would increase the affinity of Ca^{2+} binding ($K_{dO} < K_{dC}$), giving rise to the positive cooperativity. Because the open state has a higher Ca^{2+} affinity than the closed state, the Ca^{2+}-bound open state is energetically more favorable than the Ca^{2+}-bound closed state with the same number of Ca^{2+} ions. Therefore, Ca^{2+} binding promotes channel opening, and the C–O transition is altered by factor C ($C = K_{dO}/K_{dC}$) for the binding of each additional Ca^{2+} (Fig. 3B).

Properties of BK channels are consistent with the key features of the MWC model. First, BK channels can open without binding to Ca^{2+}. Although earlier studies had observed BK channel activities with low open probability at very low Ca^{2+} concentrations [20, 40, 41, 186, 187], this property was explicitly demonstrated only after the cloning of Slo1. Expression of cloned BK channels in *Xenopus* oocytes and mammalian cells made it possible to study macroscopic BK channel currents at extremely low Ca^{2+} concentrations (0.5 ~ 100 nM). It was shown that at 0.5 nM Ca^{2+}, mSlo1 channels open at least 200 times faster than the diffusion limit for Ca^{2+} binding; thus the channels must open prior to Ca^{2+} binding to the channel [133]. In addition, the P_O or activation rate of the channel does not change with Ca^{2+} concentration in the range between 0.5 and 100 nM [132, 133], consistent with the idea that the channel opens without sensing the low Ca^{2+} concentrations. Furthermore, in the low Ca^{2+} concentrations, the channel can be activated to its maximum P_O [133], demonstrating that channel opening is not limited by Ca^{2+} binding. Second, channel opening follows a two-state model (C–O) at different Ca^{2+} concentrations. This is supported by the activation and deactivation kinetics, which are well fitted with an exponential time course, after a brief delay due to voltage-sensor activation, over a wide range of voltages (–350 ± 300 mV) and Ca^{2+} concentrations (0–1000 μM) [133, 143, 179]. Single-channel measurements revealed multiple open and closed states, and the transitions among open or closed states are dependent on Ca^{2+} concentration [146, 180, 181, 183], consistent with the two-tiered MWC model (Fig. 3B). Third, the Hill coefficient of Ca^{2+}-dependent activation depends on voltage, increasing with membrane depolarization [133, 160, 180]. This indicates that the cooperativity among Ca^{2+} binding sites increases with voltage, even though Ca^{2+} binding itself may not be voltage-dependent and the interaction between Ca^{2+} binding and voltage-sensor activation is weak [133, 179]. This result is consistent with the idea that, in conjunction with channel opening, the conformation of the Ca^{2+} binding site changes to increase Ca^{2+} affinity: at more depolarized voltages the open probability is higher, and thus it is more likely for Ca^{2+} binding sites to change conformation. More discussion on the allosteric mechanisms of Ca^{2+}-dependent activation of BK channels may be found in an insightful review by Karl Magleby [95].

Studies on the molecular mechanism of the coupling between Ca^{2+} binding and gate opening in BK channels have been predominately influenced by the studies of MthK from the laboratories of MacKinnon and Jiang. A recent review by Christopher Lingle provides an excellent account of these studies [97]. Here we will briefly summarize the key features of Ca^{2+}-dependent activation of MthK channels and then compare these to the results obtained in BK channel studies. The gating ring of the MthK channel comprises eight identical RCK domains (Fig. 2C), four of which derive from the intact MthK subunit and covalently link to the tetrameric pore with short

peptides, while the additional four RCK domains are generated from the MthK gene by a second start site downstream from the pore domain. The Ca^{2+} binding site in each RCK domain is strategically located adjacent to the hinge of the flexible interface between the neighboring subunits, allowing Ca^{2+} to influence its conformation [110]. Ca^{2+} binding changes the flexible interface such that the two RCK domains open like a clamshell [188]. As a result of the changes in the flexible interface, the gating ring expands to increase in diameter [188]. It is proposed that such an expansion will directly pull the pore open through the peptide linker between the pore and the RCK domains [110, 188].

In the following, we compare BK and MthK channels in terms of the location of Ca^{2+} binding sites, the location and role of the flexible interface, and coupling between the cytoplasmic domain and the pore. Differing from the MthK channel, the two putative RCK domains in BK channels are not identical. Consequently, the Ca^{2+} binding sites and flexible interfaces differ in the RCK1 and RCK2 domains of BK channels.

