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The LRRC family of BK channel regulatory subunits: potential roles in health and disease

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Abstract

Large conductance K⁺ channels, termed BK channels, regulate a variety of cellular and physiologic functions. Although universally activated by changes in voltage or [Ca²⁺]_i, the threshold for BK channel activation varies among loci of expression, often arising from cell-specific regulatory subunits including a family of leucine-rich-repeat (LRR)-containing gamma subunits (LRRC26, LRRC52, LRRC55, and LRRC38). The “founding” member of this family, LRRC26, was originally identified as a tumor suppressor in various cancers. An LRRC26 KO mouse model recently revealed that LRRC26 is also highly expressed in secretory epithelial cells and partners with BK channels in the salivary gland and colonic goblet cells to promote sustained K⁺ fluxes likely essential for normal secretory function. To accomplish this, LRRC26 negatively shifts the range of BK channel activation such that channels contribute to K⁺ flux near typical epithelial cell resting conditions. In colon, the absence of LRRC26 increases vulnerability to colitis. LRRC26-containing BK channels are also likely important regulators of epithelial function in other loci, including airways, female reproductive tract, and mammary gland. Based on an LRRC52 KO mouse model, LRRC52 regulation of large conductance K⁺ channels plays a role both in sperm function and in cochlear inner hair cells. Although our understanding of LRR-containing BK channels remains rudimentary, KO mouse models may help define other organs in which LRR-containing channels support normal function. A key topic for future work concerns identification of endogenous mechanisms, whether posttranslational or via gene regulation, that may impact LRR-dependent pathologies.

Graphical Abstract

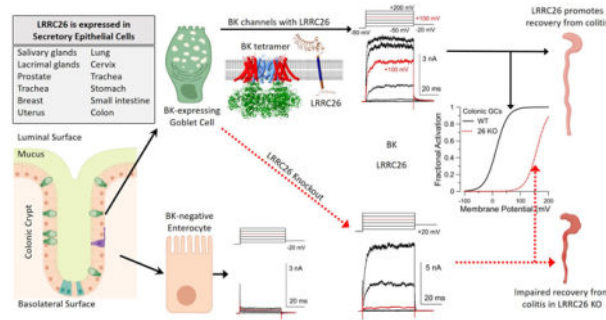
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Author contributions.

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Competing interests

The authors declare that they have no competing financial interests.



A major function of colonic crypts is the generation of a protective mucus layer separating gut contents from epithelial cells. Two major colonic epithelial cells are mucus-secreting goblet cells (GCs) and absorptive enterocytes. GCs specifically express BK channels containing the LRRC26 regulatory subunit, which shifts BK channel activation so that, at 0 cytosolic Ca^{2+} , BK channels are activated at membrane potentials (V_m) as negative as -40 mV. In mice lacking LRRC26, GCs also express BK currents, but they are not activated with 0 Ca^{2+} until V_m positive to $+50$ mV. Such LRRC26 KO mice exhibit enhanced sensitivity to colitis induced by dextran sodium sulfate. LRRC26 is expected to regulate BK-mediated K fluxes in a variety of secretory epithelial cells.

Introduction

Potassium channels of large conductance (BK or KCa1.1 , encoded by the *KCNMA1* gene (Butler *et al.*, 1993)) are ubiquitously expressed and contribute to rapid repolarization in excitable cells or K^+ fluxes in non-excitable cells (Contet *et al.*, 2016; Bailey *et al.*, 2019). A notable property of these channels is that they can be activated independently by two physiological signals: increases in submembrane Ca^{2+} , even in the absence of depolarization, or depolarization in the absence of Ca^{2+} . In a given cell type, it is the intrinsic properties of a BK channel, that is, the range of V_m and Ca^{2+} over which a particular channel is activated, that defines the circumstances under which it functions. Considering the variety of cell types in which BK channels is expressed and the functional roles they play, it is probably safe to assert that BK channels exhibit broader functional diversity than any other voltage-dependent K^+ channel (Gonzalez-Perez & Lingle, 2019; Dudem *et al.*, 2021).

Diversity in BK channel function arises from three general categories of mechanism: 1) alternative splice variants in the BK pore-forming α subunit (Shipston & Tian, 2016); 2) post-translational modifications of the BK α subunit (Shipston & Tian, 2016); and 3) three families of structurally distinct regulatory subunits, a β subunit family ($\beta 1$ – $\beta 4$), a γ subunit family of leucine-rich-repeat containing (LRRC) proteins ($\gamma 1$ – $\gamma 4$ with gene names, respectively, of *LRRC26*, *LRRC52*, *LRRC55*, *LRRC38*), and a recently described four member LINGO subfamily (LINGO1–4) (Dudem *et al.*, 2020) of which the LINGO1 subunit has been shown to mediate very rapid BK current inactivation. Although alternative splicing and post-translational modifications are important contributors to BK regulation, the tissue-specific expression of regulatory subunits probably accounts for most of the distinctive functional diversity of BK channels in native cells, conferring inactivation

behavior, affecting gating kinetics, and producing large gating shifts. The impact of β subunits on BK function and their potential physiological roles have been extensively investigated for over 35 years and recently reviewed (Li & Yan, 2016; Gonzalez-Perez & Lingle, 2019), while the LINGO subfamily is just beginning to be investigated. Here, the focus is progress that has been made pertinent to understanding physiological roles of LRRC/ γ subunits and potential pathologies that may be associated with LRRC subunits. Specifically, the combination of biophysical studies of effects of LRRC subunits on BK channels together with development of animal models in which LRRC26 (γ 1) and LRRC52 (γ 2) have been knocked out suggest that LRRC subunits may play critical roles in maintaining normal function in a variety of epithelial tissues and glands and, when mis-regulation occurs, may contribute to tissue pathology.

This presentation is organized in three sections. First, we summarize the initial identification and description of functional properties of subunits containing LRR (Leucine-Rich-Repeat) domains that are pertinent to BK channels. Since LRRC subunits of BK channels have been included in other recent reviews (Zhang & Yan, 2014; Li & Yan, 2016; Gonzalez-Perez & Lingle, 2019; Dudem *et al.*, 2021), here we highlight those properties of the subunits that we think are important for understanding their physiological roles and may provide clues to LRRC-dependent pathologies. Second, we summarize recent work with LRRC26 and LRRC52 that has addressed the role of LRRC proteins in native cells, with particular emphasis on what KO of LRRC subunits may be revealing about potential pathological consequences related to the LRRC subunits. Third, we consider how some of the functional results summarized in section I may point to processes that might underlie LRRC-dependent pathology highlighted in section II.

I. Identification and functional impact of the LRRC family of BK γ subunits

A. Overview of LRRC proteins.

LRRs (leucine-rich-repeats) are discrete structural domains found in an enormous number of proteins and thought to mediate protein-ligand or protein-protein interactions with functional roles ranging from innate immunity to nervous system development (Kobe & Kajava, 2001; Dolan *et al.*, 2007; Ng *et al.*, 2011). Whether as extracellular or cytosolic domains of proteins, the shared feature of LRR domains is that they contain some number of LRR repeats of 19–29 amino acids bracketed by characteristic N-terminal (LRRNT) and C-terminal (LRRCT) regions. The stacked arrangement of the LRR repeats generates a horseshoe-shaped structure, but the number of repeats in a given LRRC family of LRR-containing proteins, what other modules may be imbedded in the protein, e.g., immunoglobulin domains, and the topology in regard to transmembrane segments can vary.

