Annexin IV (AIV), a $Ca^{2+}$-dependent membrane-bind- ing protein, is expressed in many epithelia. Annexin IV modifies membrane bilayers by increasing rigidity, reducing water and $H^+$ permeability, promoting vesicle aggregation, and regulating ion conductances, all in a $Ca^{2+}$-dependent manner. We have characterized a mouse in which a gene trap has been inserted into the first intron of annexin IV. Processing of the primary transcript is disrupted. Northern blot and immunoblot data indicated that annexin IV expression was eliminated in many but not all tissues. Immunohistochemical analysis, however, demonstrated that annexin IV expression was eliminated in some cell types, but was unaltered in others. 5′-Rapid amplification of cDNA ends analysis of intestinal and kidney RNA revealed three transcripts, AIVa, AIVb, and AIVc. AIVa is widely distributed. AIVb is expressed only in the digestive tract. AIVc expression is very restricted. A selected number of epithelial cells of unique morphology demonstrate high concentrations. All three transcripts produce an identical annexin IV protein. The different tissue and cell-specific expression profiles of the three transcripts suggest that regulation of both the annexin IV gene expression and the cellular role of the protein are complex. The AIVa +− mouse may become a valuable model to further study transcription and the physiological role of annexin IV.

Annexins are a family of structurally related eukaryotic proteins characterized by the property of binding, or annexing, anionic phospholipid membranes in a calcium-dependent manner. The mammalian protein family is derived from at least 12 unique genes. Each family member has distinct tissue expression and subcellular localization patterns. Annexins provide novel, $Ca^{2+}$-dependent cellular mechanisms to reversibly modify membrane properties including fluidity and permeability, anchoring of cytoskeletal elements, aggregation of vesicles, and the regulation of ion conductances (1, 2). Annexin IV is expressed in many tissues and is associated with polarized epithelia (3, 4). Whole cell patch clamp studies have demonstrated annexin IV to regulate a calmodulin-dependent kinase II-activated chloride conductance via ClC-3 (5). Annexin IV also aggregates membrane vesicles in a $Ca^{2+}$-dependent manner, which is prevented by protein kinase C phosphorylation (6).

Annexin binding also causes the membrane leaflet to become more rigid, reducing water and proton permeability, and lateral lipid diffusion in the bilayer, all in a $Ca^{2+}$-dependent manner (7). Studies of the structural organization of annexin genes have shown that many are comprised of 12−15 exons. Most annexins have >10-kb introns between the first two or three exons. Many of the annexins have been shown to contain multiple transcripts, produced either through different exon usage or alternative splicing, which may amplify the functional variability within the family as a whole (2). Only one annexin IV transcript, 1961 bp, has been reported in mouse (accession number NM.013471) (8). The murine annexin IV gene, located on chromosome 6, has 13 exons and extends for ~57 kb (Ensembl gene ID number ENSMUSG00000029994). 3′ The region, consisting of an untranslated exon “a,” is followed by an extensive 27.7-kb intron 1. The translation initiation codon, ATG, is located at the 3′ terminus of exon 1. We have characterized a mouse containing a “gene trap” that has been inserted into intron 1 at a position 5′ to the first coding exon. Comparative analysis of annexin IV trapped and wild type mice revealed that there are three transcripts of annexin IV. Each transcript differs in the first exon of the 5′-untranslated region (5′-UTR), 4 which indicates alternate promoter usage. Each transcript has unique tissue and cell-specific expression.

MATERIALS AND METHODS
Northern Blot, RT-PCR, and 5′-RACE Analyses—Total RNA was isolated from mouse tissues using TRIzol reagent (Invitrogen). Total RNA (20 μg) was fractionated by 1% agarose gel and blotted onto Hybond-N+ nylon membranes (Amersham Biosciences). Blots were hybridized with 25 ng of 32P-labeled probe in QuikHyb solution (Stratagene) at 65 °C for 1 h, sequentially washed with 2× SSC, 0.1% SDS and 0.1× SSC, 0.1% SDS. The blot was exposed to a phosphorscreen and analyzed on a Amersham Biosciences PhosphorImager and analyzed using ImageQuant software (Amersham Biosciences).