1) Ca^{2+} binding sites. In MthK, the Ca^{2+} binding site is located at the C-terminal end of βD (D184) and βE (E210 and E212), which places the bound Ca^{2+} at the hinge of the flexible interface. In contrast, in the RCK1 of mSlo1, D362 (at the C-terminus of αA) and D367 (at the αA-βB loop) are located at the opposite side of the β-sheets, far away from the hinge of the putative flexible interface. M513 (C-terminus of αG), however, is located at the expected flexible interface. In the homology model, the side chain of D362 makes direct contact with that of M513 [177], suggesting that the binding of Ca^{2+} to the RCK1-related site may be able to affect the putative flexible interface. The location of the Ca^{2+} bowl in relation to the putative RCK2 domain differs in published alignments (Table 1), and there is no data to suggest whether Ca^{2+} binding to the Ca^{2+} bowl would affect the flexible interface.

2) The flexible interface. In the RCK1 of mSlo1, residues 487–498 and 501–511 correspond to helices αF and αG, respectively, which are part of the flexible interface in the MthK channel (Table 1). However, no experimental data have been published to show whether these residues are part of an interface between structural domains or whether they are important for Ca^{2+}-dependent gating. The residues corresponding to αF and αG in the RCK2 of mSlo1 differ among published alignments (Table 1), and similarly, no experimental data are available to verify the prediction of their involvement in any interactions with the RCK1 domain or in Ca^{2+}-dependent activation. Kim et al. found a mutation in RCK2 that altered the gate opening in the absence of Ca^{2+} binding and voltage-sensor activation (Table 1) [125]. This residue, located between the putative βA and αA, was proposed to be part of the flexible interface with RCK1 [125]. However, this mutation did not affect Ca^{2+}-dependent activation; thus its role in Ca^{2+}-dependent activation may not be compatible with that of the flexible interface in MthK.

3) The coupling between the cytoplasmic domain and the pore. The S6 transmembrane segment of BK channels is connected to the RCK1 domain with a peptide linker of 16 amino acids. Niu et al. found that changes to the linker length by either deleting or adding a different number of amino acids alter channel open probability both in the absence and presence of Ca^{2+} [154, 177]. Shortening the linker increases channel activity, and lengthening the linker decreases channel activity. This result is consistent with the hypothesis proposed for the MthK channel [110] that the cytoplasmic domain tugs the linker during Ca^{2+}-dependent activation.

While the mechanism of Ca^{2+}-dependent activation in MthK is a prevalent model for the coupling between Ca^{2+} binding and gate opening in BK channels, some results have suggested features of Ca^{2+}-dependent regulation of BK gating that may be outside the framework of the MthK model. Krishnamoorthy et al. found that the dSlo1 BK channel has a higher Ca^{2+} sensitivity in Ca^{2+}-dependent gating than mSlo1 [189]. By studying the chimeras between the two homologous channels, the N-terminal half of the RCK1 corresponding to βA – αC (thus named the AC region) was identified to be responsible for the phenotypical difference. A molecular dynamics simulation based on the structure of the MthK RCK domain suggested that the AC region of dSlo1 has a more tightly packed structure and less flexible dynamics than that of mSlo1. These results suggest that, unlike a rigid-body motion of the entire RCK domain in the clamshell model of MthK channels, conformational changes of a subdomain in RCK may be important for Ca^{2+}-dependent gating in BK channels. Interestingly, it has been shown that the AC region is located close to the membrane-spanning domain, and the two domains make multiple physical contacts [118, 129]. These results suggest that, in addition to the direct pulling through the peptide linker between S6 and RCK1, the interactions among residues in the membrane-spanning domain and the AC region may also contribute to the coupling between Ca^{2+} binding and channel opening.

Mg^{2+}-dependent activation of BK channels

Mg^{2+} binding sites. Mg^{2+} effects on BK channel activation were discovered in the late 1980 s [190, 191]. However, until 2001, it was not clear whether Mg^{2+} activates the channel by modulating Ca^{2+}-dependent activation or via a separate mechanism. In 2001, two independent studies of the cloned BK channel illustrated that millimolar Mg^{2+} activates the BK channel via a low-affinity metal binding site that is independent from Ca^{2+}-dependent activation [80, 81]. One of the studies [80] measured BK channel activities in a broad range of Ca^{2+} and Mg^{2+} concentrations and found that the results could be fitted by a model including a high-affinity Ca^{2+} binding site and a lower-affinity Mg^{2+} binding site. The other study showed that at both zero and saturating Ca^{2+} concentration (110 μM), Mg^{2+} activated the channel similarly with a binding affinity in the millimolar range, indicating that Ca^{2+} binding was not involved in Mg^{2+}-dependent activation [81]. In addition, by comparing the chimera channels between the Mg^{2+}-sensitive mSlo1 and Mg^{2+}-insensitive mSlo3, Shi and Cui found that the low-affinity Mg^{2+} binding site resided at a different location from the Ca^{2+} bowl [81], demonstrating that the low- and high-affinity metal binding sites are different.