The LRR-containing proteins under consideration here are termed LRRC subunits (Yan & Aldrich, 2012), reflecting their protein names (LRRC26, LRRC52, LRRC55, and LRRC38, given in order of their ability to influence BK channel activation, as summarized in Table 1). These four BK-related LRRC subunits consist sequentially of an extracellular N-terminal signal sequence followed by an LRRNT segment with four conserved cysteine residues, six LRR repeats with a consensus LxxLxLxxN sequence, and then a LRRCT segment also with four conserved cysteines ending the LRR domain. The LRR domain is followed by a

linker that connects to the single transmembrane (TM) segment followed by a short cytosolic sequence of 17–42 residues that contains little sequence conservation among the 4 proteins. At present, there is no functional role that has been determined for the LRR domain of any LRRC subunit.

Potential loci of expression of each of the BK-related LRRC subunits surround BK and LRRC26 structures in Figure 1. Evidence in support of some loci remains weak, particularly for LRRC55 and LRRC38 (summarized in Table 1). The first LRRC protein to be associated with BK channels, LRRC26, was originally found to be enriched in several tumor cell lines including prostate, colon, and breast (Egland *et al.*, 2006; Liu *et al.*, 2012). A C-terminal myc tag attached to LRRC26, when expressed in tumor cells, localized in a fibrous network with cytokeratin just under the plasma membrane. This led to its designation as a Cytokeratin-Associated Protein in Cancer (CapC (Egland *et al.*, 2006)). We will return to this topic below.

At present, it is unknown where LRRC subunits bind within the BK channel complex. For illustrative purposes, the center of Figure 1 displays the structure of the Ca^{2+} liganded human BK channel (Tao & MacKinnon, 2019) and an LRRC26 structure defined by AlphaFold (Jumper *et al.*, 2021). Two bits of functional data suggest some constraints on where LRRC26 may be positioned in a BK channel complex. First, LRRC26 and BK β subunits can coassemble within single BK channels (Gonzalez-Perez *et al.*, 2015) and, second, LRRC26 is known to influence voltage-sensor domain (VSD)/pore-gate domain (PGD) coupling (Yan & Aldrich, 2010). This suggests that LRRC binding is not occluded by simultaneous occupancy by β subunits, and may also interact with some elements in both the VSD and PGD.

B. LRRC26 and BK channels.

The existence of BK channels with unusually leftward shifted activation ranges was initially reported in prostate tumor cells (Gessner *et al.*, 2005) and inner hair cells (IHCs) of the cochlea (Thurm *et al.*, 2005). In such cases, even with nominally Ca^{2+} -free cytosolic solutions, BK currents were activated at membrane voltages much more negative than could be accounted for by any combination of heterologously expressed BK α and β subunits. Taking advantage of prostate tumor cell lines, Yan & Aldrich undertook a proteomic screen of BK-associated peptides that led to identification of a protein, LRRC26, with a predicted single pass transmembrane (TM) segment with an extracellular LRR domain (Yan & Aldrich, 2010). In comparison to BK β regulatory subunits which can also produce negative shifts in BK gating of about -80 mV but only with elevated cytosolic Ca^{2+} (Cox & Aldrich, 2000), LRRC26, when coexpressed with BK α subunits, produces a shift in the voltage of half activation ($V_{1/2}$) of the BK current conductance/voltage (G/V) relationship of about -140 mV (Fig. 2A) (Yan & Aldrich, 2010). Most importantly, this gating shift also occurs at low cytosolic Ca^{2+} , a property distinct from effects of β subunits (Yan & Aldrich, 2010). LRRC26 essentially converts BK channels from a high-voltage activated (HVA) K^+ channel to a low voltage-activated (LVA) K^+ channel, even in the absence of Ca^{2+} . This provides a potential explanation for how BK channels might contribute to K^+ fluxes under physiological conditions, i.e., negative membrane potentials and low

resting intracellular Ca^{2+} , in many non-excitabile cells. Confirming that LRRC26 was likely responsible for gating shifts in native cells, knockdown of LRRC26 in LNCaP prostate tumor cells abolishes the gating shift (Fig. 2A) (Yan & Aldrich, 2010). Similarly, knockout of LRRC26 in mice abolishes the gating shift in salivary gland acinar cells (Fig. 2B (Yang *et al.*, 2017)) and colonic goblet cells (Fig. 2C (Gonzalez-Perez *et al.*, 2021)). Subsequently, heterologous expression of other LRR subunits with BK subunits revealed that LRRC52 produces substantive shifts in BK gating (up to about -90 mV), LRRC55 produces a modest negative shift (about -50 mV) at 0 Ca^{2+} , but no shift at elevated Ca^{2+} , while effects of LRRC38 are less clear (Yan & Aldrich, 2012). It was proposed that LRRC26, LRRC52, LRRC55, and LRRC38 be considered a BK γ subunit family, $\gamma 1$ to $\gamma 4$, respectively, in order of their respective abilities to shift BK gating leftward (Yan & Aldrich, 2012). Here, we primarily use the gene names. We now review several studies that have probed functional aspects of LRRC26-mediated gating shifts, which may be informative about pathological changes in regulation by LRRC26.

A single LRRC26 subunit produces a full LRRC26-mediated gating shift, although four LRRC26 subunits can assemble in a BK channel.—An usual aspect of LRRC26-produced shifts in BK gating is that, as one increases the fractional occupancy of BK α channels with LRRC26 subunits, the BK currents appear composed of two populations, one exhibiting the expected -120 mV gating shift and another behaving like BK channels without any LRRC26 subunits (Gonzalez-Perez *et al.*, 2014) (Fig. 3A). Thus, as the fraction of injected LRRC26 cRNA is increased relative to that for BK α , the fraction of channels exhibiting the full gating shift increases, until essentially all appear fully shifted. Thus, LRRC26-mediated gating shifts occur in an all-or-none fashion, being either shifted or unshifted. This behavior differs markedly from that observed with BK β subunits for which, as one increases the number of β subunits in a BK channel from 0 up to 4, each β -subunit incrementally shifts BK gating leftward (Wang *et al.*, 2002). For BK $\beta 2$ subunits, the stoichiometry was revealed by examination at the single channel level of gating shifts as a function of inactivation rate. The cytosolic N-terminus of a $\beta 2$ subunit behaves as a mobile peptide segment that blocks the channel, independent of other $\beta 2$ N-termini. Since inactivation rate varies linearly as a function of the number of inactivation domains in a channel (1–4), the distribution of single channel inactivation rates into four components unambiguously revealed that 1, 2, 3, or 4 subunits could be present in a $\beta 2$ +BK α channel (Wang *et al.*, 2002). The LRRC26 stoichiometric problem is more complex. The unusual all-or-none behavior might occur from a number of potential LRRC26/BK assemblies, e.g., all BK channels might only contain a single unit of LRRC26 subunits (either 1, 2 or 4) or, alternatively, perhaps multiple LRRC26 subunits can assemble in a channel, but only one LRRC26 subunit is necessary for the full functional effect. By putting lanthanide tags on LRRC26 subunits and an emission acceptor on the BK blocker, iberiotoxin, it was shown that LRRC26 can associate with BK α in a maximal stoichiometry of 1:1 (Carrasquel-Ursulaez *et al.*, 2018). Similarly, Forster Resonance Energy Transfer between fluorescently tagged LRRC26 and BK α subunits led to a similar conclusion (Gonzalez-Perez *et al.*, 2018). However, to address the basis for the all-or-none behavior, a chimeric $\beta 2$ /LRRC26 construct with a $\beta 2$ inactivation domain as a functional reporter was utilized to count number of inactivation domains in a single channel while also measuring

the LRRC26-mediated gating shift (Gonzalez-Perez *et al.*, 2018). It had been previously shown that a similar $\beta 1$ /LRRC26 construct retained the LRRC26-like ability to shift BK gating (Li *et al.*, 2016). Use of the $\beta 2$ /LRRC26 construct revealed that single channels containing this chimeric subunit exhibited one of four linearly related inactivation rates, but all such channels exhibit the identical full gating shift expected with intact LRRC26. Thus, one LRRC26 subunit appears sufficient for a full gating shift. The mechanistic basis for this all-or-none shift remains undefined, as is the question of whether the unique stoichiometric properties of LRRC26:BK α assembly may have physiological implications.