The 5′-RACE system kit (Invitrogen) was used for 5′-rapid amplification of cDNA ends analysis. Total RNA (5 μg) was reverse transcribed into first strand cDNA with the AIV exon 5-specific primer AIV-544R following the manufacturer’s protocol. Terminal deoxynucleotidyltransferase-tailed cDNA was amplified with an abridged anchor primer (provided in the kit) and a nested AIV exon 4-specific primer, AIV4R (Table 1). PCR products were cloned into pCR2.1 vector (Invitrogen) and sequenced by the DNA Core Facility at the University of Cincinnati. Immuno blot—Monospecific affinity purified annexin IV antibodies were prepared as described by Kaetzel et al. (3, 4). Tissues were freshly harvested and immediately homogenized in 50 mM Tris buffer (pH 6.8) containing 5% SDS, 10 mM EDTA, 20 mM 2-mercaptoethanol, 1 μg/ml leupeptin, and 0.1 mg/ml phenylmethylsulfonyl fluoride at 60 °C and denatured further at 90 °C for 10 min. The total protein

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‡ The abbreviations used are: UTR, untranslated region; RT, reverse transcriptase; RACE, rapid amplification of cDNA ends; PGK, phosphoglycerate kinase-1 promoter; BTK, Bruton’s tyrosine kinase; LTR, long terminal repeat; NEO, neomycin resistance gene.

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concentration was determined by using the Pierce Protein assay reagent kit. Samples were aliquoted and stored at -80 °C until applied to gels. SDS-PAGE was performed on 12% polyacrylamide gels. For each set of samples, an initial gel was stained with Coomassie Blue to confirm equal loading among samples. The proteins were electrophoretically transferred from the gel to nitrocellulose membranes. After postfixation by immersion in 10% buffered formalin for 48 h followed by cold 10% buffered formalin. Individual tissues were removed and frozen by 15 s via the abdominal aorta, followed by 60-min block in 5% nonfat dry milk, 0.1% Tween 20 in phosphate-buffered saline (pH 7.4) for 15 s. Sites of antibody-antigen reaction were visualized by using enhanced chemiluminescence (Amersham Biosciences) before exposure to x-ray film.

**Immunohistochemistry**—Animals were perfused with cold phosphate-buffered saline (pH 7.4) for 15 s via the abdominal aorta, followed by 60-min block in 5% nonfat dry milk, 0.1% Tween 20 in phosphate-buffered saline, the membranes were incubated overnight at 4 °C with primary antibodies and then exposed to secondary antibody conjugated with horseradish peroxidase for 1 h at room temperature. Sites of antibody-antigen reaction were visualized by using enhanced chemiluminescence (Amersham Biosciences) before exposure to x-ray film.

**RESULTS**

**Disruption of the Annexin IV Gene**—A retrovirus-mediated gene trap strategy that randomly integrates into genomic DNA was used to generate annexin IV-disrupted mice. The construct introduces heterologous splice acceptor and donor sites that disrupt the normal splicing of the trapped gene (9). An inverse PCR method was used to determine the gene trap integration site in the mouse genome. An ES cell clone containing a single gene trap insertion in the annexin IV gene was identified from Omnimbank (Omnimbank number OST134768). By comparing the genomic flanking sequences with the mouse annexin IV genomic sequence (Ensembl Gene number ENSMUSG00000029994), the gene trap was determined to be inserted into the first intron of the annexin IV gene at nucleotide position 24,790, 3′ downstream of exon a (10001) and 5′ upstream of exon 1 (37788) (Fig. 1A). The AIVa-targeted ES (129SvEv) cells were injected into 3.5 days postcoitum C57BL/6 albino blastocysts. Male chimeric mice were mated with wild type C57BL/6 albino females.