Characterization of more chimera channels between mSlo1 and mSlo3 further pinpointed the Mg^{2+} binding site to the N-terminus of the RCK1 domain [121]. Three residues in this region, E374, E399 and Q397, were proposed as putative Mg^{2+} coordination sites based on systematic mutagenesis and structural simulation [121] of a homology model based on the RCK domain of the *E. coli* K^+ channel [111]. At the same time, the Lingle group independently identified E399 as an essential residue for millimolar Mg^{2+}/Ca^{2+} binding [122], thereby clearly separating the contribution of this putative low-affinity Mg^{2+}/Ca^{2+} binding site from that of the other two putative high-affinity Ca^{2+} binding sites, the Ca^{2+} bowl and the D362/D367 site, to channel activation [122]. Further mutagenesis studies suggest that the side chains of E374 and E399 may coordinate Mg^{2+}, while the side chain of Q397 does not. Instead, Q397 is located close to the binding site and affects Mg^{2+} binding [128]. This conclusion was largely based on two lines of evidence. First, the carboxylate groups of E374 and E399 are required for Mg^{2+} sensing. Other side chains on either of these two residues completely abolished Mg^{2+} sensing. Second, adding positive charges to residue 397 by mutagenesis or chemical modification reduced but did not abolish Mg^{2+} binding. On the other hand, adding negative charge to residue 397 increased Mg^{2+} sensitivity. The opposite charge effects on Mg^{2+} sensing indicated that Q397 is close to the Mg^{2+} binding site and charges at this residue affect Mg^{2+} sensitivity through electrostatic interaction with the bound Mg^{2+}.

Owing to its chemical properties, Mg^{2+} is predominantly coordinated by six oxygen atoms from the side chains of oxygen-containing residues, main chain carbonyl groups in proteins or water molecules [168]. Besides E374 and E399, what other oxygen-containing residues might participate in Mg^{2+} binding? A recent study [118] addressed this question. Based on the homology model of the BK channel RCK1 domain, E374 and E399 are located on the top surface of the RCK1 domain, spatially close to the VSD [118, 128, 129]. Since the octahedral geometry of the Mg^{2+} binding site constrains the distances among its coordinates, the authors postulated that other protein ligands, if there are any, can only come from the top surface of the RCK1 domain and/or the intracellular portion of the membrane-spanning domain. Through systematic mutagenesis scanning of the oxygen-containing residues in these potential regions, the side-chain oxygens of two residues in the voltage-sensor domain were found to be essential for Mg^{2+} binding. These residues are D99 at the C-terminus of the S0–S1 loop and N172 in the S2–S3 loop.

Attribution of D99 as another Mg^{2+} coordinate is based on the following evidence [118]. First, the mutations of D99 that remove side-chain oxygen completely abolished Mg^{2+} sensitivity, while the mutations that preserve oxygen with carbonyl or carboxylate groups retained partial Mg^{2+} sensitivity. In addition, these mutations specifically affected Mg^{2+} sensing without changing voltage or Ca^{2+} dependence. Second, the D99A mutation abolished the Mg^{2+} effects, but did not abolish channel activation by a positive charge that was covalently added in the vicinity of the Mg^{2+} binding site to mimic Mg^{2+} in activating the channel. Thus, D99A only abolished Mg^{2+} binding but not the coupling mechanism that opens the channel. Third, the formation of a disulfide bond between two cysteine residues that replace D99 and Q397 indicated that D99 in the voltage-sensor domain is located close to the Mg^{2+} binding site in the RCK1 domain and thus can be part of the binding site. Evidence also suggested the contribution of N172 to Mg^{2+} coordination. Mutations of N172 to positively charged residues (R or K) abolished Mg^{2+} sensitivity; while mutations to negatively charged residues (D or E) increased Mg^{2+} sensitivity. More important, mutation N172D rescued Mg^{2+} sensitivity that had been abolished by mutating other Mg^{2+} binding residues (D99A, E374A or E399N), indicating that the carboxylate group on the side chain of residue 172 may directly contribute to Mg^{2+} coordination to compensate for the loss of coordination at other positions.