Gating shifts produced by LRRC26 can be labile.—Co-expression of LRRC26 with BK α causes a -120 mV shift in the GV curve compared to BK alone, whether G/V curves are compared at 0 or 10 μ M cytosolic Ca $^{2+}$. However, when excised patches are held at -140 mV while bathed in zero Ca $^{2+}$ between generation of G/V curves (at 10 μ M Ca $^{2+}$), the G/V curve undergoes a rightward shift with time (Fig. 3B). Returning to elevated Ca $^{2+}$ for extended periods of time does not reverse the gating shift. When patches were held at $+80$ mV, a condition which favors channel activation despite the 0 Ca $^{2+}$, this gradual shift did not occur (Fig. 3C), suggesting a state-dependence to the loss of the gating shift (Gonzalez-Perez *et al.*, 2014). At intermediate times during the shift, G/V curves for BK channel activation were described by double Boltzmann's, with one component corresponding to shifted, LVA LRRC26-containing BK channels and the other behaving like HVA BK α alone channels (Fig. 3B). On average, the transition to HVA gating behavior occurred with a time constant of about 7.5 min. Although it is possible LRRC26 remains associated with BK α but has become inactive, another potential explanation for this phenomenon is that LRRC26 dissociates from BK channels. However, to date this phenomenon has not been noted in recordings of LRRC26-containing BK channels in excised inside-out patches from prostate tumor lines or with overexpression in HEK cells (Yan & Aldrich, 2010, 2012). Similarly, whole-cell recordings from parotid gland cells dialyzed with cytosolic 250 nM Ca $^{2+}$ (Yang *et al.*, 2017) do not reveal any time-dependent shifts in G/V curves. Yet, channels expressed in oocytes arising from coassembly of LRRC26 and $\beta 2$ subunits with BK α also do not exhibit the loss of LVA gating behavior when maintained in 0 Ca $^{2+}$ (Gonzalez-Perez *et al.*, 2015), perhaps suggesting that cell-specific components may impact on the maintenance of the LRRC26 regulatory effect.

The LRRC26 TM segment and cytosolic tail contribute to gating shifts and may also influence affinity between LRRC26 and BK α subunits.—Potential determinants of the ability of LRRC26 and other LRRC subunits to produce gating shifts have been functionally and biochemically probed in three papers: 1. a series of deletion mutants through the entire LRRC26 subunit (Yan & Aldrich, 2010); 2. chimeras generated from different segments of the four LRRC subunits (Li *et al.*, 2015); and 3. a $\beta 1$ /LRRC26 chimeric construct with only TM and cytosolic tail from LRRC26, along with mutational analysis of key residues through TM and the tail (Li *et al.*, 2016). Overall, the results indicate that the TM segment and a set of 4 basic residues just at the beginning of the LRRC26 cytosolic tail contain key determinants of the ability of a LRRC subunit to produce gating shifts. However, a complexity in the interpretation of the loss of gating shifts is whether a mutated construct is altering the functional effect, or disrupting the ability of

a construct to coassemble properly with BK. Some features of the results suggest that, although some mutations may specifically alter the ability of LRRC26 to shift gating while still being associated with the channels, some mutations may affect the affinity of interaction of LRRC26 with BK α . For the first case, mutation of 4 (4R) basic residues at the beginning of the cytosolic tail of LRRC26 tail markedly reduces the gating shift, while coimmunoprecipitation of 4R with BK α yields bands in western blots similar to WT LRRC26 (Yan & Aldrich, 2010). In contrast, two mutations in the LRRC26 TM segment may reflect weakened affinity of interaction. For these two mutations, P270F and F273S, G/V currents are best described by two components (Fig. 3D), an LVA population of about 75% with the remainder being HVA (Li *et al.*, 2016). Yet, despite the fact that about 75% of channels exhibit the full gating shift, co-immunoprecipitation of these mutated constructs from the BK channel complexes is reduced to much less than half of that of WT LRRC26 coassembly with BK α . Although densitometry was not used to assess this quantitatively, the reductions appear close to less than 10–20% of WT controls. In later work, densitometric measurements confirmed that association of F273S-LRRC26 with BK α is reduced to 20% of that for WT LRRC26 (Guan *et al.*, 2017). Mutation of these residues together fully abolishes the LRRC26-induced gating shift. On balance, the Co-IP experiments strongly support the idea that the affinity of interaction between LRRC26 and BK has been weakened by some TM mutations. Yan and colleagues suggest that their results are consistent with the all-or-none gating shift model in which a substantially reduced occupancy of BK channels by LRRC26 is still sufficient to permit most channels to exhibit LVA gating behavior.

The idea that double Boltzmann's in G/V curves can arise from altered affinity of interaction between LRRC26 and BK is also supported by results with the BK activator, mallotoxin, a potent BK activator (Zakharov *et al.*, 2005; Almassy & Begenisich, 2012). Mallotoxin (MTX) shifts BK gating about –120 mV at 0 cytosolic Ca²⁺ (Zakharov *et al.*, 2005; Almassy & Begenisich, 2012). Intriguingly, LRRC26 abolishes the ability of MTX to shift BK gating (Fig. 3E, (Guan *et al.*, 2017)). This has been interpreted to indicate that the presence of LRRC26 occludes the ability of MTX to reach its binding site. However, for the LRRC26-F273S TM mutation, which results in a double Boltzmann behavior, in the presence of MTX the LVA component of BK gating disappears and the resulting G/V is comparable to that arising from MTX effects on BK α alone (Guan *et al.*, 2017). With washout of MTX, the G/V then becomes similar to BK α alone. This has been interpreted to indicate that weakened binding of F273S-LRRC26 subunit to BK may not completely occlude the ability of MTX to reach its site (Fig. 3F), such that once MTX is bound it further destabilizes interaction of F273S-LRRC26 with the BK α subunit, promoting dissociation of the F273S-LRRC26 subunit from the channel. Supporting this view, MTX reduces F273S-LRRC26 co-IP with BK channels, while having no effect on WT LRRC26 in BK channels (Guan *et al.*, 2017).

Together these results, while pointing out the importance of particular LRRC26 residues in defining the gating shift, also support the idea that the intrinsic binding affinity that underlies LRRC26 subunit interaction with BK subunits may be poised to be more readily disrupted, potentially allowing down (or up) regulation of the fraction of LRRC26 subunits in the BK population. Clearly, additional work is necessary to evaluate this premise, but the set

of results raises the possibility that association of LRRC26 with BK α may be labile and perhaps subject to dynamic regulation, at least in some cellular environments.

C. LRRC52 (γ 2): a regulatory subunit of both Slo3 and BK channels.

Channel regulation by LRRC52 also reveals some interesting insights into LRRCs. The closest homolog of the BK channel is a voltage- and pH-regulated K⁺ channel, termed Slo3 (encoded by KCNU1), which is abundantly expressed in testis (Schreiber *et al.*, 1998). As expected, a voltage- and alkalization-activated K⁺ current was later identified in native mouse sperm (Navarro *et al.*, 2007), but the native sperm current was activated at more negative voltages than currents from heterologously expressed Slo3 (Zhang *et al.*, 2006). Yet, KO of Slo3 abolished all voltage- and alkalization-activated K⁺ current in mature sperm (Zeng *et al.*, 2011). Subsequently, LRRC52 was then shown to be present in mammalian sperm and to shift Slo3 gating to more negative voltages, potentially accounting for the differences between heterologously expressed Slo3 and native sperm K⁺ current (Yang *et al.*, 2011). Supporting a critical role of LRRC52 in sperm K⁺ function, genetic KO of LRRC52 produced fertility deficits in mouse sperm while also shifting sperm K currents to be more comparable to properties of heterologously expressed Slo3 alone (Zeng *et al.*, 2015).