Disruption of the annexin IV gene. A, representation of the gene trap insertion into the first intron of the annexin IV gene. The gene trap contains LTR, POG (active only in ES cells), NEO (neomycin gene with poly(A)), BTK (DNA, SD (splice donor sequence), and SA (splice acceptor sequence). The schematic depicts three transcripts, exon a-exon 12, exon a-NEO, and BTK-exon 12. The LTR is engineered in the reversed orientation and has no promoter activity. The nucleotide positions of exon a, the gene trap, exon 1, and exon 12 of the annexin IV gene are shown. B, PCR genotyping of annexin IV gene-interrupted mice. Dashed line arrows represent PCR primer binding sites for genotyping. Two sets of PCR reactions were performed from each mouse tail genomic DNA using primer sets AIV-24741F/AIV-25580R and LTR2/AIV-25580R (Table I). Wild type mice produce an 839-bp PCR product only from primers AIV-24741F/AIV-25580R. Heterozygous mice produce 839- and 840-bp PCR products from primers AIV-24741F/AIV-25580R and LTR2/AIV-25580R, respectively. AIVa/− mice only produce the 840-bp PCR product from primers LTR2/AIV-25580R. C, RT-PCR analysis of intestinal mRNA transcripts. The solid line arrows in panel A represent RT-PCR primer binding sites. The complete annexin IV transcript of exons a–12 is present only in wild type animals. The fusion transcript of annexin IV exon a and NEO, using the annexin IV promoter, was detected using primers AIVaF/NeoR in AIVa/− intestine. Primers AIVaF/AIV4R confirmed that the AIV exon a transcript existed in the AIVa/− intestine but was lost in the AIV+/− mice. In ES cells only, the integration produces two fusion transcripts, one containing exon a and NEO, and the other containing BTK and exons 1–12 of annexin IV. No fusion transcripts of BTK and 3′ downstream annexin IV exons, using the PGK promoter, were detected with primers BTKF/AIV4R in AIVa/− mouse.

**A**

**B**

**C**

![Image](image-url)
The Annexin IV Gene Produces Three Transcripts

Table I

| Primers used in PCR, 5'-RACE, and RT-PCR analyses |
|-----------------------------------------------|
| Sense primer | Antisense primer |
| PCR primer | Wild type AIV genomic identification |
| AIV-24741F | AIV-25580R |
| LTR2 | AIV-25580R |
| AIVaF | AIVaR |
| AIVbF | AIVbR |
| AIVcF | AIVcR |
| BTKF | NEO |

The Annexin IV Gene Produces Three Transcripts

5'-RACE analysis of total RNA from wild type small intestine and kidney revealed two products from both tissues (Fig. 3A). Each fragment was isolated, cloned, and sequenced. The sequence data revealed that the two mRNAs from AIVa-/- intestine have identical coding regions with distinct 5'-UTRs.

Transcriptional Characterization of the Gene Trap Insertion—The gene trap has two functional units. The first requirement is identification of the individual recipient genes in the initial gene bank of ES cells. This part of the trap consists of the phosphoglycerate kinase-1 (PGK) promoter, the structural Bruton’s tyrosine kinase (BTK) gene, and a synthetic consens splice donor sequence (Fig. 1A). In ES cells, the PGK promoter produces a fusion transcript of BTK with the 5’ downstream exons of the recipient gene. The recipient gene is identified by sequencing of 3'-RACE of the BTK-recipient gene fusion transcript (9). An essential feature of the trap is that the PGK promoter is silent in mice because heterologous promoters internal to a retrovirus are inactive in differentiated cells (9, 10). In addition, the LTR was engineered in the reversed orientation to eliminate promoter activity (9).

The second functional unit consists of a splice acceptor sequence fused to a neomycin resistance gene (NEO) followed by a polyadenylation sequence. The promoter of the trapped gene drives transcription of the exons that are 5’ upstream of the trap insertion. These exons splice onto NEO, which disrupt the appropriate transcriptional processing of the trapped gene (9). Gene trap insertion into exon 1 of the annexin IV gene utilizes an annexin IV promoter to generate a fusion transcript of exon a and NEO (Fig. 1A). This aberrant splicing produces a “dead-end” transcript because the NEO lacks a splice donor sequence.

