Distribution of epithelial ankyrin (Ank3) spliceoforms in renal proximal and distal tubules

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Distribution of epithelial ankyrin (Ank3) spliceoforms in renal proximal and distal tubules. Am. J. Physiol. 274 (Renal Physiol. 43): F129–F138, 1998.—In diverse cell types, ankyrin tethers a variety of ion transport and cell adhesion molecules to the spectrin-based membrane skeleton. In the whole kidney, epithelial ankyrin (Ank3) is the predominantly expressed ankyrin and is expressed as distinct spliceoforms. Antibodies against a portion of the Ank3 regulatory domain detected four major spliceoforms at 215, 200, 170, and 120 kDa. Immunoblotting of the renal cortex, which is 80% proximal tubule (PT), detected all four spliceoforms but showed significantly diminished Ank3200/215. To determine the Ank3 spliceoforms present in the mouse PT cells, PT fragments were purified to 100% from the renal cortex. Isolation was performed by incubating cortical tubule segments with fluorescein and isolating the fluorescein-laden PT fragments or fluorescein-deplete non-PT (distal) fragments under fluorescence microscopy. Distal tubule (DT) fragments displayed abundance of the Ank3200/215 but no Ank3170 or Ank3120. Isolated PT segments contained all four spliceoforms but dramatically diminished Ank3200/215. These larger spliceoforms bind Na-K-ATPase in diverse cell types. Densitometric analysis of Ank3200/215 and Na-K-ATPase abundance measured a lower Ank3200/215-to-Na-K-ATPase ratio in the PT vs. the renal cortex. These proximal vs. distal differences in Ank3 spliceoforms were displayed in LLC-PK1 cells, a proximal cell line, and MDCK cells, a distal cell line. The lower PT content of Ank3200/215 suggests Na-K-ATPase in PT may be organized differently than in DT. Likely reflecting their cell-specific organization, regulation, and function, these studies indicate the different renal cell types express distinct Ank3 spliceoforms.

Ank3 is the most widely distributed ankyrin, with expression observed in a variety of epithelia, liver, muscle, testes, and macrophages (12, 16, 28). In the kidney there is no Ank1 expression, little if any Ank2 expression, but abundant Ank3 expression (27, 28). Furthermore, Ank3 expression exists in multiple Ank3 spliceoforms including proteins of 215, 200, 170, 120, 119, and 105 kDa (4, 28).

The best-described role of ankyrin in the kidney is the retention of Na-K-ATPase along the basolateral membrane of distal tubular (DT) segments. In the DT segments, ankyrin colocalizes with Na-K-ATPase along the tortuous infoldings of the basolateral membrane of thick ascending limb (TAL) cells (6, 15). In Madin-Darby canine kidney (MDCK) cells, a renal DT cell line, ankyrin colocalizes with Na-K-ATPase along the basolateral membrane, coprecipitates with Na-K-ATPase, and increases the retention half-life of Na-K-ATPase in the membrane (11). All of these observations were performed using immunoreagents directed against Ank1 which detects a 190/210-kDa protein. Ank1 antibody reactivity with proximal tubule (PT) cells has been relatively poor, leaving the presence and function of ankryins within the PT cells largely unstudied (2, 5).

In the present study, antibodies directed against an Ank3-specific region of the regulatory domain, termed Ank3-R1, detected the 215-, 200-, 170-, and 120-kDa spliceoforms. Immunocytochemistry with this antibody specifically stained all tubule segments including the PT cells (28, 29). The distribution of the individual spliceoforms within the different nephron segments is unknown. The present studies demonstrate that DT segments express the known Na-K-ATPase-binding Ank3200/215 spliceoforms. These segments showed no other spliceoforms as detected with Ank3-R1. Conversely, PT segments contained predominantly the Ank3200/215 and Ank3170 spliceoforms with comparatively little Ank3200/215. As a result, the PT cells had lower ratios of Ank3200/215 to Na-K-ATPase than the whole cortex. The cell type-specific spliceoform expression and lower Ank3200/215-to-Na-K-ATPase ratio were also retained in LLC-PK1 and MDCK cells, cultures of proximal and distal origin, respectively.