Surprisingly, although initially considered to be sperm-specific, LRRC52 was subsequently found to be critical to defining the functional properties of BK channels in inner hair cells (IHCs) of the mouse cochlea (Lingle *et al.*, 2019). Remarkably, KO of LRRC52 produced a positive 210 mV gating shift of BK channels in IHCs, shifting the native IHC G/V curve to be similar to that of BK channels lacking regulatory subunits. The fact that heterologously expressed LRRC52 shifts BK gating about -90 mV suggests that LRRC52 may help stabilize some other as-yet-unidentified factor that contributes to the enormously shifted IHC BK current. Furthermore, LRRC52 KO disrupts the high-density clustering of BK channels between the stereocilia and above the nuclei, suggesting that LRRC proteins may help stabilize macromolecular BK ion channel complexes.

The discovery that LRRC52 can partner either with Slo3 or with BK α raises the question, to what extent are LRRC subunits promiscuous in their ability to assemble and regulate Slo family channels? In the initial characterization of the ability of LRRC52 to alter Slo3 function, Slo3 was also shown to coassemble with LRRC26, but only weakly with LRRC38 and LRRC55, although even for LRRC26 functional effects on Slo3 were minimal at elevated pH (Yang *et al.*, 2011). There are no reports of whether any LRRC subunits may interact with and regulate either of the two Na-activated Slo2 subunits, KCNT1 and KCNT2 (Salkoff *et al.*, 2006). The coupling of LRRC52 and BK α in IHCs also points out two challenges that are faced in attempts to understand the roles of LRRC proteins. First, because of the large single channel currents associated with BK channels, only a few channels need to be activated or even expressed in a cell in order to produce important physiological effects. Second, LRRCs may partner in very circumscribed cellular loci where the total message and protein abundance might not be readily revealed in some screening methods. Relevant to that possibility, message (Chen *et al.*, 2021a, b) and protein (Limbutara *et al.*, 2020) for LRRC52 has been recently identified in distal collecting tubule segments and cells of the kidney. Yet, message or protein for BK α was not observed in the same

segments, although BK channels have been unambiguously recorded from other locations in the kidney, including from intercalated cells of collecting tubule and cortical collecting duct cells (Palmer & Frindt, 2007). It is possible that levels of both message and protein for BK α are too low for the methodologies employed.

D. LRRC55 (γ 3) and LRRC38 (γ 4)

LRRC55 is intriguing because of robust expression of *LRRC55* message in brain (Yan & Aldrich, 2012); *in situ* hybridization has demonstrated elevated *LRRC55* expression in adult brain in medial habenula nucleus, cerebellum, and pons (Zhang *et al.*, 2018). Despite the apparent absence of gating shifts at elevated Ca²⁺, the ability of LRRC55 to produce a -50 mV gating shift at 0 Ca²⁺ might potentially have unique effects on neuronal excitability (Yan & Aldrich, 2012). As yet, there is no information about LRRC55-containing BK channels in native neurons. Recently, evidence supporting the idea that LRRC55 can impact on properties of BK channels in native tissues appeared in a study of podocytes in kidney (Hu *et al.*, 2021). Specifically, *Lrrc55* message and LRRC55 protein was found to be present in glomerular tissues and expression was increased in podocytes of patients with focal segmental glomerulosclerosis, diabetic nephropathy, and membranous nephropathy. Similarly, *Lrrc55* expression was increased in podocytes during angiotensin II induced podocytes injury in mice (Hu *et al.*, 2021). Knockout of LRRC55 ameliorated the pathology. Podocytes were shown to express a BK current, which was enhanced by angiotensin II, while LRRC55 KO abolished the angiotensin II-induced enhancement of BK current. Although these results do not reveal a specific effect of LRRC55 on BK gating properties, LRRC55 dramatically increases total BK channel current density in podocytes, with resulting pathological consequences.

Effects of LRRC38 on BK channels are quite modest (Yan & Aldrich, 2012; Kshatri *et al.*, 2017) and LRRC38 also has no effect on Slo3 gating (Yang *et al.*, 2011). As yet, whether LRRC38 can even coassemble with BK channels has probably not been satisfactorily addressed. Any role of LRRC38 on BK or other Slo family channels remains to be revealed.

II. LRR subunit regulation of channels in native cells, physiological roles, and potential implications for pathology.

A. A general role for LRRC26-containing BK channels in secretory epithelial cells.

Secretory epithelial cells provide a supportive barrier to mucosal surfaces through the production and secretion of peptides, mucus, fluid, bicarbonate, and/or salts. Regardless of location or secretory constituents, ion channels and transporters regulate the function of epithelial cells. An important gap in knowledge remains the full identification of all the sets of ion channels and transporters and their specific membrane localization (apical vs. basolateral) that work together to fulfill the particular requirements of a given epithelial layer or gland. A possible contribution of BK channels to K⁺ conductance in epithelial cells has been known for some time (Lomax *et al.*, 1996; Butterfield *et al.*, 1997; Linley *et al.*, 2014). However, at membrane potentials and cytosolic Ca²⁺ concentrations thought to be typical of inexcitable cells, channels arising from known BK splice variants and β regulatory subunits would not be expected to contribute to resting K⁺ fluxes in such cells.

This conundrum was resolved by the discovery of LRRC26 and its ability to shift BK activation leftward by over 120 mV even at 0 Ca²⁺.

With the identification of LRRC26 as a BK regulatory subunit, potential contributions of LRRC26-containing BK channels were quickly established in salivary gland acinar epithelial cells (Almassy & Begenisich, 2012) and airway epithelium (Manzanares *et al.*, 2014; Manzanares *et al.*, 2015). Parotid gland cells express BK currents with G/V properties similar to heterologously expressed LRRC26-containing BK channels and also exhibit resistance to stimulation by mallotoxin (Almassy & Begenisich, 2012). In airway epithelial cells, an interferon- γ (IFN- γ)-stimulated reduction of BK channels expression has been implicated in mucociliary dysfunction. Stimulation by IFN- γ is also associated with reduction in LRRC26 expression and the appearance of mallotoxin-activated transepithelial currents, which are absent in untreated tissue (Manzanares *et al.*, 2014), supporting the idea that LRRC26-containing BK channels are present in airway epithelium. Negative effects of transforming growth factor beta1 on airway function have also been shown to be associated with reduced epithelial cell BK activity arising from reduction of LRRC26 expression (Manzanares *et al.*, 2015). The latter results are intriguing because they point out that normally occurring signaling pathways can regulate LRRC26 expression.

A primary role of LRRC26-containing BK channels in essentially all secretory epithelial cells was given support with the development of a LRRC26 general KO mouse in which the *Lrrc26* promoter controlled expression of *LacZ* (Yang *et al.*, 2017). Essentially all secretory epithelia that were examined express *Lrrc26* message and/or exhibit Blue-Gal staining indicative of *Lrrc26*-promoter activity. Furthermore, in lacrimal glands and various salivary glands (parotid and submandibular gland), comparison of currents in cells from WT and LRRC26 KO animals directly demonstrated that KO of LRRC26 shifted BK gating in such cells from low-voltage activated to the high-voltage activated currents typical of BK channels without regulatory subunits (Fig. 3B), consistent with the effect of LRRC26 observed when coexpressed with BK α subunits.

For salivary glands, the involvement of BK channels in K secretion is well-established (Catalan *et al.*, 2014). The concentration of K⁺ in saliva is typically 5–10 fold that in plasma, but KO of BK channels markedly reduces K⁺ secretion in saliva. In contrast, KO of the KCa3.1 (IK1) Ca²⁺-activated, voltage-independent K channel was without effect on K⁺ secretion (Nakamoto *et al.*, 2008). Consistent with the importance of LRRC26 in defining properties of salivary gland BK channels, LRRC26 KO produces a reduction of K⁺ content in saliva (Yang *et al.*, 2017) similar to that occurring with full BK KO (Nakamoto *et al.*, 2008).