To characterize the transcripts produced by the wild type and annexin IV gene trapped animals, RT-PCR was performed on AIVa+/+ and AIVa-/- small intestine RNA. Three sets of primers were designed. AIVaF (specific to exon a) and AIVaR (specific to exon 4) confirm wild type transcript processing. AIVaF and NEOA (specific to NEO) verify trapping. BTKF (specific to BTK) and AIV4R confirm that the PGK promoter is silent in differentiated tissues (Table I and Fig. 1A). RT-PCR revealed a 296-bp product with primers AIVaF/AIV4R only from the AIVa+/+ mice. A 737-bp fusion transcript, exon a-NEO, was identified with primers AIVaF/NeoR only in AIVa-/- mice. No product was produced with primers BTKF/AIV4R, demonstrating that the PGK promoter was not active (Fig. 1C). These results confirmed that gene trap insertion into the intron between exon a and exon 1 of the annexin IV gene resulted in splicing of exon a onto the NEO gene, which, in turn, interrupted the appropriate processing of this AIV transcript.

Identification of Multiple AIV Transcripts—To determine whether annexin IV gene expression was disrupted, Northern blot and immunoblot analyses were performed from tissues of wild type and AIVa-/- mice. The Northern blots (Fig. 2A) showed that while the expression of AIV mRNA was markedly reduced in heart, kidney, liver, and lung, it remained abundant in the digestive tract. The Northern blots indicated a single transcript of ~2 kb. Immunoblot analysis (Fig. 2B) showed that wild type animals expressed lower levels of annexin IV in heart, muscle, and testis, intermediate levels in kidney, liver, and lung, and high levels in the digestive tract. In annexin IV gene trapped animals, the expression of annexin IV in heart and lung was not detectable (Fig. 2B). However, low amounts of annexin IV remained in kidney, liver, and testis, and high amounts remained in duodenum, jejunum, ileum, and colon of the AIVa-/- mouse. This result suggested more than one transcript of annexin IV.

5'-RACE analysis of total RNA from wild type small intestine and kidney revealed two products from both tissues (Fig. 3A). Each fragment was isolated, cloned, and sequenced. The sequence data revealed that the two mRNAs from AIVa-/- intestine have identical coding regions with distinct 5'-UTRs.
One 5′-UTR is 48 bp and the other is 166 bp in length (Fig. 3B). The 5′-UTRs were identified as sequences of the annexin IV gene. The 48-bp 5′-UTR is identical to exon a of the reported annexin IV gene (8). The 166-bp non-coding sequence was mapped as a newly identified exon, “b” (Sim 4-cDNA and genomic DNA alignment program,3 located at nucleotide position 28,482, which is 18 kb 3′ from exon a and 3.7 kb 3′ of the gene trap (Fig. 3B). Both exons a and b are spliced onto coding exon 1 (Fig. 3B).

Sequencing of the two products from wild type AIVa+/+ kidney confirmed that the −300-bp product was from the exon a containing message (AIVa). Sequencing of the −400-bp product, however, revealed that it was produced from an additional unique mRNA, AIVc. The 5′-UTR of AIVa starts at nucleotide position 37,681 and has an extended 5′ sequence (107 nucleotides) fused to exon 1 and mapped as a single 154-nucleotide exon (Fig. 3B). In all three mRNAs, translation starts with AUG on exon 1, which results in an identical full-length annexin IV protein.

In comparison, 5′-RACE analysis of the total RNA isolated from small intestine and kidney of gene trapped AIVa−/− mice revealed that each tissue contained a −400-bp product, AIVb in intestine and AIVc in kidney (Fig. 3, A and B). These results demonstrated that mRNA processing of AIVa was interrupted, whereas the gene trap did not affect AIVb and AIVc transcriptional splicing.

