CALL FOR PAPERS | Protein and Vesicle Trafficking, Cytoskeleton

Lin-7 targets the Kir 2.3 channel on the basolateral membrane via a L27 domain interaction with CASK

Christine Alewine, Bo-young Kim, Vandana Hegde, and Paul A. Welling

Department of Physiology, University of Maryland School of Medicine, Baltimore, Maryland

Submitted 24 July 2007; accepted in final form 24 September 2007


Renal potassium secretion is made possible by the asymmetric distribution and operation of the basolateral membrane Na⁺-K⁺-ATPase and several types of K⁺ channels in the distal nephron principal cell (13, 34). The major basolateral K⁺ channels are encoded by different inwardly rectifying (Kir type) potassium channel genes (14, 31, 49) and exhibit completely different properties than Kir 1.1 (15, 24, 29, 30) and large-conductance, Ca²⁺-activated K⁺ channels (2, 36, 37, 52) at the apical membrane. On the basis of biophysical characteristics (17, 28) and immunolocalization (1, 51) studies, it has been logically argued that the predominating basolateral channel in the rat is encoded, at least in part, by Kir 4.1-Kir 5.1 channel genes (14, 28), whereas the product of the Kir 2.3 gene (51) seems to be a likely candidate in the mouse collecting duct (31). Because the basolateral channels exhibit stronger inwardly rectifying properties than Kir 1.1 at the apical membrane, they function to maintain an electronegative membrane potential while limiting potassium efflux at the basolateral membrane. Consequently, they play an important role to ensure net potassium secretion.

Present evidence indicates that basolateral targeting of Kir 2.3 (21, 22, 32) and Kir 4.1 (47, 48) may rely on similar mechanisms. Both require COOH-terminal sorting sequences and type I PDZ-binding motifs. Basolateral sorting of newly synthesized Kir 2.3 in the biosynthetic pathway is driven by an autonomous sorting signal that neighbors or overlaps with a PDZ-binding site. A comparable COOH-terminal structure in Kir 4.1, distinct from the PDZ-binding motif, also has been reported to coordinate basolateral trafficking (47, 48). The PDZ-binding motif likely acts to stabilize or retain channels at the basolateral membrane. Unlike mutations in the Kir 2.3 biosynthetic sorting signal, which cause apical mistargeting, alterations of the PDZ-binding site cause the channel to localize within reactivating endosomes. Similarly, the PDZ-binding motif in Kir 4.1 channels is important for controlling plasmalemma membrane clustering rather than polarized delivery (16).

We previously identified two PDZ proteins, mammalian Lin-7 (mLin-7) (32) and tax interacting protein 1 (TIP-1) (1), as PDZ-dependent binding partners of Kir 2.3. Both proteins are expressed in the collecting duct, where they likely have opposing activities. Lin-7, originally identified in Caenorhabditis elegans as a member of a basolateral PDZ protein complex (Lin-7/Lin-2/Lin-10) (42), is believed to have an evolutionarily conserved function in the mammalian kidney. Current evidence suggests that mLin-7 is a chief PDZ adapter responsible for stabilizing basolateral membrane expression of Kir 2.3, as well as its other known PDZ binding partners, including Kir 4.1 (25), BGT-1 (35), and ErbB-2/HER2 (41). TIP-1, by contrast, functions as a negative regulator of Kir 2.3 surface expression; it competes for binding with Lin-7 and causes Kir 2.3 to accumulate within an endocytic compartment, similar to mutant channels lacking the PDZ ligand motif (1).

The mechanism by which mLin-7 drives basolateral expression of Kir 2.3 has not been systematically evaluated but may depend on an NH₂-terminal structure that is not found in TIP-1 called an L27 domain. First recognized by in silico analysis as a structure that is common to Lin-7 and its C. elegans basolateral membrane PDZ protein partner, Lin-2 (10), the L27 domain is now appreciated to be a conserved protein-protein interaction module that is found in several different PDZ
proteins. Recent studies indicate that the mLin-7 L27 domain specifically interacts with the COOH-terminal L27 domain of calcium/calmodulin-dependent serine protein kinase (CASK) (12), the mammalian orthologue of Lin-2 (8). Because basolateral localization of mLin-7 is conferred by an NH2-terminal structure that includes the L27 domain (44), L27-dependent association of mLin-7 with the CASK scaffold could also underlie PDZ-dependent stabilization of Kir 2.3 at the basolateral membrane. In the present study, we test this hypothesis.