B. LRRC26-containing BK channels and colitis.

Recently, the just-described LRRC26 KO mice were used to probe the potential role of LRRC26-containing BK channels in the colonic epithelium (Gonzalez-Perez *et al.*, 2021). The single layer of colonic epithelial cells organize in crypts to form a physical and biochemical barrier between the host and the environment while promoting mucosal homeostasis (Peterson & Artis, 2014). Colonic epithelium consists of multiple cell types, with the primary cell types being 1. absorptive enterocytes comprising more than 75% of the

cells, 2. mucin2-secreting goblet cells, which are responsible for formation of the protective mucus layer, and 3. enteroendocrine cells. Although BK channels have been known to be present in colonic epithelium and BK channels are thought to be associated with colonic K^+ loss during secretory diarrhea (Sandle & Hunter, 2010), the precise cellular membranes, the cellular loci, and circumstances of activation of BK channels in colonic epithelium have remained incompletely resolved. Somewhat surprisingly, genetic deletion of *LRRC26* renders mice sensitive to colitis induced by dextran sodium sulfate (DSS) (Gonzalez-Perez *et al.*, 2021), a standard model of colitis primarily involving the distal colon. In response to DSS, *LRRC26* KO mice exhibited irreversible loss of body weight, high disease severity indices, and examination of distal colon revealed loss of crypts and GCs. By crossing *LRRC26* KO mice with mice that express a transgene encoding mCherry attached to *Mucin2* under control of the *Mucin2* promoter (Birchenough *et al.*, 2016), direct visualization of mCherry fluorescence in GCs allowed electrophysiological recordings from unambiguously identified fluorescent GCs and adjacent non-fluorescent enterocytes from both WT and *LRRC26* KO mice. This showed that *LRRC26*-containing BK channels were only present in the mCherry-identified GCs, while the non-fluorescent enterocytes exhibited no BK current at all. The specific mechanism underlying the *LRRC26* KO-induced colitis, i.e., whether the loss of BK function leads to increased sensitivity to colitis via a deficit in mucus formation, deficient stem cell proliferation or growth, or deficient healing and recovery, remains to be determined.

Given the critical role of GCs in maintaining the protective mucus layer of the colon (Nystrom *et al.*, 2021), these results point to a potential role of BK channels in helping to sustain the generation of normal mucus layer properties. However, a number of ion channels of the colonic epithelium, including CFTR (Gustafsson *et al.*, 2012) and bestrophin2 (Yu *et al.*, 2010), are also known to be important. The present results support the idea that BK channels on the luminal side of some epithelial cells (Sandle & Rajendran, 2012) may be critical for supporting anion fluxes essential for mucus secretion and maturation. The recent work (Gonzalez-Perez *et al.*, 2021) now shows clearly that dysfunction in BK channel properties may also increase susceptibility or perpetuation of colonic disease. Given the presence of *LRRC26*-containing BK channels in most other secretory epithelial cells (Yang *et al.*, 2017), *LRRC26*-containing BK channels may play a general role in a number of tissues in helping maintain normal secretory function, whether fluid, peptides, or mucus.

Although *LRRC26*-containing BK channels are suited to play a prominent role in secretory epithelial cells, our understanding of how such fluxes are critical to normal physiology remains incomplete. One issue is that in the recent work a specific colitis-inducing stimulus, DSS, was required to unmask a critical role of *LRRC26*-containing channels. However, the idea that some naturally occurring diminution in *LRRC26* expression might be a contributing factor to development of pathology is worth consideration. Related to this possibility, analysis of gene-expression profiles of colon from IBD patients has revealed a reduced expression of *LRRC26* in patients with active ulcerative colitis (UC) in comparison to patients with inactive disease (Gonzalez-Perez *et al.*, 2021). Yet, somewhat contrary to this idea, it has also been reported that colonic BK α subunit message is increased in UC patients (Sandle *et al.*, 2007). However, it is unknown whether this is a compensatory change to the underlying pathology or causative. Given that loss of BK function predisposes to

colitis sensitivity, we would suggest that the increase in BK subunit expression is part of a compensatory process.

C. LRRC26 and smooth muscle

A potential role of LRRC26-containing BK channels has also been proposed for rat cerebral arteries (Evanson *et al.*, 2014), mouse uterine artery (Lorca *et al.*, 2018), and mouse bronchiole smooth muscle cells (mBSMCs) (Noda *et al.*, 2020). A role of LRRC26-containing BK channels in smooth muscle would be of high impact because of expected profound effect on vascular tone (Dopico *et al.*, 2018). As highlighted, the primary distinguishing feature of LRRC26-containing BK channels is their marked -120 to -140 mV leftward gating shift, which is clearly apparent both from heterologously expressed channels and also native cells for which WT and LRRC26 KO animals are compared. Unfortunately, for some native cells, whole-cell measurements of G/V relationships can be difficult to define. Furthermore, as yet, there is no demonstration of a useful antibody for LRRC26 immunohistochemistry that meets the criteria of differential staining between WT and KO tissues. However, a recent paper that compares BK currents and the resulting G/V curves in mBSMCs to those in mouse aortic SMCs (mASMCs) provides some good support for a role of LRRC26-containing BK channels in some vascular myocytes (Noda *et al.*, 2020). With 10 nM cytosolic Ca^{2+} , the V_h for activation of currents from BSMCs was similar to that ($V_h \sim -15$ to -25 mV) for BK α +LRRC26 expressed by the same authors in HEK cells (Noda *et al.*, 2020). In contrast, BK currents from mASMCs exhibited a V_h similar to that of either BK α expressed alone or BK α + β_1 in HEK cells ($V_h \sim +130$ - $+150$ mV).

D. LRRC26 and cancer.

The *LRRC26* gene was originally identified from a cDNA library prepared from human breast and prostate cancer cell lines (Egland *et al.*, 2006). The abundance of this message in a number of different cancer cell lines and its cellular localization in close juxtaposition with sub-plasma membrane cyokeratin led to its designation as CAPC (Cytokeratin-Associated Protein in Cancer) (Egland *et al.*, 2006). In general, both message and protein for LRRC26 are elevated in cancer cell lines or cancer specimens in comparison to normal tissue, leading to the proposal that LRRC26 may serve as a tumor marker and may influence cancer cell behavior. Although evaluation of the role of LRRC26 in tumor initiation and development is beyond the scope of this commentary, some results are worth mentioning. In both prostate and breast cancer cell lines, knockdown of *LRRC26* expression by siRNA enhances tumor growth, while overexpression negatively regulates NF- κ B activation and suppresses tumor growth and metastasis. This parallels the observation that triple negative breast cancer (TNBC) is associated with downregulation of *LRRC26* expression associated with epigenetic hyper-methylation of *LRRC26* promoter sequence upstream of the LRRC26 open reading frame (Miyagawa *et al.*, 2018). The specific molecular cascade by which LRRC26 may impact on tumor cell growth remains unknown.