METHODS

The kidney is a highly organized organ with the transitions of the nephron segments defining the different regions of the kidney (Fig. 1). The dissection of the regions of the kidney and isolation of tubule segments from within those regions was used to determine the presence, absence, and relative abundance of epithelial ankyrin spliceoforms. By volume, PT...
and finely ground under liquid nitrogen. A sample was perfusion. The kidneys were removed, frozen in liquid nitrogen (DME-F12, 50 mg collagenase (Warthong Biochemicals), 5 mg deoxyribonuclease, and 50 mg bovine serum albumin (BSA)) and incubated at 37°C with rapid shaking. After 10 min, the suspension was drawn into a pipette 5–10 times and the suspended tubules are rescued from the chunks. The chunks were resuspended in collagenase solution, and the process was repeated two to four times. The total PT suspension was washed at 150 g for 2 min and then again at 100 g for 2 min.

PT segments are the densest tubular segments. To further concentrate the PT segments, the tubules were resuspended in 25 ml of 45% Percoll solution (22.5 ml Percoll, 2.25 ml 10× Dulbecco’s phosphate-buffered saline (PBS), 38.4 mg N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 25 ml DME-F12). The viable PT (~95% PT) were separated at 25,000 g for 30 min and washed twice at 150 g for 2 min. To minimize the potential for DT contamination, only tissue migrating ≥90% the length of the Percoll gradient was retained. For DT isolation, the tubules were resuspended in 25 ml of 35% Percoll solution (22.5 ml Percoll, 2.25 ml 10× Dulbecco’s PBS, 38.4 mg HEPES, 25 ml DME-F12). The viable DT were separated at 25,000 g for 30 min and washed twice at 150 g for 2 min. To increase the distal content without including nonviable cells, all segments migrating ≥50% the length of the Percoll gradient were retained. The suspensions were maintained in ice-cold DME-F12 until needed.

### Fluorescence Loading and Isolation of PT

In contrast to all other cell types within the kidney, the PT possesses an avid capacity to transport and accumulate organic acids, including fluorescein (31). This concentrating capacity was exploited to load PT fragments with fluorescein and isolate fluorescein-laden PT or fluorescein-deplete DT under fluorescence microscopy. Percoll-purified PT fragments were resuspended in DME-F12 containing 2 mM fluorescein and incubated for 25 min at room temperature, and ~1 ml was transferred into ice-cold test tubes to allow the tubules to settle out. The supernatant was then aspirated from a single test tube, and the segments were resuspended in DME with 1% goat serum. Tubules were placed in a petri plate at low density and, under fluorescence microscopy (×100 magnification), aspirated into a hemocytort tube and transferred into a microcentrifuge tube containing DME-goat serum and 4 mM ethylene glycol-bis(β-aminoethylether)-N,N,N’,N’-tetraacetic acid, and 2× protease inhibitor cocktail (20 µM leupeptin, 4 µg/ml aprotinin, 400 µM PMSF, and 20 µM pepstatin A). Following the collection of the tubule segments, the tubules were pelleted at 2,000 g and solubilized in 5× PAGE buffer. The inclusion of serum or BSA was required to inhibit isolated tubules from sticking to the plates and tubes. This inclusion disallowed the accurate measurement of total isolated protein.

### Cell Culture Maintenance and Preparation

MDCK and LLC-PK₁ (clone 4) were passaged and maintained as previously described (9, 20). Cell monolayers on semipermeable supports were grown for five days following the establishment of confluence. The cells were solubilized by the addition of 1.0 ml of warmed 5× PAGE and reduced as described above.