These studies indicate that the Mg^{2+} binding site may comprise residues from two different domains: D99 and N172 from the VSD, and E374 and E399 from the cytoplasmic RCK1 domain.

Yang et al. further demonstrated that in each Mg^{2+} binding site D99/N172 may not come from the same subunit as E374/E399, but rather from a neighboring subunit [118]. While individual mutations D99R, N172R, E374R and E399C abolished Mg^{2+} sensitivity, hybrid channels resulting from the co-expression of the single mutations from different domains (for example, D99R from VSD and E374R from RCK1) retained some Mg^{2+} sensitivity. The retention of Mg^{2+} sensitivity cannot be explained by an intrasubunit binding site model in which all four Mg^{2+} binding residues come from the same subunit, because in this model each Mg^{2+} binding site would contain a mutation that abolished Mg^{2+} sensitivity. Instead, this result suggests that D99/N172 in the VSD and E374/E399 in the RCK1 domains from neighboring subunits form an intersubunit Mg^{2+} binding site. Thus, according to binomial distribution of the single mutations in the tetrameric channels, some of the Mg^{2+} binding sites may contain two mutations, leaving other Mg^{2+} binding sites intact. The retained Mg^{2+} sensitivity measured in experiments can be fitted well with the contribution from these intact sites. Therefore, the Mg^{2+} binding site of BK channels may comprise D99/N172 in the VSD of one subunit and E374/E399 in the RCK1 domain of the neighboring subunit.

Besides this low-affinity divalent cation (Mg^{2+}/Ca^{2+}) binding site in the RCK1 domain, BK channels may contain divalent cation binding sites with even lower-affinity (K_d ~ 40 mM), termed as very low affinity sites [166]. These very low affinity sites might be responsible for the additional shifts of the G–V relation induced by increasing $[Mg^{2+}]_i$ or $[Ca^{2+}]_i$ from 10 to 100 mM [80, 122, 166], partially because the shifts are too large to be solely attributed to surface charge screening [166]. In addition, these very low affinity sites are distinct from the two high-affinity Ca^{2+} and the low-affinity Mg^{2+} binding sites because mutations that eliminate the three binding sites do not affect the G–V shifts induced by high concentrations of Ca^{2+} or Mg^{2+} [166]. A recent study further illustrated that these very low affinity sites activated BK channels allosterically by affecting both the opening of the activation gate (L) and voltage-sensor movement (J) (Fig. 3A) [192]. At present, the location, number and physiological significance of the very low affinity sites is still not clear. However, importantly, at Mg^{2+} concentrations around 10 mM the contribution of these very low affinity sites to channel activation overlaps with that of the Mg^{2+} binding site in RCK1, which interferes with the study of Mg^{2+}-dependent activation through the Mg^{2+} binding site in RCK1. Nevertheless, the separation of the activation effects by these distinct sites can be achieved by a simple subtraction [118, 128, 129, 166] because studies show that these sites affect channel activation independently [166].

Coupling between Mg^{2+} binding and channel activation. Similar to Ca^{2+}-dependent activation of BK channels, millimolar Mg^{2+} increases open probability and shifts the G–V relation to more negative voltages [80, 81]. Likewise, these properties can also be described by an allosteric MWC gating model. However, unlike micromolar Ca^{2+}, which accelerates the activation rate and reduces the deactivation rate of the channel, millimolar Mg^{2+} only reduces the deactivation rate but has no effect on the activation rate [80, 81, 164, 166, 192], implying that the underlying molecular mechanism of Mg^{2+}-dependent activation may differ from that of Ca^{2+}-dependent activation.