Expression of Annexin IV Transcripts—RT-PCR analysis using forward primers specific to exons a, b, and c and a common reverse primer to exon 4 (Table I and Fig. 3B) revealed that the three messages had distinct tissue distributions in wild type animals (Fig. 4). AIVa is expressed in many tissues, low in heart, liver, and lung, intermediate in kidney and stomach, and high in the small and large intestines. AIVb is restricted to high expression in the digestive tract. AIVc is expressed in kidney, liver, and the digestive tract. RT-PCR results from AIVa−/− tissues confirmed that AIVa was not produced and that AIVb and AIVc expression profiles were comparable with wild type animals. The levels of annexin V were also not affected.

Histology—Tissues are comprised of many cell types with distinct functions. Often the analysis of total tissue RNA or protein does not reflect the respective expression in specialized cells. Immunohistochemical evaluation can focus more directly on the individual cell types that express annexin IV and provide insight into physiological function. Immunohistochemistry of wild type and AIVa−/− animals was performed to evaluate the differential expression of the three transcripts. The morphology and immunohistochemistry of the organs of the digestive tract displayed no differences between wild type and the AIVa−/− animals (Fig. 5). In the small intestine, annexin IV is present in Paneth cells in the crypts and the epithelia lining the lumen. As the cells migrate up the villus, nuclear localization is apparent. Most striking are the intensely staining narrow columnar epithelial cells that are dispersed between the goblet and absorptive cells.

Annexin IV is expressed throughout the kidney, including the juxtaglomerular apparatus and tubule segments of the nephron of wild type animals. Annexin IV is highly concentrated in the medullary collecting ducts and the transitional epithelium lining the calyx (Fig. 6, a and c). This pattern is distinctly different from the AIVa−/− kidney in which only AIVc is expressed (Fig. 6, b and d). Annexin IV localization is restricted to a scarce number of tubules in the cortical region. This epithelium has a subpopulation of cells that contain high concentrations of annexin IV throughout the cytoplasm (Fig. 7). Examination of cross-sections of the ductus efferens of the AIVa−/− mouse also reveals cells that express very high levels of annexin IV, interspersed in the epithelium (Fig. 7).

DISCUSSION

Weber et al. (11) first sequenced the annexin IV protein. The bovine cDNA was cloned and sequenced by Hamman et al. (12). Tait et al. (13) reported human annexin IV cDNA. Mouse and rat annexin IV cDNA were more recently sequenced (8, 14). Few vertebrate annexins have been subjected to detailed promoter or transcriptional regulation analysis (2). The human (Ensembl gene number ENSG00000115980) and mouse annexin IV genes (Ensembl gene number ENSMUSG0000029994) have similar genomic structures. Each has been reported to have a total of 13 exons. Twelve are coding exons. The ATG initiation codon starts at the 3′ terminus of exon 1. The first 5′ non-coding exon is separated from the first coding exon by a large intron, −40 kb in human and −27 kb in mouse. Similarly, human
and chicken annexin V (15, 16) and human and mouse annexin XI (17) have an untranslated exon 1 preceding 12 coding exons.

The annexin IV gene has been reported to have a single transcript (8, 13). Our study reveals that there are three transcripts for the mouse annexin IV gene. These multiple transcripts suggest that the transcriptional and translational regulation of the mouse annexin IV gene is complex in regard to its function. Characterization of annexin transcripts in other species has revealed variability. Preliminary studies using 5'RACE analysis of rat intestine RNA produced two transcripts containing distinct 5'-UTR 5'RACE analysis of human intestine RNA, however, produced a single transcript. Differences between human and mouse annexin IV transcripts are consistent with other members of the annexin family. For example, the human annexin XI has three transcripts that differ in their 5'-UTR, whereas mouse annexin XI, produces a single transcript (17). Human annexin V has one transcript, whereas the rat annexin V gene expresses multiple transcripts (15).