### Immunoblotting

Gel electrophoresis was performed as previously described (5, 8). Quantities of 20–60 µg protein of the 5× PAGE

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**Fig. 1.** Nephron segments of the mouse kidney. Renal epithelia of the nephron and collecting system are highly organized and define the regions of the kidney. These regions include the cortex, outer medulla, which is subdivided into the outer stripe (OS) and inner stripe (IS), and the inner medulla. Proximal tubule (PT) and distal tubule (DT) segments were isolated from the renal cortex (see Figs. 2–4, and 6). Immunofluorescence micrographs (see Fig. 6) are a cross-section of a cortical ray, which contain PT, thick ascending limbs (TAL), and collecting ducts (CD). G, glomerulus; TL, descending and ascending thin limbs; DCT, distal convoluted tubule; CT, connecting tubule.
dissolved tissues were run on a 3.5%-14% polyacrylamide gradient gel (200 mV, 3.5 h). The gels were then transferred onto nitrocellulose (300 mV; 3.25 h).

Western blotting was performed on the transblots as previously described (32). The blots were washed once in blot buffer [130 mM NaCl, 10 mM Na2HPO4, 1 mM EDTA, 0.2% Triton X-100, and 1 mM NaN3; pH 7.4], once in blot buffer with 4% BSA for 20 min or 0.5% dry milk for 60 min, once with blot buffer, and then incubated with the primary antibody for 60 min at room temperature. The blot was then washed four times (4 min/wash) and incubated with a peroxidase-labeled anti-immunoglobulin G (anti-IgG) secondary antibody (1: 25,000 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA). The reaction products were detected using enhanced chemiluminescence detection (ECL, Amersham).

The Ank3-R1 antibody is specific for the regulatory domain of mouse Ank3. It is used to assess the relative abundance of Ank3 spliceoforms in specific cell types of the mouse kidney. The differences in immunoblot band intensities should represent differences in abundance and not differences in antibody affinities, because the proteins are denatured and directed against antigenic sites generated from the identical gene sequence. Since the sequence of the sites in the different species has not been identified, intensity differences between species are only considered for their qualitative value.

Immunofluorescence

Rat and mouse kidneys were fixed, stained, and examined by immunofluorescence as previously described (5). The kidneys were flushed free of blood with ice-cold PBS and then perfused with 3% paraformaldehyde in PBS. The kidneys were excised and further fixed for 60 min. The cortex was then cut into pyramidal blocks, incrementally infused with 5% (60 min), 10% (60 min), and 25% (overnight) sucrose, and then plunged into liquid nitrogen-cooled isopentane. The blocks were then sectioned (5 μm) and stained. Staining was done by blocking nonspecific interactions (1% BSA and 10% normal goat serum), reducing the block (0.1% BSA and 1% normal goat serum), incubating with the appropriate antibody, washing, incubating with a rhodamine-labeled goat anti-rabbit IgG, washing, postfixing, and mounting in 60% glycerol with 10% diazobicyclooctane. Nonimmune serum replaced the primary antibodies in control sections. The sections were visualized on a Zeiss LSM confocal microscope.

Antibodies and Reagents

Polyclonal antibodies directed against a segment of the regulatory domain of Ank3 (epithelial ankyrin; Ank3-R1) were developed and characterized as previously described (28). Dilutions for immunoblotting were 1:500; dilutions for immunohistochemistry were 1:250. The polyclonal antibody against Ank1 (erythrocyte ankyrin) was graciously provided by Dr. Vann Bennett (Duke University). Dilution for immunohistochemistry was 1:1,000. The polyclonal antibody against aquaporin-2 (AQP2) and bumetanide-sensitive Na-K-ATPase levels. Although the renal cortex showed specific staining for AQP2, the isolated PT had no detectable AQP2 reactivity. Similarly, although the signal from mouse cortical tissue was weak, no BSC-1 reactivity was detectable in isolated PT samples (data not shown).

