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Regulation of Potassium Channel Trafficking in the Distal Nephron

Paul A. Welling

Department of Physiology, University of Maryland Medical School, Baltimore, MD 21201

Abstract

Purpose of review—Potassium channels in the distal nephron are precisely controlled to regulate potassium secretion in accord with physiological demands. In recent years, it has become evident that membrane trafficking processes play a fundamental role. This short review highlights recent developments in elucidating the underlying mechanisms.

Recent findings—Novel sorting signals in the renal potassium channels, and the elusive intracellular trafficking machinery that read and act of these signals have recently been identified. These new discoveries reveal independent signals sequentially interact with different intracellular sorting, retention, internalization machineries to appropriately ferry the channels to and from the apical and basolateral membrane domains in sufficient numbers to regulate potassium balance.

Summary—A new understanding of the basic mechanisms that control potassium channel density at polarized membrane domains has emerged, providing new insights into how potassium balance is achieved and how it goes awry in disease.

Keywords

ROMK; BK; Kir channel; clathrin; AP clathrin adaptor; PDZ protein

Introduction

Potassium homeostasis is largely controlled by regulating potassium transport within the aldosterone-sensitive distal nephron (ASDN). Regulatory mechanisms ensure potassium secretion and reabsorption are precisely adjusted so that urinary K⁺ excretion matches dietary potassium intake. Ultimately, this depends on the regulation of different types of potassium channels. Apical potassium channels act as the final-regulated components of the renal potassium secretory machinery, while basolateral membrane potassium channels set a favorable membrane potential for the electrogenic potassium secretory process. Channel localization, number and activity are precisely controlled to appropriately tune potassium secretion to meet the physiological demand. In recent years, it has become evident that

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Author of correspondence. Paul A. Welling, Department of Physiology, University of Maryland Medical School, 655 W Baltimore St, Baltimore, MD 21201, 410-706-3851, pwelling@umaryland.edu.

Conflicts of Interest

There are no conflicts of interest to disclose.

membrane trafficking processes play a fundamental role. This short review highlights recent developments in elucidating the underlying mechanisms.

Polarized Distribution of Potassium Channels in the Distal Nephron

A robust potassium secretory process depends on the polarized trafficking processes that send the “right” channels to the correct membrane domain. Indeed, disparate types of potassium channels localize to opposite membrane domains of the potassium-secreting cells in the ASDN for different physiological duties. ROMK channels (aka Kir 1.1, product of the KCNJ1 gene) are specifically expressed on the apical membrane[1]. Because the ROMK exhibits a near linear current-voltage relationship and high open probability, the channel allows avid efflux into the tubule lumen[2,3] for robust and constitutive potassium secretion. Calcium-activated “BK” channels provide an additional conduit for potassium flux across the apical membrane, controlling flow-dependent potassium secretion[4]. By contrast, the basolateral K⁺ channels, encoded by different inwardly-rectifying (aka Kir-type) potassium channel genes (Kir2.3[5], Kir4.1[6], Kir5.1[7]) carry much less potassium out of the cell (hence their name, inward-rectifying potassium channels). Consequently, basolateral membrane K⁺ channels set the negative membrane potential, while limiting significant potassium efflux back to the interstitium. The importance of the basolateral Kir 4.1 channel is underscored by the recent discovery that loss of function mutations in the gene cause a human disease that is characterized, in part, by a distal tubulopathy[8,9].

Polarized Trafficking Mechanisms, General Considerations

According to present understanding, polarized targeting is governed by trafficking signals that are embedded within the structures of polarized membrane proteins. Some of these drive protein sorting into apical- and basolateral-destine transport vesicles at the trans-Golgi apparatus, and recycling endosomes. Other trafficking signals control directed basolateral delivery from the sorting stations. Once shipped to appropriate membrane domains, retention signals dictate residence at the right polarized locale. In recent years, different sorting and retention signals in potassium channels have been identified. Together with discoveries about the intracellular machineries that act on these signals, an understanding of the fundamental mechanisms of polarized targeting has begun to emerge.

Polarized Trafficking of Basolateral Channels (Figure 1)

The strongly inwardly rectifying “Kir 2.x” channels provide a foundation for understanding the nature of polarized trafficking signals and how they operate. Recent exciting new studies have begun to reveal how several independent trafficking signals can sequentially interact with different intracellular machineries to drive basolateral-directed sorting, traffic and retention.