Identification of AIVb and AIVc explains the annexin IV expression in the intestine, kidney, and testis of the AIVa<sup>−/−</sup> mouse (Fig. 2, A and B). In wild type animals, all three mRNAs, AIVa, AIVb, and AIVc, are produced. In the gene trapped AIVa<sup>−/−</sup> animals, transcription of AIVa is disrupted, whereas transcription of AIVb and AIVc remains unaffected. The gene trap disrupts processing of the exon a-containing message because of the introduction of heterologous splice acceptor and donor sites between exons a and 1 (Fig. 1A). The annexin IV expression detected by Northern blot and immunoblot (Fig. 2, A and B) in AIVa<sup>−/−</sup> mice is because of the expression of AIVb and AIVc transcripts. The three transcripts have similar sizes, 1968 bp for AIVa, 2086 bp for AIVb, and 2075 bp for AIVc. The ~1.5-kb band detected by Northern blot in the AIVa<sup>−/−</sup> mouse using probes spanning exons 2–5 includes all three transcripts (Fig. 2A). The message detected by Northern blot analysis in the digestive tract includes both AIVb and AIVc in the AIVa<sup>−/−</sup> mouse. Because AIVc is only expressed in a restricted number of cells in the kidney, it was not detectable by Northern blot analysis using total kidney tissue RNA of AIVa<sup>−/−</sup> mice (Fig. 2A).

The three mouse annexin IV mRNA variants, AIVa, AIVb, and AIVc, each have distinct 5'-UTRs. In all three mRNAs, translation starts with a Kozak sequence (AGCAUGG) located at the 3' terminus of exon 1, which results in an identical full-length annexin IV protein product. mRNA variants produced by differences in the 5'-UTR or 5' non-coding exons have been reported in several other proteins, including neuronal nitric-oxide synthase (18–20), human reduced folate carrier gene (21, 22), human estrogen receptor-a (23), excitatory amino acid transporter 2 (24), and human glucocorticoid receptor (25). These studies suggest that differences in the first exons of the 5'-UTR are because of alternative promoter usage.

Although translation of the three mRNAs of annexin IV results in an identical protein, three uniquely regulated pro-
motors provide a cell-specific mechanism to respond to changes in physiological demands. Protein expression can be regulated by activation or suppression of alternative promoters of a single gene in a cell-specific manner. As shown by our present study, alternative promoter usage in annexin IV results in tissue and cell-specific expression. AIVa is expressed in various amounts in most tissues; the highest levels are in intestine, stomach, and kidney. AIVb is expressed only in the epithelia of the digestive tract. Although the expression of AIVc appears low relative to AIVa and AIVb by RT-PCR analysis, immunohistochemistry demonstrates that AIVc expression, whereas restricted to cells interspersed in the epithelium of, for example, certain renal tubules and the ductus efferens, is found at very high cellular concentrations (Fig. 7).

The differential cellular expression of annexin IV suggests that the gene is regulated at both the transcriptional and translational levels. The putative promoter regions, 1000 bp upstream of the three annexin IV transcripts, were compared with other reported promoter elements. None of the annexin IV sequences contained TATA, CCAAT, or GC boxes. A CpG island (0.5 kb), however, is present in the putative promoter region near the 5′/H11032 end of exon a. The TATA-less, GC-rich promoter has been identified in human annexin V (16) and human annexin XI (17). CpG islands are characteristic of many constitutively expressed genes (26). Both human annexins V and XI have wide expression using a TATA-less, GC-rich promoter (16, 17). The broad expression of AIVc is also consistent with these gene motifs. Putative binding sites for transcription factors GATA, PDX1, and HNF1 are present in the potential promoter region of the AIVb transcript. These transcription factors are involved in regulating gene expression in the digestive tract (27–29).

Differences in 5′-UTR structure affect mRNA processing, localization, stability, and translation efficiency (30–32). Regulation of protein translation by the upstream open reading frame can be cell-specific (33, 34). One of the features within the AIVc 5′-UTR is a pair of upstream open reading frames in which the upstream start codon (uAUG) is followed by in-frame stop codons that would produce peptide products of 6 and 27 amino acids, respectively. The presence of upstream open reading frames in AIVc could contribute to the cell-specific expression pattern.