Are the effects of LRRC26 in tumor cells specifically linked to BK channels? The negative impact of LRRC26 overexpression on NF- κ B activation may be independent of BK channels, since knockdown of BK α by siRNA did not abolish or reduce the

LRRC26-mediated reduction in NF- κ B activation in MCF7 cells nor in LNCaP cells which abundantly express LRRC26 (Liu *et al.*, 2012). Furthermore, if the impact of LRRC26 on tumor growth was associated with BK channels, one might expect that the consequences of manipulations of BK expression might parallel those of LRRC26. Yet, reduction of BK expression is associated with increased tumor free survival in mice while both in breast cancer and prostate cancer cell lines increases in BK expression lead to more metastatic tumor growth (Bloch *et al.*, 2007; Khaitan *et al.*, 2009; Oeggerli *et al.*, 2012). Although the impacts of various ion channels on tumor growth remain inadequately understood (Lang & Stourmaras, 2014; Prevarskaya *et al.*, 2018), both the failure of BK knockdown to remove the LRRC26-mediated effects on NF- κ B activation and the dissociation of BK channel effects on tumor growth from LRRC26 effects would seem to suggest LRRC26 may have functions independent of its role as a BK regulatory subunit. Clearly, this requires additional consideration. Furthermore, irrespective of the underlying mechanism by which LRRC26 impacts on tumor growth, the pronounced suppression of breast tumor metastasis by LRRC26 overexpression (Liu *et al.*, 2012) certainly warrants additional investigation, particularly in regards to tumors recalcitrant to other treatment strategies, e.g., the TNBCs.

E. Summary

The above sections highlight the prominent contributions of LRRC26-containing BK channels in both glandular acinar epithelium and layered epithelium, and point out the likely role of such channels in essentially all secretory epithelium, and perhaps in some vascular smooth muscle cells. For salivary glands, airways, and colon, loss of LRRC26 or downregulation by naturally occurring signals leads to or is associated with profound pathological changes in those tissues, presumably in some way related to changes in K⁺ flux or other aspects of secretion. At present, we are really in the early stages of unveiling the potential roles this category of BK channel can play in different tissues. With appropriate tests, similar pathological consequences of LRRC26 loss might be expected in other tissues. A number of questions must be addressed. Why do LRRC26 KO mice not exhibit clear spontaneous phenotypic deficits? Full KO's grow and breed normally as far as has been observed. In the absence of the DSS manipulation, no obvious colitis phenotype has been noted out to 12 weeks. In other systems for which LRRC26-containing secretory cells play a central role, might a particular assault, e.g., a uterine infection, an airway irritant, or other xenobiotic factor that enters the lumen surrounded by the vulnerable epithelium, trigger pathological consequences?

III. Possible underpinnings of LRR-associated pathology

We now turn to a consideration of potential mechanisms that might underlie naturally occurring LRR-associated disease. Results cited above indicate clearly that loss of LRRC26 from specific epithelial tissues can result in pathological function. Although gene KO may point to a potential role of a protein in particular pathologies, it does not reveal how changes in expression occur normally. Here we consider two kinds of mechanism that might influence the availability of LRRC26 for assembly in BK channels: first, factors that may influence the affinity of the LRRC26-BK α interaction and, second, regulatory mechanisms that may alter LRRC26 expression.

A. Implications of low affinity interactions of LRRC26 with BK channels.

We have highlighted several results suggesting that the intrinsic affinity of the LRRC26-BK interaction may be poised such that subtle changes in that affinity may shift BK channels to high voltage-activated. First, the unusual stoichiometric relationship in which a single LRRC26 subunit is sufficient to produce a full gating shift permits a complete shift from LVA to HVA gating by loss of a single subunit (Gonzalez-Perez *et al.*, 2014; Gonzalez-Perez *et al.*, 2018). This would be most impactful in cases where the average LRRC26 occupancy in a channel population is low. It will be interesting to see whether the double Boltzmann behavior observed with partial LRRC26 occupancy may be a feature of currents in some native cells or in disease states. Second, the shift from LVA to HVA behavior observed for LRRC26-containing BK channels expressed in oocytes when patches are exposed continuously to 0 Ca²⁺ under conditions that favor closed channels suggests (Gonzalez-Perez *et al.*, 2014) that Ca²⁺ or state-dependent mechanisms can influence LRRC26-BK affinity. Third, point mutations in the LRRC26 TM produce gating shifts consistent with weakened affinity, supporting the idea that the native affinity may be poised at a level permissive for regulation (Li *et al.*, 2016). Fourth, mallotoxin can irreversibly drive the gating of the F273S-LRRC26 construct to become fully HVA with associated biochemical evidence that mallotoxin reduces F273S-LRRC26 coassembly with BK channels. (Guan *et al.*, 2017).

Together these results suggest that the physical interaction between LRRC26 and BK α , might be subject to modulation, perhaps by cell-specific factors, that may alter the fractional occupancy of BK channels by LRRC26. At present, no such regulatory components are known, although the LRRC26 cytosolic C-terminus contains both consensus phosphorylation sites and a cluster of basic residues near the membrane that might be a target for phospholipid interaction. A labile LRRC26-BK interaction would not only be physiologically important, but also might lead to pathological consequences, as illustrated by the impact of LRRC26 KO on colitis. It must be kept in mind that, in order for a loss of LRRC26 to be physiologically impactful in regards to association with BK, it probably requires that almost all channels lose LRRC26 subunits, since even a small fraction of LVA channels might be expected to dominate physiologically.

B. Potential mechanisms of down-regulation of expression of LRRC26

Results cited above implicate two distinct signaling pathways in the down-regulation of *LRRC26* message and protein in airway epithelial. In one case, the cytokine, IFN- γ , reduces BK function in airway epithelial via downregulation of LRRC26 expression (Manzanares *et al.*, 2014). In the other case, in cystic fibrosis, transforming growth factor β 1 (TFG- β 1) is upregulated and induces down-regulation of LRRC26 and reduction in BK function underlying deficits in mucociliary clearance (Manzanares *et al.*, 2015). Presumably these effects arise from regulation of transcription.

An additional mechanism suggested above that may underlie down regulation of LRRC26 expression is suggested from an RNA-sequencing analysis of triple-negative breast cancer (TNBC) specimens along with analysis of The Cancer Genome Atlas TNBC dataset (Miyagawa *et al.*, 2018). The *LRRC26* sequence is remarkable in the extent to which it

contains GpC islands that are the candidate loci for methylation. This analysis identified frequent downregulation of *LRRC26* expression in TNBC tissues (Miyagawa *et al.*, 2018). Further evaluation revealed the *LRRC26* was frequently silenced in both TNBC tissues and cell lines as a consequence of promoter methylation. Intriguingly, in a TNBC cell line that lacks *LRRC26* expression, *LRRC26* expression was restored by an inhibitor of methylation. Although the analysis just summarized focused on the potential importance of *LRRC26* expression in tumor growth regulation, that *LRRC26* expression can be influenced by epigenetic methylation of the *LRRC26* promoter region (Miyagawa *et al.*, 2018) indicates that mechanisms are available that could lead to pathologies similar to those that may be associated with *LRRC26* KO. Methylation and demethylation are cell and tissue specific processes (Lanata *et al.*, 2018). Furthermore, although it typical to think of methylation as irreversible, this is not uniformly the case (Lanata *et al.*, 2018). Although a detailed examination of methylation pertinent to *LRRC26* is beyond the summary here, such a mechanism has two key features that might merit its consideration as a contributor to *LRRC26* based pathologies, namely tissue specificity and reversibility. Although identification of genes that show up- or down-regulation by methylation in response to different diseases and xenobiotic agents is a major cottage industry that largely just identifies associations, it is interesting to note *LRRC26* is one of a set of 22 methylated CpGs in 21 gene loci associated with Ischemic Stroke (Soriano-Tarraga *et al.*, 2020).