According to the current textbook understanding, polarized expression of many proteins, including the basolateral Kir channels[10,11], is initiated soon after synthesis by signal-dependent sorting processes at the trans-Golgi network (TGN). Although this general explanation is widely appreciated, it has lacked a definitive mechanistic understanding because the sorting machineries that read, interpret and act on the sorting signals have

remained highly elusive. Clathrin was recently found to be a key coat component of many (but not all[12]) basolateral membrane-destined TGN export carriers[13], but how these select proteins are actually marked for inclusion in these coated vesicles has been a major unresolved question until several recent discoveries.

Work with the Kir2.1 channel [14] revealed a unique Golgi export signal that is specifically recognized by AP-1A, a clathrin adaptor that predominately localizes to the TGN. AP-1A binds to the Kir 2.1 sorting signal while simultaneously interacting with clathrin to drive the channel into clathrin-coated vesicles for export from the TGN. AP-1A had been thought to exclusively select endosomal and lysosomal-bound proteins for transport from the TGN to endosomes. Some proteins travel from the TGN to the basolateral cell surface in a circuitous fashion through endosomes[15], but observations with Kir2.1 indicated something much different. Live-cell imaging measurements revealed that Kir2.1 channels travel rapidly, if not directly, from the Golgi to the cell surface. The observation and a subsequent discovery, revealing AP-1A mediates the direct targeting of surface receptors to somatodendritic sites in neurons[16], expanded the function of AP1A to include sorting of polarized plasma membrane-bound proteins at the TGN.

Soon after AP-1A was found to mediate Golgi-to-surface sorting, Gravotta et al reported the AP-1A clathrin adaptor controls basolateral polarization of many commonly studied basolateral proteins, including the Vesicular Stomatitis Virus G protein (VSV-G) and the LDL receptor[17], highlighting AP-1A as a key basolateral sorting machine. The discovery provides an especially satisfying explanation for how a major class of basolateral sorting signals is decoded. Many classically studied basolateral membrane molecules rely on basolateral trafficking signals that share a high degree of similarity with clathrin-dependent endocytic sorting signals. For example, tyrosine-based “YXX Φ ” (Y, tyrosine; Φ , hydrophobic residue) and dileucine-containing signals, which typically mark proteins for clathrin-dependent internalization from the cell surface, also drive basolateral sorting of the VSV-G protein and the IgFc receptor[18], respectively. Because these signals are appreciated to bind to structurally defined docking sites on AP-type clathrin adaptors, the involvement of AP-1A in the basolateral membrane-sorting step at the TGN is easily rationalized.

Other basolateral sorting signals are unrelated to endocytic motifs, yet appear to rely on AP-1A. Such is the case with Kir2.x. Unlike conventional trafficking signals, which are typically comprised of short linear peptide sequences, the Golgi exit signal in Kir2.1 is dictated by a patch of residues embedded within its tertiary structure[14]. The unique topological organization of the signal, which forms when channels acquire proper conformation, is thought to provide a quality control mechanism, allowing only appropriately folded channels to exit the Golgi. Because the signal patch is highly conserved in Kir2.3, Kir2.2, Kir4.1 and Kir 5.1, it seems likely that the AP-1A signal recognition event at the TGN provides a general sorting mechanism for basolateral Kir channels.

The AP-1A dependent sorting step does not appear to be entirely sufficient for basolateral sorting of Kir channels, however. Early studies with the Kir2.3 channel revealed two additional signals are required to guide efficient basolateral targeting[10,11]. Both of them, a

canonical type I PDZ binding motif and an upstream site, are embedded within the last ~40 residues of the cytoplasmic C-terminal tail.

The proximal signal is required to drive an early basolateral trafficking operation, being decoded soon after channel synthesis. Without the signal, Kir2.3 becomes mis-localized to the apical membrane. Because the signal does not influence AP-1A binding, it is likely that it works to coordinate an AP-1A independent operation at the TGN, such as directed delivery to the basolateral membrane. Significantly, the signal exhibit similarities with signals identified in the GAT-2 GABA transporter[19] and the Kir4.1 channel[20,21], which also neighbor archetypal PDZ binding motifs, raising the possibility of a novel class of basolateral delivery signals for Kir channels and other basolateral proteins.

PDZ-based Basolateral Retention

The last four carboxyl-terminal amino-acids of the Kir 2.x and Kir 4.1 channels form archetypal type I PDZ binding motifs, which direct interaction with a variety of different PDZ-domain containing molecules, including the Lin-7/CASK complex[10,22], MAGI[23], syntrophin[24,25], and MUPPP[26]. PDZ proteins usually contain multiple protein-protein interaction domains, allowing them to act as molecular scaffolds that recruit and organize their binding partners into macromolecular protein complexes to facilitate signal transduction, trafficking, and/or compartmentalization at specific subcellular locales. In renal epithelia, basolateral retention seems to be the prevailing function of the PDZ-binding site in Kir2.x and Kir4.1. Consistent with this, mutations in the PDZ binding motif destabilizes residence on the basolateral membrane, causing the channels to accumulate into intracellular vesicles, largely recycling endosomes[21,27].