MATERIALS AND METHODS

DNA constructs. The hemagglutinin (HA) epitope-tagged mLin-7b construct was created as described previously (32). mLin-7a and mLin-7c, the kind gift of Dr. David Bredt, were modified with an NH2-terminal HA epitope tag. A COOH-terminal, Myc epitope-tagged mLin-7c was generated by placing the mLin-7c insert into the pcDNA3.1(H11001)/mycHisB vector (Invitrogen). The myc-tagged mLin-7c was subsequently subcloned into pShuttleCMV (Stratagene) for incorporation into adenovirus. The ΔL27mLin-7 construct was created by engineering unique restriction sites in the myc-tagged mLin-7c, removing the region encoding the L27 domain, and then religating in-frame. The TIP-1 construct was created as described previously (1). To create mLin-7ΔL27/TIP-1, a HindIII restriction site was engineered into mLin-7c just 3′ to the coding sequence of the L27 domain. The NH2 terminus of mLin-7c was subsequently subcloned in-frame into pShuttleCMV/c-myc-TIP-1, upstream of the TIP-1 insert, by using KpnI and HindIII. The CASK-L27 constructs were generated from the IMAGE clone AI028046 (Open Biosystems) by PCR, and inserts were then ligated in-frame into pGEX5x-1 (Amersham) to produce NH2-terminal glutathione S-transferase (GST) fusion constructs. The CASK-L27nc fragment was also inserted into pcDNA3.1(+)/mycHisA to add a COOH-terminal myc epitope tag and was subsequently subcloned into pShuttleCMV for adenoviral expression. In all cases, mutagenesis reactions were performed by using the QuickChange site-directed mutagenesis kit (Stratagene). All sequences were verified by using dye termination DNA sequencing.

Cell culture and adenoviral infection. COS, HEK, and MDCK/Kir 2.3-vesicular stomatitis virus (VSV) cells were grown as described previously (1). CASK-L27nc and PDZ protein adenoviruses were produced by using AdEasy adenoviral vector system (Stratagene), which relies on homologous recombination to insert the gene of interest into a plasmid containing a modified adenoviral genome. Amplification, purification, and infection of adenoviruses were performed as described previously (1). Transepithelial monolayer resistance was assessed by use of a voltmeter (Evohm, World Precision Instruments).

Cell lysis, immunoprecipitation, and immunoblotting. Cells were washed with ice-cold modified Ringer solution (5 mM HEPES, 144 mM NaCl, 5 mM KCl, 1.2 mM NaH2PO4, 5.5 mM glucose, 1 mM MgCl2, and 1 mM CaCl2, pH 7.4) and were then lysed in HEENG (20 mM HEPES, pH 7.6, 25 mM NaCl, 1 mM EDTA, 1 mM EGTA, and...
L27 domain-dependent targeting of Kir 2.3

10% glycerol) (25, 26) containing a protease inhibitor cocktail. Protein concentration of the lysates was assessed by using Bradford assay reagent (Bio-Rad). Immunoprecipitations with rabbit anti-myc antibodies and immunoblotting were performed as described previously (1).

GST pull-down. GST-CASK-L27nc, -L27n, and -L27c recombinant proteins were produced in Escherichia coli and were purified by using well-established methodologies, as described previously (1). The purified GST proteins were incubated with GS4B beads for 1 h, washed with PBS, and incubated overnight with lysates from COS cells transiently transfected with HA-mLin-7a, -b, or -c. Beads were washed with TEE (50 mM Tris-HCl (pH 7.5), 1 mM EDTA, and 1 mM EGTA) and were then eluted by boiling in SDS-sample buffer.