As described above, the Ank3200 band intensity in renal cortical samples was 18% ± 5% of the Ank3200 band intensity. Isolated mouse PT showed the prominent Ank3120 and Ank3170 bands (Fig. 3A). However, the Ank3200/215 bands were markedly reduced, only seen on blot overexposure, and below the linear range of densitometric analysis. In contrast to the PT, isolated mouse DT displayed prominent Ank3 bands at 200 and 215 kDa and did not display either the Ank3120 or the Ank170 spliceoforms (Fig. 3B).

RESULTS

As previously described (28), the mouse kidney contains a number of epithelial ankyrin spliceoforms including a prominent band at 120 kDa and lower intensity bands at 170, 200, and 215 kDa. The 105-kDa band observed in whole kidney crude membranes and macrophages of other mouse strains was only faintly detected in the present study (12, 28). Immunoblots of the mouse renal cortex showed the presence of the same spliceoforms but the intensities of the 200- and 215-kDa bands were relatively lower in the cortex vs. the whole kidney. In paired mouse kidney and cortical samples, densitometric analysis showed the 200 kDa-to-120 kDa ratio was significantly higher (P < 0.05, paired t-test) in the whole kidney (0.38 ± 0.11, n = 4) than in the renal cortex (0.18 ± 0.05, n = 4). The lower cortical content of the 200/215-kDa spliceoforms in a region of the kidney containing predominantly PT segments suggests that the diminished Ank3200/215 content reflects a PT property. To ascertain the segment-specific distribution of the Ank3 spliceoforms directly, PT and DT segments were individually isolated.
Ankyrin Immunofluorescence

To corroborate the relative Ank3<sub>200215</sub> abundance in the distal segments and paucity in proximal segments, mouse cortical sections were fixed and stained for the Ank3<sub>200215</sub> spliceoforms in the mouse kidney. For ease of comparison, sections of cortical rays containing straight PT and TAL (Fig. 1) are shown. Although the Ank3-R1 antibody detects several spliceoforms, the Ank1 antibody cross-reacted with only the Ank3<sub>200215</sub> spliceoforms and failed to detect the Ank3<sub>120</sub> and Ank3<sub>170</sub> spliceoforms (Fig. 4A). The immunolocalization of the Ank3 spliceoforms, as detected by the Ank3-R1 antibody, has been previously described in detail (28, 29).

Antibodies with spliceoform-specific reactivity would definitively demonstrate the segmental and intracellular distribution for the spliceoforms. The Ank1 antibody, which reacted specifically with only the Ank3<sub>200</sub> and Ank3<sub>215</sub> spliceoforms, was used in this manner. As previously described in rats (5), the Ank1 antibody strongly stained the TAL and collecting duct cells (Fig. 5B). In mouse PT cells, Ank1 antibody staining was faint but specific. The weakness of the Ank3<sub>200215</sub> signal in the PT cells corroborates the relative decrease of these spliceoforms observed in the immunoblots of the isolated PT. In contrast to the DT segments, the staining was not only along the basolateral membrane but also along the terminal web of the PT cells. Although fodrin also exists within the terminal web region (5, 30), the protein(s) being tethered by ankyrin in this region is unknown.

The Na-K-ATPase staining intensities of the different cortical segments correlate with the known Na-K-ATPase activity levels within these segments (Fig. 5C) (14). TAL cells, which have high Na-K-ATPase activities, stained intensely. Staining of collecting duct and PT cells, which have comparatively lower Na-K-ATPase activities, were comparatively less intense. In TAL cells, Na-K-ATPase colocalized with ankyrin along the basolateral membrane (15). In the mouse PT, Na-K-ATPase was observed along the basolateral membrane. For comparison, the TAL immunofluorescence intensities after Na-K-ATPase and Ank1 antibody staining were adjusted to similar levels. In these sections, Na-K-ATPase staining intensity of PT cells was clearly greater than Ank3<sub>200215</sub> staining intensity of PT cells. This suggested that was comparatively less Ank3<sub>200215</sub> per Na-K-ATPase in the PT vs. the TAL.