Several recent studies further demonstrate that Mg^{2+} and Ca^{2+} indeed activate BK channels through distinct molecular mechanisms [129, 140, 192]. While Ca^{2+} activates the channel largely independent of the voltage sensor [179], Mg^{2+} activates the channel by an electrostatic interaction with the voltage sensor [129, 192]. The involvement of the voltage sensor in Mg^{2+}-dependent activation is suggested by two lines of evidence. First, Mg^{2+} has no measurable effect on channel activation at negative voltages where voltage sensors are in the resting state [129, 192]; in contrast, 70 μM $[Ca^{2+}]_i$ can increase the open probability >2000-fold at similar voltages [179]. This result suggests that Mg^{2+} activates the channel only when the voltage sensor can be activated. Second, neutralization of R213, a voltage-sensing residue at the C-terminus of S4, specifically eliminated Mg^{2+} sensitivity, but did not affect Ca^{2+} sensing [140]. This result suggests that the voltage sensor, more specifically R213, is important for Mg^{2+}-dependent activation. Recently, it was shown that Mg^{2+} activated the channel by an electrostatic repulsion of R213 [129] based on the following four lines of evidence. 1) Altering the ionic strength of intracellular solutions specifically changed Mg^{2+} sensing but had little effect on Ca^{2+} sensing, indicating that Mg^{2+}-dependent activation is electrostatic by nature. 2) A positive charge at residue 397, introduced either by mutagenesis or chemical modification, mimicked Mg^{2+} effects on channel activation. Similar to Mg^{2+}, effects of this positive charge were also sensitive to ionic strength. Since residue 397 is in the vicinity of the RCK1 Mg^{2+} binding site, these results further support the involvement of electrostatic interaction in Mg^{2+}-

dependent activation. 3) 10 mM $[Mg^{2+}]_i$ reduced the amplitude and slowed the relaxation rate of off gating currents (Ig_{OFF}), indicating that Mg^{2+} stabilizes the voltage sensor in its active state. 4) R213 was found to be the only charged reside in the VSD that sensed the electric field from Mg^{2+} or charges at position 397, which is close to the RCK1 Mg^{2+} binding site. Adding back a positive charge to R213C, a mutation that abolishes Mg^{2+} sensitivity, by chemical modification, rescued partial Mg^{2+} sensitivity. This result indicates that a positive charge at position 213 is necessary and sufficient for Mg^{2+} to activate the channel through an electrostatic repulsion.

Interestingly, while Mg^{2+} affects Ig_{OFF} significantly, it has only a small effect on the on gating currents (Ig_{ON}) [129]. Since Ig_{ON} reflects voltage sensor activation when the channels are closed and Ig_{OFF} reflects the return of the voltage sensor from the active state to the resting state when channels are open, this result suggests that the electrostatic interaction between Mg^{2+} and R213 is state-dependent, i.e., the interaction is much stronger when the channel is open than closed. A fitting of the HCA model to the experimental data showed that the equilibrium of voltage-sensor movements at the open state (V_{hO}) was affected by Mg^{2+} much more than at the closed state (V_{hC}), which means that Mg^{2+} strengthens the allosteric coupling between voltage sensor and the activation gate (described by the allosteric factor *D*, see Fig. 3B) [129]. Likewise, in a recent study, Horrigan and Ma evaluated the biophysical mechanism of the electrostatic interaction and attributed Mg^{2+} effects to the ability of Mg^{2+} to stabilize channels in states with both activated voltage sensors and open gates (open-activated states or OA states) [192]. The stabilization of the OA state by Mg^{2+} results in enhancement of the coupling between voltage sensor and channel activation (the allosteric factor *D*), thereby facilitating channel activation. The state-dependent interaction suggests that the distance between R213 and Mg^{2+} differs between the open and the closed states. In other words, the cytoplasmic domain and the membrane-spanning domain may undergo a shift in their relative positions during channel opening.