Biotin labeling and immunocytochemistry. For surface biotin labeling, filter-grown MDCK/Kir 2.3-VSV cells were placed on ice and washed with ice-cold Ringer solution, and 1.5 mg/mL NHS-SS-biotin (Pierce) in Ringer solution was then added to the basolateral compartment. Cells were incubated for 30 min at 4°C before being quenched by incubation with 50 mM Tris-HCl (pH 7.5) in Ringer solution for 5 min at 4°C. Cells were immediately fixed in 4% paraformaldehyde for 15 min at 4°C and were then blocked/permeabilized for 1 h at room temperature with 0.4% saponin and 1% BSA in Ringer solution. Saponin (0.4%) was subsequently included in all wash and antibody solutions. Cells were labeled with primary antibodies overnight at 4°C, washed, and then labeled with secondary antibodies for 1 h at room temperature. AlexaFluor-conjugated streptavidin (Invitrogen) was added to the secondary antibody solution to visualize biotin labeling. Labeled cells were washed with Ringer solution, mounted onto slides with Vectashield glycerol mounting medium (Vector), and sealed with nail polish.

Antibodies. All antibodies were purchased from commercial suppliers: rabbit anti-myc and mouse anti-myc (9E10) from Santa Cruz Biotechnologies; mouse anti-VSV from Sigma; rabbit anti-Rab-11 and mouse anti-β-catenin from BD Transduction Laboratories; mouse anti-Na⁺/K⁺-ATPase-α1 from Upstate Cell Signaling Solutions; rabbit anti-Veli3 (also known as mLin-7c) and anti-ZO-1 from Zymed Laboratories; horseradish peroxidase-conjugated goat anti-mouse antibodies from Jackson Laboratories; and AlexaFluor-conjugated secondary antibodies from Invitrogen.

Imaging methods. Cells were visualized by using the Zeiss 410 confocal laser-scanning microscope under a ×63 oil immersion lens (1.40 numerical aperture). Images were processed with the use of Adobe Photoshop. Images of Kir 2.3 in MDCK cells (z plane) were constructed by using Velocity software (Improvision) and were then scored for intracellular localization (4 = most intense, 3 = strong, 2 = moderate, 1 = low, and 0 = no intracellular expression) by an observer blinded to the identity of the treatment groups. In all cases, only cells that contained positively with anti-myc antibodies to detect the Lin-7, TIP-1 or CASK constructs were scored. Scores for equal numbers of cells from three separate experiments were pooled and then averaged together to produce an “intracellular localization score” for each experimental condition.

RESULTS

L27 domain-dependent localization of Kir 2.3. Lin-7 is thought to stabilize its PDZ-binding targets at the basolateral membrane by associating with basolateral scaffolds through a shared protein-protein interaction structure (23, 38, 44) called an L27 domain (10). Here we explore this hypothesis by testing how the subcellular localization of an mLin-7 PDZ-binding partner, Kir 2.3, is affected by loss and gain of the mLin-7 L27 domain (see Fig. 1A). To test for loss-of-L27 function, the consequence of wild-type mLin-7 expression was compared with expression of a truncated mLin-7 construct, specifically lacking the L27 domain (ΔL27mLin-7). For examination of gain-of-function, expression of wild-type TIP-1 was compared with a PDZ chimera, which was composed of the Lin-7 L27 domain fused to TIP-1 (L27/TIP-1).

The PDZ protein constructs shown in Fig. 1A were first characterized biochemically after adenoviral-mediated cDNA transfer into fully confluent MDCK cells, stably expressing VSV-tagged Kir 2.3 (MDCK-Kir 2.3+cells). As assessed by immunoblot analysis with myc antibodies (Fig. 1B), the PDZ proteins were equally expressed. Each migrated as a single species at the predicted molecular mass on SDS-PAGE gels (mLin-7, 25 kDa; ΔL27mLin-7, 19 kDa; L27/TIP-1 chimera, 24 kDa; and TIP-1, 15 kDa). Coimmunoprecipitation analysis showed that the four PDZ proteins exhibit predicted binding properties. In the present study, the myc-tagged PDZ proteins were immunoprecipitated from MDCK cells with anti-myc antibodies and were then immunoblotted with either anti-VSV to detect Kir 2.3 or with anti-CASK. As shown in Fig. 1C, all four PDZ proteins associate with Kir 2.3 (top panel in each set), which verifies PDZ-dependent interaction. In contrast, only the constructs with an intact L27 domain (wild-type Lin-7 and the L27/TIP-1 chimera but not wild-type TIP-1 or ΔL27mLin-7) coimmunoprecipitate with CASK. The latter observation is consistent with the notion that the L27 domain is both necessary and sufficient for CASK interaction.