The Lin-7/CASK complex, a phylogenetically-conserved basolateral PDZ-protein complex that is expressed in the distal nephron[28], nicely illustrates a common retention mechanism. The PDZ domain of Lin-7 interacts with the channels [10], while another protein-protein interaction structure, called an L27 domain, dictates basolateral localization[29]. The L27 domain of Lin 7 binds to a cognate L27 domain in CASK (calcium/calmodulin-dependent serine protein kinase)[27]. Because CASK associates with the membrane through a web of disparate interactions[30,31], it acts as a stable basolateral-membrane anchor to retain Lin-7 interacting proteins, like Kir channels. The mechanism is a common one; PDZ proteins often function to retain their PDZ-binding partners on specific membrane domains by attaching to the membrane through other protein-protein interaction domains.

Binding between the Kir channels and PDZ retention proteins can be regulated. Phosphorylation of the critical serine in the type I binding motif of Kir4.1, for example, disrupts the PDZ protein interaction, causing the channels to become destabilized from the basolateral surface and targeted for endocytosis[23]. Swapping PDZ-protein interactions provides another regulatory modality. For example, TIP-1 operates as a scaffolding antagonist, which competes with the basolateral membrane Lin-7/CASK complex for interaction with Kir 2.3 to drive endosomal targeting of the channel[22]. TIP-1 is present in the ASDN, but further studies are required to determine if the relative abundance of TIP-1/

Lin-7 is physiologically modulated to adjust basolateral membrane potassium channel density.

Polarized Trafficking of the Apical Potassium Channels

In contrast to the ever-growing understanding of the basolateral Kir channel targeting mechanisms, far less is known about the polarized trafficking pathways that guide ROMK and BK channels to the apical membrane. Apical membrane proteins typically rely on complex trafficking signals that control lipid raft association, clustering, and post-translational modification [32]. There is reason to hypothesize that some of these processes may direct BK and ROMK to the apical surface. For example, recent observations that ROMK trafficking in the thick ascending limb is influenced by Tamm-Horsfall glycoprotein[33], a raft-associated GPI-linked protein, raise the possibility that ROMK may be delivered to the apical membrane in raft-carriers. Similarly, rafts have been implicated in apical trafficking of the BK channel [34]. An apical sorting determinant has been identified in the BK alpha subunit, but it is unknown if this structure mediates specific lipid association[35].

Controlling Apical Potassium Channel Density

Adaptive changes in the distal nephron principal cell take place in response to an increase in dietary potassium, allowing a more effective and enhanced excretion of potassium [36]. The response, called potassium adaptation, involves an increase in the number of active ROMK-type channels[37,38], and BK channels on the apical surface. In recent years, studies reveal the adaptive increase in potassium channel density involves stimulation of channel movement to the apical surface and inhibition of endocytosis.

Forward Trafficking of ROMK in the Secretory Pathway

In the basal state, ROMK channels are weakly expressed on the principal cell apical membrane, residing predominantly in the endoplasmic reticulum (ER) and Golgi [37]. A short basic-residue motif within the cytoplasmic C-terminus of the ROMK is thought to be responsible [39,40]. Similar signals in other channels serve as binding sites for the COP-1 coatamer[41,42], a trafficking complex that ferries proteins at the cis-Golgi for retrograde trafficking back to the ER. As a consequence, proteins containing COP-1 binding signals are effectively retained in the ER and cis-Golgi. A similar mechanism likely dictates residence of ROMK in the ER, but further studies are required to rigorously test this idea.

ROMK channels require a physiological stimulus to initiate their journey in the secretory pathway, remaining in the ER until they become phosphorylated at a cytoplasmic N-terminal residue, S₄₄. Phosphorylation of S₄₄ creates a separate trafficking structure [39] that effectively overrides ER/Golgi retention to drive anterograde movement to the cell surface (figure 2). SGK-1 and PKA both phosphorylate S₄₄, and initiate forward trafficking. Because both kinases become activated upon dietary potassium-loading, it is highly likely that the forward trafficking process plays an important role in the adaptive up-regulation of ROMK[43]. Significantly, several of the key residues in the forward trafficking structure (RXR) are shared by the Kir 2.1 signal patch (see above), raising the possibility that

phosphorylation of S44 induces the formation of a similar Golgi-export signal. A more detailed understanding of the phosphorylation-dependent forward trafficking mechanism awaits the identification of intracellular trafficking machineries that act on the signal.