Relationship between activation pathways

Ca^{2+}- and voltage-dependent activation. Although distinct mechanisms underlie Ca^{2+}- and voltage-dependent activation of BK channels, it is apparent that the G–V relation shifts to more negative voltages with increasing $[Ca^{2+}]_i$ (Fig. 1A), while the G–$[Ca^{2+}]_i$ dose-response curve is altered by increasing voltage to decrease EC_{50} and increase the Hill coefficient [133]. These changes, however, are primarily mediated by channel opening, rather than a direct effect of Ca^{2+} on the voltage-dependent mechanism or vice versa. First, both voltage and Ca^{2+} can activate the channel independently to increase P_O. Therefore, P_O at the same voltage is larger with higher $[Ca^{2+}]_i$, and at the same $[Ca^{2+}]_i$ is larger with a more depolarized voltage. Second, due to the allosteric coupling of the activation gate with the voltage sensor and the Ca^{2+} binding sites, channel opening facilitates voltage-sensor activation as well as Ca^{2+} binding (Fig. 3). Thus, the opening of the channel due to one stimulus (Ca^{2+} or voltage) will enhance the effect of the other via the allosteric coupling between the activation gate and the VSD or Ca^{2+} binding sites. These indirect interactions mediated by channel opening account for the majority of the Ca^{2+}-dependent G–V shift and the voltage dependent G–$[Ca^{2+}]_i$ shift [179, 185]. In addition, there is a weak direct interaction between voltage sensor and Ca^{2+} that is not mediated by channel opening that is described by an allosteric factor *E* (Fig. 3B). This interaction was shown by Horrigan and Aldrich by recording the fast component of on gating currents (Ig_{fast}) at 0 and 70 μM $[Ca^{2+}]_i$ [179]. During their recordings, the channels were all closed so that the Ca^{2+} dependence of Ig_{fast} reflected a direct interaction between Ca^{2+} binding and voltage-sensor movement that is independent of the ability of Ca^{2+} to alter channel opening. They found that the Q_{fast}–V relation shifted by –20 mV in 70 μM $[Ca^{2+}]_i$ as compared to that in 0 $[Ca^{2+}]_i$. This shift of the Q_{fast}–V relation was mainly due to the reduction in the rate of the voltage sensor returning from the active state to the resting state [179].

Ca^{2+}- and Mg^{2+}-dependent activation. While the high-affinity Ca^{2+} binding sites in BK channels favor Ca^{2+}, other divalent cations at higher concentrations can bind to these sites [164, 191]. It was shown that Mg^{2+} competes with Ca^{2+} for the Ca^{2+} binding sites, with an affinity of ~5 mM [80, 81, 166]. However, the binding of Mg^{2+} to high-affinity Ca^{2+} binding sites does not activate the channel; thus the net effect of such binding is to reduce Ca^{2+}-dependent activation at low Ca^{2+} concentrations [80, 81]. The competition of Mg^{2+} to the Ca^{2+} binding sites was more clearly demonstrated in the study of mutant channels, in which the Mg^{2+} binding site was abolished so that Mg^{2+} no longer activated the channel. Thus, the G–V relation at low Ca^{2+} concentrations shifted to more positive voltage ranges upon the addition of 10 mM Mg^{2+}, opposite to the effect of Mg^{2+} in wild-type BK channels (Fig. 1) [166]. It is not known whether Mg^{2+} selectively binds to the Ca^{2+} bowl or the RCK1-related Ca^{2+} binding site. On the other hand, the low-affinity Mg^{2+} binding

site at the interface of the VSD and the RCK1 domain is not selective for Ca^{2+} or Mg^{2+} ions. Like Mg^{2+}, Ca^{2+} at millimolar concentrations binds to this low affinity site to activate the channel [80, 81, 122]. This site is called the Mg^{2+} binding site because it binds to Mg^{2+} around the physiological concentration of intracellular Mg^{2+} [193].

The molecular mechanisms of Mg^{2+} coupling to the activation gate do not interfere with those of Ca^{2+}. The effect of millimolar $[Mg^{2+}]_i$ on channel activation is not altered regardless of the presence of Ca^{2+} [80, 81], and the effects of saturating $[Ca^{2+}]_i$ and millimolar $[Mg^{2+}]_i$ on activation are largely additive [122]. Additionally, mutations that reduce or abolish Mg^{2+} sensitivity usually do not affect Ca^{2+} sensitivity [118, 121, 122, 128, 129, 140, 166]. Similarly, mutations that reduce Ca^{2+} sensitivity generally have little effect on Mg^{2+}-dependent activation [121, 122]. These results are remarkable since the putative Mg^{2+} and one of Ca^{2+} binding sites are both in the RCK1 domain and close to each other. In the homology model of the RCK1 domain in BK channels, E374 is located at the N-terminus of βB for Mg^{2+} binding, while D367, only seven amino acids away in the primary sequence, is located in the loop connecting the N-terminus of βB and αA for Ca^{2+}-dependent activation. These results illustrate an important feature in BK channel activation, i.e., each metal ion perturbs only a subset of amino acid residues to affect channel gating; these perturbations do not overlap perhaps because, as discussed above, Mg^{2+} activation is mediated via the interaction with the voltage sensor and Ca^{2+} activation is through forces applied to the linker between the gate and the cytoplasmic domain.