To test whether the Lin-7 L27 domain is also sufficient for basolateral membrane targeting, subcellular localization of the PDZ proteins was examined by using immunofluorescence and confocal microscopy. As shown in Fig. 2, myc-mLin-7 predominately localizes to the basolateral membrane in MDCK cells (Fig. 2A), which is identical to earlier observations of the endogenous protein. In contrast, the ΔL27 Lin-7 mutant localizes diffusely within the cytoplasm (Fig. 2B), taking on a pattern similar to that of wild-type TIP-1 (Fig. 2C). Just as removal of the L27 domain caused a loss of basolateral targeting, addition of the Lin-7 L27 domain to TIP-1 largely shifted the localization to the basolateral membrane (Fig. 2D).
basolateral membrane (Fig. 2D). Indeed, intracellular aggregates of L27/TIP-1 were often observed, but the chimera predominately localized to the basolateral membrane. These observations pinpoint the basolateral targeting determinant of Lin-7 to the L27 domain. Together with the binding studies above, these data indicate that Lin-7 is targeted to the basolateral membrane through a L27 interaction with CASK or related L27-containing scaffolds.

Fig. 3. The Lin-7 L27 domain prevents Kir 2.3 routing to recycling endosomes. MDCK cells, stably expressing Kir 2.3-VSV, were grown to confluence on permeable supports and were infected with the mLin-7 and TIP-1 adenoviruses described in Fig. 1A. After 28 h, cells were decorated with biotin on the basolateral membrane (BLM). A: cells were stained with anti-VSV antibodies to detect Kir 2.3 (monochrome and green), with streptavidin to mark the basolateral membrane (red), and with anti-myc antibodies to detect PDZ protein expression (not shown). Scale bar = 10 μM. Orientation of apical (Ap) and basolateral (Bl) membrane surfaces is indicated. B: z-plane images were scored for intracellular Kir 2.3 localization (4 = most intense, 3 = strong, 2 = moderate, 1 = low, 0 = none) by an observer blinded to the identity of the treatment groups. Only cells staining positive for PDZ protein expression were scored. Statistical significance was assessed by one-way randomized ANOVA followed by Dunnett’s post hoc test. Values are means ± SE (n = 48 cells and 3 separate infections; *P < 0.01). C: cells expressing ΔL27mLin-7 were colabeled with anti-VSV antibodies to detect Kir 2.3 (green) and anti-Rab-11 antibodies (red) to mark recycling endosomes. In the z-plane image, the apical surface is facing upward.
To determine whether targeting of the Lin-7 PDZ binding partner Kir 2.3 is dependent on the Lin-7 L27 domain, steady-state localization of the channel was examined in MDCK/Kir 2.3 cells following adenoviral-mediated delivery of the PDZ proteins. Representative images are shown in Fig. 3A, and the results of quantification are presented in Fig. 3B. In contrast with expression of wild-type mLin-7, which did not alter the basolateral localization of Kir 2.3, expression of the ΔL27Lin-7 mutant caused a dramatic intracellular accumulation of Kir 2.3. Similar to the pattern of channel mislocalization in TIP-1-expressing cells (1), partial colocalization of Kir 2.3 with a Rab-11-positive compartment was evident following the expression of ΔL27mLin-7 (Fig. 3C). In contrast, no channel mislocalization was observed when the L27 domain was transplanted on TIP-1 (L27/TIP-1 chimera). Taken together, the data indicate that the Lin-7 L27 domain is required for Lin-7 to target its PDZ binding partner, Kir 2.3, on the basolateral membrane.

L27-interacting scaffold is required for basolateral targeting of Kir 2.3. To test whether Lin-7 stabilizes Kir 2.3 at the basolateral membrane by binding to the cognate L27 domain in CASK, we determined whether expression of the isolated CASK L27 domains (CASK-L27nc) affects polarized localization of Lin-7 and Kir 2.3 in MDCK cells (see Fig. 4).