ROMK Endocytosis

Clathrin-dependent endocytosis also controls the density of ROMK channels at the apical surface (Figure 3). In states of dietary potassium deficiency [44–46], endocytosis is enhanced to down regulate the channel, and limit urinary potassium loss. By contrast, ROMK internalization is suppressed in states of dietary potassium loading to increase ROMK density at the apical membrane[47]. Exaggerated endocytosis of ROMK contributes to urinary potassium retention and hyperkalemia in pseudo-hypoaldosteronism type II[48]. Recent studies have revealed the mechanism.

An unusual variant of the tyrosine-based “NPXY” endocytosis signal marks ROMK for endocytosis[47]. Compared to the canonical internalization signal, which was first described in the LDL receptor (LDLR) [49,50], the signal in ROMK contains a phenylalanine in place of the highly conserved tyrosine (NPXF). Also unlike canonical NPXY signal, the NPXF motif in ROMK has a highly selective binding preference [47]. It serves as a binding site for a single clathrin-associated sorting proteins (aka CLASP[51]), called ARH (Autosomal Recessive Hypercholesterolemia)[47].

ARH is a member of a subfamily of CLASPs, characterized by the presence of a phosphotyrosine binding domain. The unusual PTB domains in CLASPs prefer binding substrates that do not contain a phosphotyrosine, and thus are ideally suited to recognize “NPXY” internalization signals. The PTB domain in ARH is even more unusual because it has the capacity to interact with signals, like the one first described in ROMK, that contain a phenylalanine in place of the “critical” tyrosine. Other domains in ARH simultaneously interact with clathrin and the AP-2 clathrin adaptor, allowing it to mark the channel as cargo for inclusion into clathrin-coated pits at the apical surface (figure 3).

Studies in mice revealed that ARH is necessary for the physiologic regulation of ROMK surface expression[47]. ARH protein abundance increases to stimulate ROMK endocytosis in states of potassium-deficiency and decreases in states of potassium excess to reduce ROMK endocytosis[47]. ARH knockout mice are unable to down-regulate ROMK, and consequently maintain a high level of channel expression at the apical membrane regardless of potassium intake[47].

Conclusion

Studies in the last decade have begun to reveal the fundamental molecular mechanisms that control the location and number of potassium channels in the ASDN. The identification of trafficking signals and the discovery of the intracellular machinery that act of them provides a new understanding of how potassium balance is achieved.

Acknowledgments

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Key Points

- An efficient potassium secretory process depends on the polarized trafficking of different types of potassium channels to the apical and basolateral membrane.
- Basolateral membrane targeting is hierarchical and dependent on several types of trafficking signals.
- The clathrin adaptor AP-1 sorts channels into basolateral bound vesicles at the TGN, while PDZ-proteins retain the channels on the basolateral channel.
- ROMK channels are delivered to the apical surface in a phosphorylation-dependent manner. Phosphorylation of a forward trafficking signal overrides a separate ER retention signal.
- ROMK channels contain a unique internalization signal that specifies interaction with a single clathrin adaptor, ARH. ARH levels are increased in dietary potassium restriction to target ROMK for endocytosis.

Figure 1. Basolateral Targeting of Kir Channels is Hierarchical

1) The AP-1A clathrin adaptor binds to the “Signal Patch” to drive channel export from the Golgi in basolateral-bound vesicles; 2) A C-terminal biosynthetic signal likely specifies a directed delivery process, ensuring vesicles emanating from the TGN are targeted to the basolateral membrane (BLM), 3) Interactions with PDZ proteins retain the channels at the BLM. The prototypical basolateral PDZ retention complex, Lin-7/CASK is shown.

Figure 2. Phosphorylation-Dependent Trafficking of ROMK

Phosphorylation of an N-terminal residue, S44, by SGK-1 or PKA creates a forward trafficking signal, allowing newly synthesized channels to be exported from the Golgi. A separate trafficking signal in the cytoplasmic C-terminus effectively retains the channel in the endoplasmic reticulum when S44 is not phosphorylated.

Figure 3. ROMK is targeted for endocytosis by ARH

ARH interacts with the NPXF motif on ROMK, while simultaneously binding to clathrin and AP-2, so as to recruit the channel into clathrin coated pits at the apical surface. ARH levels are physiologically regulated, increasing in dietary potassium restriction to target ROMK for endocytosis. The process limits urinary potassium loss.