The actin-binding proteins adseverin and gelsolin are both highly expressed but differentially localized in kidney and intestine

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SUMMARY

To understand the distinct functions of the closely related actin-severing proteins adseverin and gelsolin, we examined the expression of these proteins in detail during mouse and human development using a new highly sensitive and specific set of antibody reagents. Immunoblot analysis demonstrated that adseverin was highly expressed in mouse kidney and intestine at all stages of development and in human fetal and adult kidney. In contrast and as reported previously, gelsolin was expressed much more widely in both murine and human tissues.

Immunohistochemistry on murine kidney sections revealed a predominantly differential localization of adseverin and gelsolin. Adseverin was expressed in peripolar cells, thin limbs, thick ascending limbs, and principal cells of cortical and medullary collecting ducts where it was diffusely localized in the cytoplasm. Gelsolin was expressed in the distal convoluted tubule, intercalated cells and principal cells of cortical and medullary collecting ducts, and in ureter. In the distal convoluted tubule, gelsolin showed a diffuse distribution and in principal cells of collecting ducts a localization at the basolateral pole. In intercalated cells, gelsolin localization was heterogeneous, either at the apical pole or diffusely in the cytoplasm. In human fetal and adult kidney, adseverin was expressed only in collecting ducts whereas gelsolin was expressed in thick ascending limbs and collecting ducts.

In mouse and human intestine adseverin was expressed in enterocytes with a gradient of increasing expression from the duodenum to the colon, and from the crypt to the villus. The observations indicate high level expression of adseverin in specific cells of the kidney and colon, and suggest a previously unrecognized function of adseverin in epithelial cell function.

Key words: Actin, Adseverin, Gelsolin, Kidney, Intestine, Epithelial cell

INTRODUCTION

Actin occurs in cells both as a monomeric globular protein (molecular mass 42 kDa) and polymerized into actin filaments. The actin cytoskeleton is responsible for many of the structural and viscoelastic properties of cells, and dynamic changes in the actin filament architecture are critical for cell motility (Lauffenburger and Horwitz, 1996; Mitchison and Cramer, 1996; Hartwig and Kwiatkowski, 1991), division (Wong et al., 1997; Dutartre et al., 1996), adhesion (Fath and Burgess, 1993; Fath et al., 1993). These physiologically important processes involving actin remodelling are determined or regulated by a large number of actin-binding proteins as well as by small GTPases of the ras superfamily (Macara et al., 1996) and actin-related proteins (Frankel and Mooseker, 1996; Moreau et al., 1996).

Gelsolin (Yin and Stossel, 1979) is an 83 kDa protein which in vitro regulates the dynamics of actin filament assembly in a calcium-dependent manner by severing of preexisting filaments, capping of the (+)-end of the newly generated filament after severing, and nucleating actin filament assembly from monomers. These functional activities are common features among a family of proteins which bear structural and functional homology to gelsolin (Weeds and Maciver, 1993; Hartwig and Kwiatkowski, 1991). Family members include villin (Breitscher and Weber, 1980), adseverin/scinderin (Bader et al., 1986; Maekawa et al., 1989; Rodriguez del Castillo et al., 1990), flightlessI (Campbell et al., 1993), and the gelsolin-like proteins described for the invertebrates Drosophila melanogaster (Heintzelman et al., 1993) and Homarus americanus (Lueck et al., 1995).

We have performed a series of studies implicating a crucial role for gelsolin in fibroblast motility (Azuma et al., 1998; Witke et al., 1995; Cunningham et al., 1991) where it is moderately highly expressed (~0.1% of total cell protein). Gelsolin null fibroblasts have reduced motility in multiple assays and overexpress the GTPase Rac1 5-fold. Transfection of gelsolin into NIH3T3 cells or gelsolin null fibroblasts results in an increase in motility that correlates with gelsolin...
expression level (Azuma et al., 1998; Cunningham et al., 1991) and reverts the overexpression of Rac1 in the gelsolin null cells. In mammalian tissues and cultured mammalian cells, gelsolin is widely expressed (Kwitowski et al., 1988a,b), whereas villin is found only in cells that form microvilli (Bretscher et al., 1981).

Adseverin, also termed scinderin, is another member of the gelsolin family and has been identified in several bovine tissues with secretory activity (Tchakarov et al., 1990) as well as some neuroendocrine and brain tissues (Sakurai et al., 1990), where it is expressed at low levels. Adseverin predominantly colocalizes with F-actin in bovine adrenal medulla chromaffin cells whereas gelsolin is diffusely localized in these cells (Vitale et al., 1991). Moreover, treatment of these cells with either 10 μM nicotine or 56 mM K+ triggers exocytosis and disruption of the cortical F-actin network with redistribution of adseverin. Under these conditions, gelsolin distribution remains unchanged. Thus, adseverin is postulated to have an important role in exocytosis (Vitale et al., 1991; Trifaro et al., 1993).

Here we report a comprehensive analysis of adseverin and gelsolin expression during murine and human development with a detailed examination of the in situ expression of these proteins in the tissues with the highest level of adseverin expression, kidney and intestine. The high expression of both adseverin and gelsolin in these tissues and their predominant complementary localization in resorptive epithelial cells suggest that adseverin and gelsolin, despite their structural similarity, might serve important but distinct roles in these cells.