The CASK-L27nc construct was first characterized biochemically to confirm that the isolated CASK L27 domain retains the ability to associate with Lin-7. In the present study, CASK-L27nc was fused in-frame to the COOH terminus of GST (Fig. 4A), and in vitro binding assays were performed with extracts of COS cells, which were separately transfected with HA epitope-tagged Lin-7a, -b, and -c. As shown in Fig. 4B, we found that GST-CASK-L27nc bound to all three Lin-7 isoforms. Because the Lin-7 L27 domain is thought to preferentially oligomerize with the COOH-terminal L27 domain of CASK (11, 12, 23), we tested interaction specificity by evaluating Lin-7 binding to the individual CASK L27 domains. As expected, HA-mLin-7 exclusively bound to GST-CASK-L27c. The CASK-L27n domain did not associate with any of the HA-mLin-7 isoforms (Fig. 4B). Identical results were observed by using purified, recombinant Lin-7 proteins (data not shown), which verifies that CASK-L27nc and CASK-L27c directly bind to all Lin-7 isoforms.

Characterization of the CASK-L27 domains in MDCK-Kir 2.3+ cells is shown in Fig. 5. Initial studies showed that the isolated CASK-L27n and CASK-L27c domains were not as efficiently expressed as the CASK-L27nc construct. We speculate that single L27 domains are unstable and are subjected to rapid degradation in mammalian cells since similar difficulties were encountered with the isolated mLin-7 L27 domain (data not shown) and have recently been observed by others (45). Therefore, only the CASK-L27nc construct was studied. Following adenoviral-mediated gene transfer, the CASK-L27nc protein was detected as a ~19-kDa band corresponding to the predicted molecular mass of the myc-tagged protein (Fig. 5A). No proteins were detected in mock-infected control cells (Fig. 5A). As observed by immunocytochemistry and confocal mi-
croscopy with anti-myc antibodies, CASK-L27nc localized diffusely to the cytoplasm (Fig. 5B), which reinforces the idea that basolateral localization of CASK is not mediated by its L27 domains (23) but instead relies on interactions with the actin cytoskeleton and extracellular matrix receptors (9).

As observed by immunocytochemistry, lateral membrane distribution of β-catenin was unaffected by expression of CASK-L27nc, which demonstrates that the adherens junctions remain intact (Fig. 5C, green). ZO-1 was largely detected at the apex of the lateral membrane, as expected for a tight junction marker, but increased intracellular localization was often observed in cells expressing CASK-L27nc (Fig. 5C, red). Despite this, junctions were competent enough to prevent biotin, added to the apical compartment, from labeling the lateral membrane (Fig. 5D, blue). Furthermore, a normal distribution of the basolateral membrane marker Na+/K+ -ATPase (Fig. 5D, green) was observed in CASK-L27nc-expressing cells. Expression of CASK-L27nc did, however, make MDCK cells prone to detachment. Presumably as a consequence, cell packing density was reduced, and remaining cells frequently appeared wider and shorter.

In contrast with the normal localization of Na+/K+ -ATPase and β-catenin, we found that CASK-L27nc expression caused extensive accumulation of mLin-7 (Fig. 6A) and Kir 2.3 (Fig. 6B) into large subapical vesicles, which is reminiscent of the...
mLin-7 and Kir 2.3 to recycling endosomes.

**DISCUSSION**

Here we provide a structural framework to explain how Lin-7 localizes its PDZ binding partner, Kir 2.3, on the basolateral membrane. Our data support the concept that Lin-7 uses two disparate protein-protein interaction motifs to bridge its PDZ targets to L27-containing basolateral membrane anchoring proteins (Fig. 7). We found that removal of the L27 domain from Lin-7 abrogated interaction with CASK but not Kir 2.3. Consequently, expression of the ΔL27Lin-7 mutant prevented the channel from engaging the endogenous Lin-7/CASK complex at the basolateral membrane, and it caused Kir 2.3 to accumulate within endosomes, just as observed with mutant channels that lack a PDZ-binding motif and are unable to interact with Lin-7 (32). A similar Kir 2.3 endosomal sorting phenotype was observed following expression of the isolated CASK-L27nc domain, which prevented endogenous Lin-7 from associating with CASK. In contrast, adding the Lin-7 L27 domain onto the small PDZ protein, TIP-1, conferred CASK association and abrogated endosomal targeting of Kir 2.3 that is observed with wild-type TIP-1 (1). Taken together, these observations indicate that basolateral targeting of Lin-7 and Kir 2.3 is made possible by L27 dependent oligomerization with CASK or related proteins.