Relative Na-K-ATPase and Ank3<sub>200</sub> Contents

To quantitatively assess this observation, the immunoblot densities of Na-K-ATPase and Ank3<sub>200</sub> in paired isolated PT and renal cortex samples were measured (Fig. 4B). Figure 4B shows an immunoblot of serially diluted renal cortex and isolated PT samples dual-stained for Ank3 and Na-K-ATPase. Ank3<sub>200215</sub> bands of the concentrated PT sample were roughly equivalent to those from 54 µg of loaded cortical sample. From the same blot, Na-K-ATPase was much greater in the isolated PT sample vs. the 54 µg cortical sample. The ratio of Ank3<sub>200</sub> to Na-K-ATPase in cortical and isolated PT samples was roughly equivalent to those from 54 µg of loaded cortical sample. From the same blot, Na-K-ATPase was much greater in the isolated PT sample vs. the 54 µg cortical sample. The ratio of Ank3<sub>200</sub> to Na-K-ATPase in cortical and isolated PT samples was quantified by densitometry. In cortical samples, the paired sample Ank3<sub>200</sub>-to-Na-K-ATPase ratio from dual-stained immunoblots was 3.3 ± 1.2 (n = 3). In isolated PT cells, this ratio was 0.8 ± 0.2 (n = 3). These values were statistically significant (P < 0.05) as determined by a paired t-test. Isolated PT had signifi-
cantly less Ank3200 per unit of Na-K-ATPase than was measured in the renal cortex.

Ank3 Spliceoforms in Proximal and Distal Cell Lines

To verify the observations made in the kidney and assess potential models to study the functions of renal ankysins, the Ank3 spliceoforms were examined in LLC-PK1 cells, a proximal cell line, and in MDCK cells, a distal cell line. Much like the isolated mouse distal segments, MDCK cells displayed a prominent ankyrin. As previously observed with Ank1 antibodies, these ankysins presented as a doublet around 200 kDa. Unlike the isolated distal segments, MDCK cells also displayed an ankyrin which migrated around 175 kDa. This ankyrin migrated slower than the 170-kDa ankyrin in the mouse kidney.

Similar to the mouse PT cells, LLC-PK1 cells only showed the presence of the Ank3200 doublet after overexposure of the blot (data not shown). Previously, with use of Ank1 antibodies, LLC-PK1 cells were shown to contain an Ank3200 doublet (5). The lower Ank3200-to-Na-K-ATPase level measured in isolated PT cells was even more apparent in the LLC-PK1 cell vs. MDCK cell cultures. In the bottom of Fig. 6A, Na-K-ATPase levels were essentially identical between LLC-PK1 and MDCK cells. Ank3200, however, was distinctly prominent in the MDCK sample but unobserved in LLC-PK1 sample.

Ank3 Spliceoforms Vary Between Species

Unlike the mouse PT cells, LLC-PK1 cells did not display an ankyrin at 120 and 170 kDa but, instead, had a prominent ankyrin at 145 kDa. To assess whether the difference in LLC-PK1 cells, derived from porcine kidney, was a product of speciation or arose after culture, Ank3 spliceoforms were compared in mouse, rat, and rabbit kidneys (Fig. 6B). Rat kidneys, phylogenetically close to the mouse, expressed the same Ank3 spliceoforms as the mouse, although the Ank3170 spliceoform was dramatically reduced. In contrast, rabbit kidneys, which are phylogenetically more distant from mice than rats, showed distinct differences. The rabbit kidneys had a higher-molecular-weight ankyrin spliceoform that migrated distinctly from the Ank3200/215 seen in mice and rat kidneys and similar to ankyrin observed in MDCK cells. Like LLC-PK1 cells, rabbit kidneys had a prominent ankyrin around 145 kDa and the complete absence of the 120- and 170-kDa ankyrin.
spliceoforms. The 145-kDa rabbit ankyrin spliceoform migrated to a distance similar to that of the LLC-PK₁ band. Although sequence and functional analysis are ultimately required, these observations suggest that across species, the same cell types express distinct spliceoforms to serve similar functions.