Activation gate

Energy provided by voltage, Ca^{2+} and Mg^{2+} binding will propagate to the activation gate of BK channels to initiate ion conduction through the pore. In canonical K^+ channels, the selectivity filter with the signature 'GYG' sequence determines ion selectivity and permeation [102, 103, 194], the hydrophobic residues at the C-terminus of S6 (or inner helix) form the inner mouth of the pore to restrict K^+ ion flux when channels are closed [195], and the Gly hinge in the middle of S6 [196] or the Pro kink(s) [197] at the C-terminus of S6 swings the gate to open on stimulation. However, several unique features of the BK channel indicate that, while the selectivity filter of the BK channel may be highly conserved, its pore and activation gate may differ somewhat from this picture. First, BK channels exhibit much larger single-channel conductance (~100–300 pS) than other K^+ channels while still maintaining high selectivity to K^+ ions. Several elegant studies indicate that rings of negative charges at the intracellular entrance of S6 (composed of eight Glu residues) [198, 199] and the extracellular outer pore [200, 201] of BK channels partially determine the large conductance of BK channels. These negatively charged rings attract K^+ ions, resulting in a severalfold increase in local $[K^+]$ [199], thereby speeding up K^+ flux. In addition, a large inner vestibule and a wide intracellular entrance to the vestibule might also contribute to the large conductance. In a series of experiments carried out by the Magleby [202] and Aldrich groups [203–205], chemicals of various sizes were used to probe the size of the inner vestibule and the cytoplasmic entrance of the pore. Based on the changes of the K^+ diffusion rate from bulk intracellular solution to the inner vestibule induced by sucrose interference, the inner mouth of BK channels was estimated to be twice (~20Å) as large as that of the Shaker K^+ channel [202]. Consistent with this, quaternary ammonium (QA) ions, such as tetrabutylammonium (TBA) and decyltriethylammonium (C_{10}), showed much faster block and unblock kinetics in BK channels than in canonical K_V channels. The time-independent blockade indicates that BK channels may have an enlarged inner vestibule and a widened intracellular entrance [203, 205], so that large QA ions can access their binding site from the cytoplasmic solution with less restriction. All these features together may contribute to the large conductance of BK channels.

Second, different from canonical K^+ channels, the inner mouth of the closed BK channel pore may not obstruct small hydrated ions, such as K^+ and QA ions. Instead, the permeation gate may be near or in the selectivity filter, similar to several other ligand-gated ion channels such as CNG (cyclic nucleotide-gated) channels [206] and SK (small conductance, Ca^{2+}-activated K^+) channels [207]. The Aldrich group showed that C_{10} and TBA did not slow the deactivation kinetics of the gate, indicating that the closure of the BK channel permeation gate was not hindered by the presence of these blockers. These experiments excluded the classic open-channel block mechanism ('foot in the door'), but left unresolved whether the blockers are trapped inside the inner vestibule when the gate closes (the trapping model), or can freely access their binding site inside the vestibule regardless of the state of the gate (the state-independent free-access model). These two possibilities could not be distinguished using these QA ions due to their very fast block/unblock kinetics. Subsequently, a different strategy was devised by Willkens and Aldrich who used a TBA derivative containing a bulky benzoyl-benzoyl group (bbTBA) to study steady-state and

kinetic behavior [205]. Unlike TBA and C_{10}, bbTBA blocks BK channels in a time-dependent fashion. The slow block kinetics allowed the investigators to study bbTBA blockade using a classic trapping experiment protocol. Their experiments showed no evidence that bbTBA was trapped inside the channel pore after gate closing. Thus, bbTBA blocks BK channels independent of gate opening and closing. Together with other careful characterizations, the authors concluded that BK channels might employ their selectivity filter as the permeation gate so that both K^+ ions and small QA ions can have relatively free access to the internal vestibule independent of channel states [205]. Consistent with the selectivity filter being the ion permeation gate, Piskorowski and Aldrich proposed that the interaction between the selectivity filter and permeating ions is critical for BK channel opening based on the effects of permeating thallium ion (Tl^+) on channel activation [208]. The authors suggested that permeating Tl^+ may stabilize a collapsed state of the selectivity filter; thereby shifting the activation curve to more positive voltages and increasing the flicker frequency at the single-channel level [208].