Our observations corroborate and extend those of previous studies that pointed to critical scaffolding functions of the Lin-7 NH2-terminal domain. Building on the pioneering work of Straight et al. (44), who discovered that the basolateral targeting determinant of Lin-7 is embedded within the NH2 terminus rather than the PDZ domain, our studies define the L27 domain as being the necessary and sufficient basolateral localization component. Recent structural studies indicate that individual L27 domains are composed of three α-helices that pair with cognate L27 domains in other proteins to form stable coiled bundles and, consequently, link proteins with matching L27 domains into macromolecular complexes (11, 27). We found that the L27 domain of all Lin-7 isoforms is necessary and sufficient for interaction with CASK. Because CASK associates with the basolateral membrane through a web of L27-independent interactions (see below), the CASK L27 domain is free to interact with the L27 domain of Lin-7 and thereby anchor Lin-7 and its PDZ target, Kir 2.3, at the polarized locale.

Consistent with this idea, basolateral localization of other Lin-7 PDZ-binding proteins is also dependent on the Lin-7 NH2-terminal domain. For example, expression of a mutant Lin-7 protein, lacking the entire NH2 terminus (ΔNmLin-7), causes apical mistargeting of two other Lin-7 interacting partners, sErbB-2/HER2 (41) and a chimeric LET-23/NGF receptor construct (43). In the chimeric receptor, loss of interaction with the endogenous Lin-7/CASK complex is thought to destabilize basolateral expression in such a way to cause endocytosis and transcytotic delivery to the apical membrane (43). Newly synthesized ErbB-2 receptors, in contrast, have been reported to require interaction with a different Lin-7 NH2-terminal structure, coined a kinase interacting domain (KID), to mature through the biosynthetic pathway and prevent shunting of the receptor to the apical membrane (41). In this special case, the Lin-7 L27 and PDZ domains are presumably free to recruit CASK and other PDZ-binding proteins that may be required for basolateral delivery of ErbB2 receptors in the secretory pathway.

Unlike ErbB2, Kir 2.3 binds to Lin-7 exclusively through a canonical PDZ interaction (32), and it does not require the Lin-7 KID domain for efficient basolateral surface expression. Moreover, we found that Kir 2.3 is mistargeted to apical endosomes rather than the apical membrane when its interaction with Lin-7 is disrupted or when Lin-7 association with CASK is prevented. Thus the Lin-7/CASK complex appears to keep the channel out of the endocytic pathway rather than to coordinate polarized delivery of Kir 2.3 in the secretory pathway. In fact, present evidence indicates that sorting and delivery of newly synthesized Kir 2.3 channels to the basolateral membrane depends on a trafficking signal that is distinct from the Lin-7-binding motif (22, 32).

A subapical compartment that partially colocalizes with Rab-11 accumulates Kir 2.3 after the channel is uncoupled from the Lin-7/CASK complex. This is accompanied by a dramatic expansion of the Rab-11 compartment, as if these endosomes become engorged with misdirected Kir 2.3 cargo. Routing of a basolateral membrane protein to the Rab-11 compartment is somewhat surprising because Rab-11 is generally considered to define the subapical recycling endosome (50), a key endocytic station for apical membrane traffic in fully polarized epithelia (6). Moreover, it should be pointed out that the subapical compartment containing Kir 2.3 is very diffuse and beyond the limits of confocal microscopy to resolve as individual endosomes. Consequently, colocalization...
studies do not permit an unambiguous identification of the intracellular compartment, containing Kir 2.3, as Rab-11-positive endosomes. Further studies are required to more precisely define the intracellular compartment that accumulates Kir 2.3 when the channel’s ties with the Lin-7/CASK complex are broken. It will also be important to map the postendocytic routing itinerary of Kir 2.3 as it travels to this final destination.

While Lin-7/CASK is poised to function as a stable anchoring complex at the basolateral membrane, it remains to be determined whether the PDZ complex also plays a role to recycle the channel back to the basolateral membrane from the endocytic pathway as has been described for other PDZ proteins, such as Na/H exchanger regulatory factor (7). In the mammalian brain, mLin-7 and CASK assemble with another PDZ protein, Mint1/X11 (known as Lin-10 in C. elegans) (4, 5, 18), which, in turn, can interact with the neuron-specific molecular motor KIF1 (40). The organization has been reported to permit vesicles, containing Lin-7 interacting cargo (such as N-methyl-D-aspartate receptors), to be vectorially transported along microtubules in neuronal dendrites. Because the Mint1 isoform that interacts with CASK is only expressed in the brain (4), other systems in epithelia may exist to allow endocytic recycling of Lin-7 interacting cargo.