DISCUSSION
Renal Ank3 Spliceoforms Showed Segmental Specificity

The present study demonstrates that Ank3 spliceoforms are heterogeneously expressed in the different renal epithelial cells. Immunoblotting of the renal cortex vs. the whole kidney with Ank3-R1 antibody provided the initial evidence for segmental specificity of Ank3. In the renal cortex, which is 80% PT by volume, the major isoforms are Ank3₁₂₀ and Ank3₁₇₀, with the Ank3₂₀₀/₂₁₅ spliceoforms significantly diminished compared with the whole kidney. This demonstrates that Ank3 isoforms are not homogeneously distributed throughout the kidney. Isolated PT segments confirmed this and demonstrated a greatly diminished concentration of the Ank3₂₀₀/₂₁₅ spliceoforms (Fig. 3). Furthermore, isolated DT segments expressed only the Ank3₂₀₀/₂₁₅ spliceoforms; Ank3₁₂₀ or Ank3₁₇₀ were absent.

These observations in freshly isolated tubule segments were mirrored in cultured cells. MDCK cells, a DT cell line, has been shown to possess a 210-kDa ankyrin (5, 25). These ankryins tether Na-K-ATPase along the basolateral membrane (25). In the present study, the Ank3-R1 antibody also detected the 210-kDa ankyrin in MDCK cells and, like the mouse DT, failed to detect proteins at 120 or 170 kDa. LLC-PK₁ cells, derived from PT, also express the 210-kDa ankyrin (5). Immunodetection with the Ank3-R1 antibody demonstrated, like the mouse PT, that expression of this spliceoform pales in comparison to other ankyrin spliceoforms in the PT (Figs. 3 and 6). Taken together, these studies clearly demonstrate that the epithelial ankyrin gene is differentially spliced in the different cell types of the kidney.
The origin of Ank3120 is well documented in macrophages (12, 28). It utilizes a start site within the last repeat of the membrane-binding domain (29 amino acids upstream of the spectrin-binding domain) and includes the entire spectrin-binding and regulatory domains. A 21.5-kDa, highly acidic, alternatively spliced exon is present within the regulatory domain of macrophage Ank3120. Presumably, the kidney Ank3120 isoform arises similarly but definitive proof by cloning and sequencing has not yet been obtained. Northern analysis confirms that kidney Ank3120 contains the spectrin-binding domain (28). Identifying the potential functions of Ank3120 in the modulation of spectrin transport, organization, or binding within PT cells awaits further inquiry.

The 170-kDa ankyrin is not well characterized, and its origins remain obscure (28). Given the enormous diversity of alternative splicing of the Ank3 gene described to date, the Ank3170 is likely to represent a true spliceoform. However, isolated kidney microsomes have a leupeptin-sensitive protease activity capable of clipping the 29-kDa COOH terminus from the regulatory domain of Ank1 (2). Thus the 170-kDa isoform of Ank3 could arise by partial proteolysis of a larger spliceoform. Further analysis will be required to elucidate the origin of Ank3170.

Functions of the Ank3 Spliceoforms in Renal Epithelial Cells

The myriad of segment-specific, polarized transport and adhesion molecules in the kidney indicate the need for specific mechanisms to develop, regulate, and maintain this polarization. The cell type-specific Ank3 spliceoform expression demonstrated above provides one mechanism by which the renal epithelial cells might direct cell type-specific polarization of transporters. Understanding of renal ankyrin spliceoform function and regulation, however, is in its infancy.