Overview.—Since recognition almost 12 years ago that a family of homologous *LRRC* proteins are potential regulators of BK channel function, we are really only at the beginning stages of understanding how such subunits may impact on normal physiological function. Yet, KO animal models are now revealing that, in the absence of particular *LRRC* subunits, major alterations in BK channel function and/or expression can occur that can result in major pathological consequences, e.g., colitis for *LRRC26*, male infertility for *LRRC52*, and perhaps nephropathy for *LRRC55*. Given the pervasiveness of *LRRC26* in a variety of critical organ systems, there are probably many more pathological consequences to be discovered. Yet, despite what can be learned from KO models, they are only useful in pointing out potential consequences of changes in subunit expression, but fail to identify mechanisms that might contribute to disease. Here, particularly in the case of *LRRC26*, we have highlighted aspects of the regulation of BK channels by *LRRC26* that may eventually help identify the molecular underpinnings of disease that may arise from mis-regulation or altered expression of *LRRC* subunits.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BK	large conductance, Ca and voltage-activated K channel
LRRC	leucine-rich-repeat-containing
GCs	goblet cells
V_m	membrane potential
G/V	conductance/voltage
MTX	mallotoxin
HVA	high-voltage-activated
LVA	low-voltage-activated
V_h	voltage of half activation

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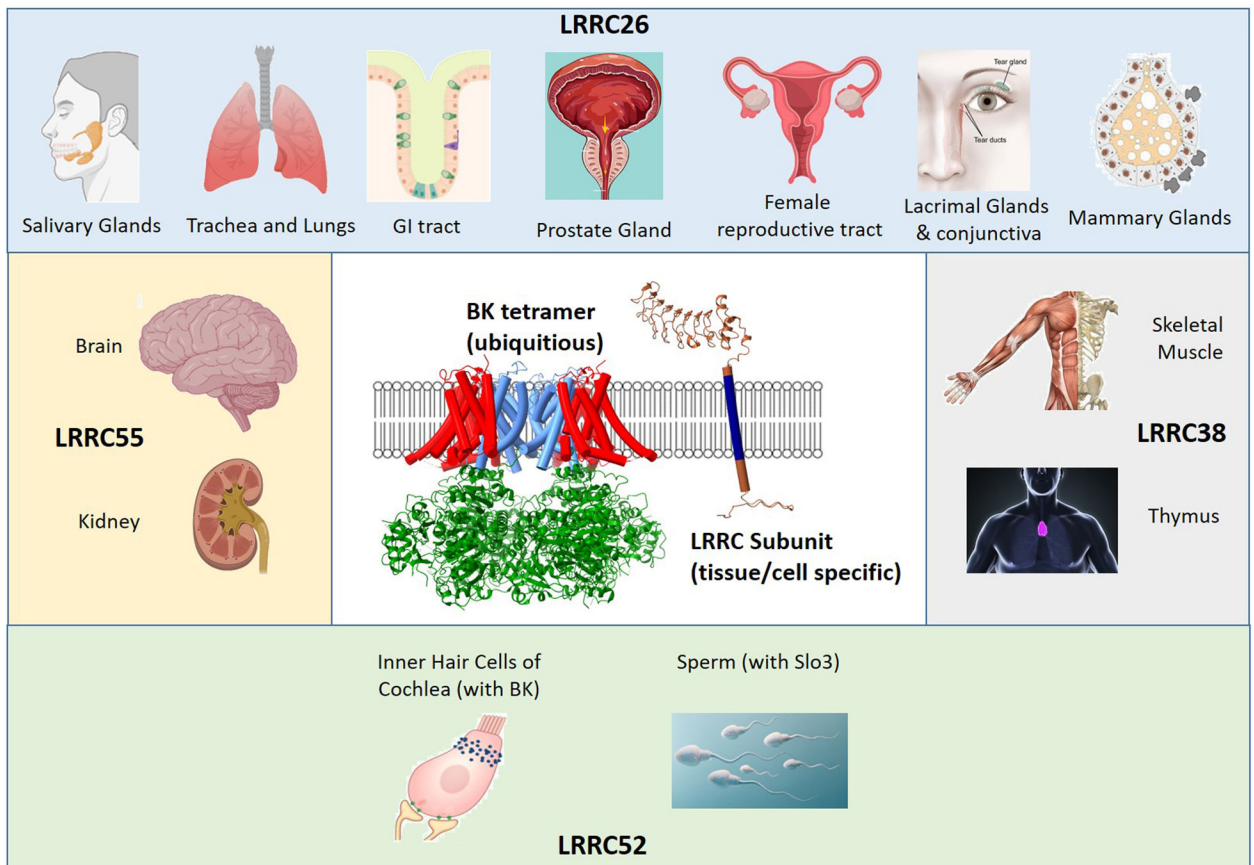


Figure 1. A family of four LRRC subunits contribute to tissue-specific regulation of BK channels and related family members.

In the center, structures are shown of a human Ca^{2+} -liganded BK channel (PDB 6V38, (Tao & MacKinnon, 2019)) along with an isolated LRRC26 subunit from Google Alpha Fold (Uniprot Q2I0M4, (Jumper *et al.*, 2021)). For the BK structure, red highlights the voltage-sensor domain (VSD), green is the ligand-sensing domain or so-called gating ring, while blue corresponds to pore-gate-domain (PGD). Around the center, for each of the four LRRC subunits, some of the potential loci of expression are displayed, which are summarized in Table 1.

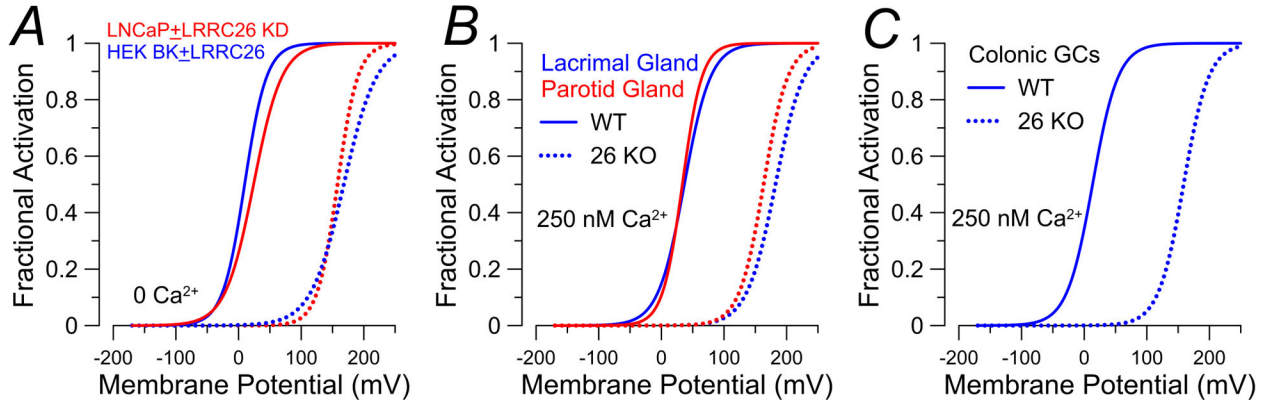


Figure 2. LRRC26 produces robust leftward BK gating shifts explaining low-voltage activation in prostate gland, lacrimal gland, parotid gland, and colonic goblet cells.

G/Vs here and in Figure 3 were generated from published Boltzmann fit values from papers cited in the text. Dotted lines correspond to G/V curves generated under conditions where LRRC26 is absent, while solid lines represent G/V curves for BK α with LRRC26. All G/Vs were generated under low Ca²⁺ conditions (either 0 (A) or 250 nM (B,C) Ca²⁺). A, G/V curves for BK current activated at 0 Ca²⁺ in LNCaP prostate tumor cells (red lines) without (solid red line) or with knockdown (KD, dotted red) of LRRC26. KD of LRRC26 shifts gating rightward making it indistinguishable from BK α currents alone. Expression of BK alone in HEK cells (dotted blue) is similar to KD of LRRC26 in LNCaP cells, while BK+LRRC26 expression in HEK cells (solid blue) shifts gating leftward (Yan & Aldrich, 2010). B, Native BK currents from acinar epithelial cells from lacrimal gland and parotid gland result in G/V curves similar to BK+LRRC26 (solid lines). In the LRRC26 KO mouse, the gating shift is abolished (dotted lines) (Yang et al., 2017). C, in identified colonic GCs, WT cells exhibit LVA BK currents (solid line), but GCs from LRRC26 KO mice exhibit HVA BK currents (dotted line) (Gonzalez-Perez et al., 2021).