Many tissues, such as the kidney, are quite complex and physiologically dynamic. Hemostatic conditions can rapidly change with regard to ingested salts and water. The tubule segments of the nephron are composed of distinct cell types that coordinate functions to maintain hemostasis. The kidney cortex contains a mixture of tubule segments dominated by the proximal convoluted tubule. The distal convoluted tubule is less abundant. The annexin IV-positive tubules in AIVa mice.

![Fig. 4. Analysis of tissue distribution of AIVa, AIVb, and AIVc in wild type and the AIVa+/− mouse by RT-PCR. Total RNA (5 μg) was first reverse transcribed using oligo(dT), then PCR amplified with primers specific for AIVa (AIVaF) (28 cycles), AIVb (AIVbF) (28 cycles), and AIVc (AIVcF) (35 cycles) and a common reverse primer on exon 4 (AIV4R) (shown in Fig. 3B). Equal amounts of PCR products were analyzed on agarose gels. Annexin V (AV) and β-actin controls showed no difference in expression between AIVa+/− and AIVa−/− mice.](image1)

![Fig. 5. Immunohistochemical analysis of annexin IV in intestine. Four-μm sections of small intestine were incubated with affinity purified sheep anti-annexin IV (4). In wild type and AIVa−/− intestines, annexin IV is expressed within the Paneth cells at the base of the crypt of Lieberkuhn, as well as the differentiated columnar epithelial cells of the villi. Brown, annexin IV; blue, hematoxylin counterstain.](image2)

![Fig. 6. Immunohistochemical analysis of annexin IV in inner renal medulla. In wild type mice, annexin IV is localized in the renal inner medulla, in the collecting ducts (CD) and the transitional epithelium lining the renal pelvis (a and c). AIV is not expressed in the AIVa−/− renal medulla (b and d). Brown, annexin IV; blue, hematoxylin counterstain.](image3)
mice are structurally and morphologically similar to distal convoluted tubules and this segment also contains cells that secrete urodilatin, a renal natriuretic peptide. Likewise, the tubules of the ductus efferens of AIVa–/– mice express annexin IV at very high levels in a restricted number of cells interspersed in the epithelium (Fig. 7). These tubules also contain the kallikrein-kinin system. Confirmation by co-localization of annexin IV with the respective hormones and paracrine factors may demonstrate that AIVc transcript expression is associated with cells that are responsible for local sensory and secretory functions within the epithelium. There are several types of gastrointestinal endocrine cells that secrete peptide hormones and paracrine factors in response to local physiological changes. Co-localization studies with gastric inhibitory peptide, secretin, neurotensin, and other gastroendocrine secretory products will provide identity of the columnar cells containing high levels of annexin IV (Fig. 5). It will be valuable to identify the specific cell types that use the AIVc promoter.

In the intestine, there is no apparent difference in morphology, annexin IV localization, or annexin IV overall expression between wild type and AIVa–/– animals. The gut may represent an instance where two transcripts, AIVa and AIVb, are expressed in the same cell type. In fact AIVb may compensate for the loss of AIVa in AIVa–/– intestines.

Lower vertebrates have been used as model systems to investigate annexin IV function. Seville et al. (35) used subtractive hybridization screens to identify annexin IV as a gene expressed in the pronephros of developing Xenopus larvae. The protein localized to the luminal surface of the developing pronephros. The temporal and spatial expression patterns of annexin IV in frog and zebrafish suggest a role in pronephric tubule development (35, 36). Morpholino injection into one-cell stage embryos is valuable to evaluate specific protein function during the early stages of larval development. For example, depletion of annexin IV in developing Xenopus, using morpholinos, produced shortened, dilated pronephric tubules (35). Our gene trapped mice are null for kidney AIVa–/– mRNA throughout fetal and postnatal development and provide an ideal model to elucidate the role of annexin IV in epithelial function and normal kidney development.

The expression of three distinct annexin IV transcripts makes the AIVa–/– mouse a restricted loss of function model. The physiological role of annexin IV in many tissues can be evaluated without the changes in the digestive tract. Disruption of the expression of AIVb and AIVc will further contribute to the elucidation of the physiological role of annexin IV.

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