Third, the movement of the pore-lining helix (S6) of BK channels might be different from canonical K^+ channels. Although BK channels may employ their selectivity filter as the permeation gate, the S6 helix may still move during channel gating. Li and Aldrich found that a Shaker ball peptide (ShBP) homologue, which has a larger size than QA ions, blocked BK channels in a state-dependent fashion, i.e., ShBP blockade depended on the activation kinetics and open probability of the channel [204]. In addition, ShBP blockade slowed the deactivation kinetics without affecting the onset of the activation kinetics [204]. Thus, the cytoplasmic entrance of the BK channel pore may indeed move from the open to the closed state to restrict the entry of large ShBP molecules but not smaller K^+ and QA ions. In another study, Guo et al. found that hydrophobicity of a residue (323 in mSlo1) at the inner mouth of the BK channel pore is important for channel gating [209]. All hydrophilic mutations of residue 323 exhibited increased subconductance levels, while the hydrophobic mutations showed prolonged open duration. The authors proposed that a reduced hydrophobicity at residue 323 would reduce cooperativity among the four subunits of the channel in opening the activation gate, thus increasing the chance of partial channel openings that result in subconductance levels. These results are also consistent with the idea that the inner mouth of the BK channel is involved in the opening of the activation gate, although the gate for ion permeation may reside in the selectivity filter.

Structural and functional studies of K^+ channels suggest that the highly conserved 'Gly hinge' at the middle of the pore-lining helix [196] and/or the Pro-Val-Pro (PVP motif) at the C-terminus [197] are two important pivot points for the movement of the activation gate. In BK channels, the PVP motif is substituted by YVP, and there are two adjacent Gly residues at the position of the 'Gly hinge'. Single Ala mutations to either one of these Gly residues shifted the G–V relation similarly to more positive voltages for about +70 mV in 100 μM $[Ca^{2+}]_i$, while the double Ala mutation shifted almost twice as much as the single mutations [210], suggesting that the flexibility around the Gly hinge is critical to the gating of BK channels. It is worth noting that in Shaker K^+ channels, an Ala mutation of the Gly hinge completely abolished gate opening without affecting the membrane trafficking of the mutant channel [211]. This is different from the Ala mutations of the Gly hinge in BK channels, as mutant channels can still be opened by voltage and intracellular Ca^{2+}. In addition, altering the side chain of a phenylalanine residue (F315 in mSlo1) four amino acids after the Gly hinge changes the gating properties of BK channels [212, 213]. F315Y greatly facilitates BK channel opening, while F315I extensively decreases channel open probability. This phenylalanine residue is not conserved in K_V channels. All the above evidence suggests that the physical motions of the activation gate in BK channels might differ from those in K_V channels. Further studies are needed to elucidate their differences.

Concluding remarks

True to their name, BK channels exhibit large single-channel conductance and a large molecular mass among K^+ channels. The massive structure contains sensors to a variety of cellular signals, including phosphorylation [86, 89], oxidation [214–218], CO [165], H^+ [130, 219], heme [89, 123, 220] and PIP_2 [221] in addition to voltage, Ca^{2+} and Mg^{2+}. BK channels respond to these stimuli by changing the P_O, thus integrating cellular signals to modulate excitability and Ca^{2+} homeostasis. The BK channel molecule comprises the membrane-spanning pore-gate domain and the voltage-sensing domain as well as a large cytoplasmic domain. Studies of channel activation in response to voltage, Ca^{2+} and Mg^{2+} reveal that interactions among these structural domains are crucial in mediating stimulation to channel opening. These findings provide principles that may also govern channel gating in response to other stimuli. In addition, the function of BK channels is modulated by their association with the accessory β subunits,

which are important for the phenotypic differences of BK channels in various tissues [39, 90]. Some of the β subunits modulate voltage, Ca^{2+} or Mg^{2+} sensitivity of BK channels [107, 132, 145, 175, 222–233], but the molecular mechanisms of such modulation remain unclear. The different mechanisms of BK channel gating reviewed here may provide a basis for further investigation in this area.

BK channels share structural and functional similarities with K_V channels and ligand-activated K^+ channels. The common principles of ion channel gating apply to these channels. However, the BK channel is unique in that it combines the structure and function of both K_V channels and ligand-gated K^+ channels. Thus, gating by one stimulus can be tuned by change in another stimulus. Elucidation of the effects of voltage, Ca^{2+} and Mg^{2+} on BK channels has clearly demonstrated that allosteric mechanisms are fundamental to the regulation of BK gating. Although in other ion channels such allosteric couplings between distinct domains of the channel protein may not be readily observable, it would not be surprising if similar allosteric mechanisms may underlie gating in all ion channels.

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