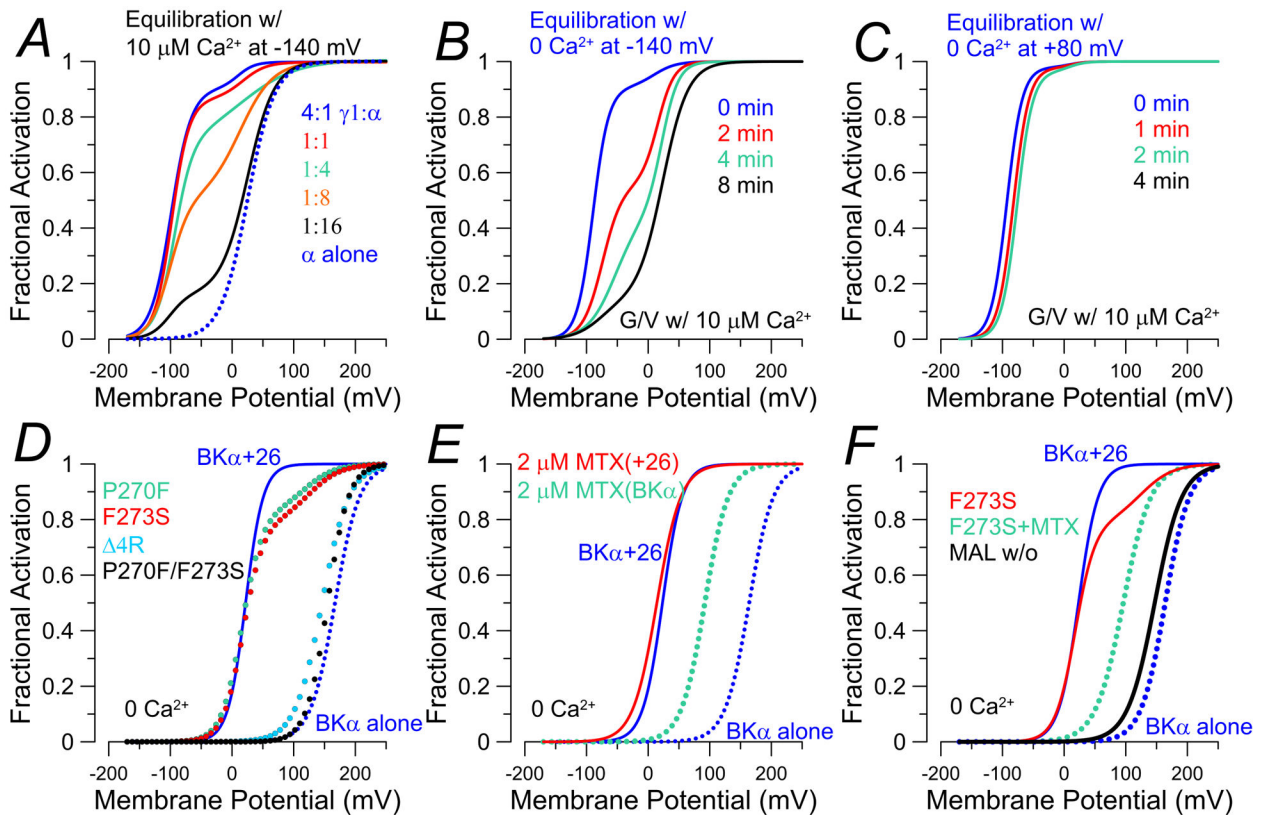


Figure 3. Factors affecting LRRC26 regulatory effects on BK G/V curves.

G/V curves were constructed from published values in the cited papers. For panels A-C, G/V curves were generated with 10 μM . *A*, BK G/V curves show loss of shifted (LVA) fraction as injected ratio of LRRC26:BK α is reduced from 4:1 to BK α alone (Gonzalez-Perez et al., 2014). *B*, for BK+LRRC26 channels expressed in oocytes, exposure of patches to 0 Ca^{2+} for the indicated times between acquisition of G/Vs with 10 μM Ca^{2+} results in gradual loss of shifted fraction of channels (Gonzalez-Perez et al., 2014). *C*, LVA behavior of BK+LRRC26 channels does not disappear if channels are held under conditions that promote channel activation (0 Ca^{2+} , +80 mV, or 10 μM Ca^{2+} , 0 mV) (Gonzalez-Perez et al., 2014). In D-F, G/V curves were generated with 0 Ca^{2+} with expression in HEK cells (solid blue lines: BK α +LRRC26; dotted blue: BK α alone). *D*, G/V curves for mutations in LRRC26 S6 TM segment that result in reductions or complete loss of LVA component of BK gating (Li et al., 2016). P270F and F273S result in loss of roughly over 80% of Co-IP with BK α , while Δ 4R produces minimal effect on assembly with BK α . *E*, The BK activator, mallotoxin (MTX), shifts gating of BK α alone channels (dotted cyan), but not BK α +LRRC26 channels (dotted red) (Guan et al., 2017). *F*, for LRRC26-F273S (red line), following application of MTX the LVA component of BK current is abolished (dotted cyan), while still exhibiting a shift comparable to effect of MTX on BK α alone. Following washout of MTX (dotted red), the G/V becomes like BK α alone (Guan et al., 2017).

Table 1.

Potential loci of expression of BK LRRC subunits

	q-rtPCR; (human tissue) $>10^{-3}$ RPLPO ¹ ; RT-PCR ⁹ ; (mouse tissue) $>10^{-2}$ β -actin ³	LRRC26 promoter activity (mouse) ³	Native Cell Recordings Appropriate gating shift (with KO confirmation in some cases)	Protein (confirmed by KO and deglycosylation) ²	V _h (Ca ²⁺ free) ¹ Heterologous expression with BK α ¹
LRRC26 (γ 1)	[Colon, aorta, trachea, prostate, salivary gland] ¹ ; [salivary gland, prostate, colon, pancreas, intestine] ⁹ ; [lacrima gland, salivary glands, vagina, prostate, trachea, glandular stomach, conjunctiva, lung, colon, spleen, mammary gland] ³ ;	Secretory epithelium (lacrima gland, salivary glands, colonic goblet cells, trachea epithelium, prostate, conjunctiva, uterine epithelium, mammary gland, vagina, oviduct, cervix)	Lacrima gland ³ , parotid gland ^{3,7} , submandibular gland ³ , colonic goblet cells ⁵ , prostate ⁶	Lacrima gland, parotid, submandibular, trachea, mammary gland lung, colon, glandular stomach.	-147 mV
LRRC52 (γ 2)	[Testis, skeletal muscle] ¹ ; [testis, kidney] ²	n.a.	With Slo3 in sperm ⁴ ; BK in IHCs ⁸	Testis and sperm ⁴	-101 mV
LRRC55 (γ 3)	[Brain, fetal brain, thymus] ¹ ; [olfactory bulb, cortex spinal cord, hippocampus] ³ ; [podocytes] ¹⁰	n.a.	Podocytes ¹⁰	n.d.	-51 mV
LRRC38 (γ 4)	[Skeletal muscle, adrenal gland, thymus] ¹ ;	n.a.	n.d.	n.d.	-19 mV

RPLPO (large ribosomal protein); n.a., not applicable; n.d., not determined

¹(Yan & Aldrich, 2012)²(Yang *et al.*, 2011)³(Yang *et al.*, 2017)⁴(Zeng *et al.*, 2015)⁵(Gonzalez-Perez *et al.*, 2021)⁶(Gessner *et al.*, 2005)⁷(Almassy & Begenisich, 2012)⁸(Lingle *et al.*, 2019)⁹(England *et al.*, 2006)¹⁰(Hu *et al.*, 2021)