Ank3200/215 association with Na-K-ATPase. With reagents developed against Ank1, the association of ankyrin with the Na-K-ATPase was described in renal epithelia, retinal pigmented epithelium, choroid plexus, and parotid gland (1, 10, 21–24). Additionally, two ankyrin-binding sites have been identified on Na-K-ATPase (3, 13). In rat TAL, immunoelectron microscopy
showed both ankyrin and Na-K-ATPase were colocalized on the basolateral membrane. The lack of specific immunoreactivity in the rat PT cells, however, left the ankyrin-Na-K-ATPase association in PT cells undetermined. In the mouse cortex, antibodies specific to the Ank3200/215 spliceoforms displayed faint but specific staining of the PT cells (Fig. 5). Similar to the TAL, the ankyrin showed basolateral staining, placing it in the proper location for retaining Na-K-ATPase in PT cells. However, ankyrin staining also appeared in the terminal web region where Na-K-ATPase is not localized. This suggests that one or both of the Ank3200/215 spliceoforms serves an alternative function than to bind Na-K-ATPase.

PT have less Ank3200/215 per unit Na-K-ATPase than the renal cortex. When cultured proximal cells were compared with cultured distal cells, the lower Ank3200/215 content was even more striking.

Several explanations would account for the lower Ank3200/215 to Na-K-ATPase ratio in PT cells. First, the DT may have a greater number of integral proteins, in addition to Na-K-ATPase, which require Ank3200/215 tethering. Second, Na-K-ATPase in PT may be tethered to fodrin by other, yet discovered, ankyrin spliceoforms. Third, PT cells may have a lower percentage of their
Na-K-ATPase tethered to the fodrin cytoskeleton. These hypotheses are currently under investigation.

Ankyrin spliceoform expression diverges between species. A species-specific Ank3 expression in kidneys was apparent with the first descriptions of Ank3 spliceoforms (16, 28). Despite their close phylogenetic relationship, Peters et al. (28) described in 1995 the presence of five Ank3 spliceoforms in the mouse kidney while Kordeli et al. (16) in 1995 reported only two Ank3 spliceoforms present in the rat kidney. The most striking difference was the absence of the relatively abundant 170-kDa spliceoform in rat kidneys. This difference did not result from a difference in applied antibodies, since identical results were obtained in the rat kidney with the Ank3-R1 antibody (Fig. 6B). The species-specific expression was even more pronounced in the rabbit kidney. More phylogenetically distant, the rabbit kidney did not display spliceoforms at 120 and 170 kDa, had a prominent band at 145 kDa, and expressed a narrow doublet around 215 kDa. This is of particular interest since the 120-kDa spliceoform, the most prevalent form in the mouse and rat kidneys, lacks the membrane-binding domain, whereas the 145-kDa spliceoform, the most prevalent form in the rabbit kidney, would be predicted from its size to contain at least a partial membrane-binding domain. Like the rabbit kidney, porcine-derived LLC-PK1 cells also display the 145-kDa band but not the 120- and 170-kDa bands. Furthermore, the 215-kDa doublet in canine-derived MDCK cells was similar to the 215-kDa doublet in rabbits. Although the functional significance remains to be determined, these studies clearly indicate there is a marked species variation in Ank3 spliceoform expression.

In summary, these studies showed that the epithelial ankyrin spliceoforms expressed in the mouse kidney are differentially expressed in the various renal epithelia. Furthermore, within specific renal epithelia, there is an apparent divergence of ankyrin spliceoform expression. Although the complete functional significance will require further study, the apparent tethering of Na-K-ATPase in the basolateral membrane by the Ank3200215 spliceoform is significantly lower in PT vs. DT cells. The continued identification and localization of novel Ank3 spliceoforms highlight the potential role of ankryns in mediating the delivery, segregation, and regulation of membrane receptors, transporters, and adhesion molecules.

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