Following the idea that CASK has an evolutionarily conserved function as a basolateral scaffolding protein (6), it seems reasonable to suggest that CASK is a major Lin-7 binding partner at the basolateral membrane in mammalian renal epithelia. Consistent with this notion, CASK is abundant in mature MDCK and collecting duct cells, exhibits a basolateral membrane localization pattern, and robustly communoprecipitates with mLin-7. Because CASK associates with the basolateral membrane through multiple protein-interaction modules (8) that do not involve its L27 domains, it is poised to act as a stable anchoring protein. Indeed, CASK interacts with actin-binding 4.1 proteins through its Hook domain while its type II PDZ domain simultaneously binds to the syndecan family of heparan sulfate proteoglycans (8, 9). These linkages free the CASK L27 domains to recruit Lin-7 and SAP-97, and their PDZ interacting proteins, to the basolateral membrane.

Since CASK was identified as the mammalian counterpart of the C. elegans basolateral membrane scaffolding protein, Lin-2 (4, 5, 18), two similar Lin-7 interacting proteins have been discovered in mammalian systems (19). Called proteins associated with Lin-7 (Pals) or 1-methyl-4-phenylpyridinium (MPP) proteins, Pals1/MPP5 and Pals2/MPP7 are members of the p55 family of membrane-associated guanylate kinases, like CASK. Because Pals1 and Pals2 contain L27 domains and localize to the lateral membrane and/or tight junction in MDCK cells (19), it is conceivable that they could support a similar Lin-7 anchoring function as CASK. Nevertheless, present evidence indicates that Pals1 and Pals2 may not have completely redundant activities as CASK. Pals1 is best known for its role as an adaptor which links Crumbs and Discs large into a complex (38) for the establishment of the tight junction and apical/basolateral polarity (39). Unlike CASK, Pals1 requires Lin-7 interaction to stabilize its expression (45), which makes Pals1 poorly suited to act as a stable Lin-7 anchoring point. In contrast, Pals2/MPP7 has recently been reported to form a complex with Lin-7 and Dlg1/SAP97 (3, 46) at the lateral membrane in MDCK cells, identical to CASK (23). Unlike our observations with CASK, however, Pals2/MPP7 has recently been reported to interact with Lin-7 in an isoform-specific manner (3), which suggests that it may have a more specific role than CASK. Considering that Lin-7 isoforms are differentially expressed along the nephron (33), it will be important to learn whether the Pals2 binding specificity translates to renal cell specific functions.

Kir 2.3 and several other members of the inward rectifier family of potassium channels can interact with the PDZ protein Dlg1/ SAP-97 as well as Lin-7 (25, 26). Similar to mLin-7, SAP97 binds to CASK through a heterotypic L27 interaction, and some SAP97 isoforms depend on this association for basolateral targeting (25–27, 53). Since the CASK-L27c construct includes the L27c domain that binds mLin-7 and the L27n domain that interacts with SAP97 (11, 27), it is likely to affect SAP97 as well as mLin-7 localization. Interestingly, SAP97/hDlg orthologues in Drosophila and C. elegans are critical for the establishment and maintenance of epithelial cell apico-basal polarity (reviewed in Ref. 39). Although the link between SAP97 and polarization is less clear for mammalian epithelial cells, SAP97 localizes to points of cell-to-cell contact and may regulate the integrity of adherens junctions (20). Future studies will be required to address whether Lin-7 and SAP97 play identical roles to target Kir 2.3 channel at the basolateral membrane through L27 interactions with CASK or Pals2/MPP7.

In summary, mLin-7 uses its L27 domain to link Kir 2.3 with CASK or related Stardust proteins. In doing so, it effectively acts as a basolateral PDZ to L27 adaptor that stabilizes basolateral localization of its PDZ binding partners.

GRANTS

This study was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grants DK-54231 and DK-63049 (to P. A. Welling) and National Heart, Lung, and Blood Institute Grant T32-HL-072751 (to C. Alewine).

REFERENCES