MATERIALS AND METHODS

Expression and purification of recombinant murine adseverin

The cDNA of murine adseverin (a gift from Drs Renaud and Louahed, Ludwig Institute for Cancer Research, Buxelles, Belgium) was subcloned into the prokaryotic expression vector pQE30 (Qiagen) using unique SacI and Sall sites. This construct encoded an N- terminal extension of 20 amino acids (MRGSHHHHHHHSACELHRGT) compared with native adseverin. Bacterial expression was achieved by growth to A600=0.6, induction with 1 mM IPTG for 3 hours, collection of cells by centrifugation and resuspension in 40 ml/l culture of ice-cold 10 mM Tris-HCl, 1 mM EGTA, 1 mM EDTA, 0.5 mM DTT, 0.5% Triton X-100, 5 mM benzamidine, 1 mM PMSF, pH 7.8. Cell lysis was achieved by sonication and after centrifugation, the supernatant of the lysate was applied to a DE52 (Whatman) column and rinsed with 10 mM Tris-HCl, 1 mM EGTA, 1 mM 2-mercaptoethanol, pH 7.8. Recombinant murine adseverin was eluted with 10 mM Ca2+ in the same buffer. Adseverin-containing fractions were pooled and dialyzed against 0.1% Tween-20, and elution with 50 mM glycine, pH 2.3. The pH of the eluate was raised to 7.5 and the eluate was dialyzed against PBS.

Affinity purified chicken anti-adseverin IgG was prepared by application of total chicken IgG to adseverin Sepharose (prepared as described by Kwiatkowski et al., 1985) followed by rinsing with PBS, 0.1% Tween-20, and elution with 50 mM glycine, pH 2.3. The pH of the eluate was raised to 7.5 and the eluate was dialyzed against PBS.

Affinity purified Ads-1 was prepared by application of serum (diluted 1:10 in PBS) to a NHS HiTrap column (Pharmacia) to which the above described peptide was coupled, following the manufacturer’s directions. Rinsing and elution were done as described above.

Preparation of tissue extracts and SDS-PAGE

Mice were killed by CO2 narcosis and tissues were dissected immediately on ice. For some experiments animals were perfused prior to organ harvest to minimize plasma gelsolin content. For perfusion, the thorax was opened and the left ventricle of the still beating heart was punctured with a 25 gauge needle and the right ventricle was opened with an incision. PBS was injected into the left ventricle until the returning circulation was clear. Human fetal and adult tissues were obtained fresh (BWH Human Research Committee approval). Different regions of the kidney were separated under a dissecting microscope. Murine and human tissues were homogenized on ice in a hand-held tissue homogenizer (volume 2 ml) in 1 ml of extraction buffer (PBS containing 1% (v/v) Triton X-100, 5 mM EGTA, 5 mM EDTA, 1 mM PMSF, 5 mM benzamidine) per gram of tissue. Insoluble material was removed by centrifugation for 5 minutes at 16,000 g at room temperature. The clear supernatant was mixed with 1/6 volume of a 6x SDS sample buffer (320 mM Tris-HCl, pH 6.8, 20% (v/v) glycerol, 40% (v/v) 2-mercaptoethanol, 10% (w/v) SDS, 20 mM EDTA, 0.05% Bromphenol Blue), and the samples were then boiled for 10 minutes at 100°C. For SDS electrophoresis, we used discontinuous gels with a 10% resolution gel and a 3.5% stacking gel. Electrophoresis was carried out at 7 V/cm using the Laemmli buffer system (Laemmli, 1970).

Immunoblotting

Proteins separated by SDS-PAGE were transferred to a PVDF membrane (Immobilon P, Millipore) with 25 mM Tris-HCl, 192 mM glycine, 20% methanol, pH 8.3, as the transfer medium in a semi-dry process (Khýsé-Anderson, 1984) for 90-120 minutes at 15 V. After transfer, the proteins bound to the membrane were stained with Ponceau S solution (3% trichloric acid, 3% sulfosalicylic acid, 0.2% Ponceau S) to monitor transfer efficiency. The Ponceau S stain was removed by washing the membrane briefly in 50 mM unbuffered Tris solution. Blocking was performed overnight at 4°C in TNT buffer (10 mM Tris-HCl, 150 mM NaCl, 0.05% Tween-20, pH 8.0) containing 5% (w/v) nonfat dry milk powder. Primary antibodies were applied to the membrane for 4 hours at room temperature in blocking buffer at the following concentrations: Ads-3, 0.5 μg/ml; Ads-1, 0.1 μg/ml; rabbit anti-murine gelsolin (Azuma et al., 1998), 0.05 μg/ml; monoclonal antibody to human gelsolin (clone 2C4) 0.1 μg/ml. For detection of human adseverin, we used Ads-3 after a concentration of 1.3 μg/ml. The membrane was washed 4x 15 minutes each in TNT buffer, and secondary antibodies (rabbit anti-chicken, HRP conjugate;...
Expression of adseverin and gelsolin in kidney and gut

3635

goat anti-rabbit, HRP conjugate, and goat anti-mouse, HRP conjugate; all Sigma) were applied at a dilution of 1:3,000 for 60 minutes in TNT buffer. After this incubation the membrane was washed 4 × 15 minutes in TNT buffer. For signal detection we used the Renaissance ECL reagent (NEN Life Science Products, Boston) and Biomax MS X-ray film (Kodak, Rochester, NY).

Immunohistochemistry on paraffin-embedded tissue sections

Dissected organs were fixed for up to 72 hours in PBS containing 4% paraformaldehyde. Paraffin embedded sections (5 μm thick) were dewaxed in xylene (2 × 5 minutes), followed by two rinses (5 minutes each) in 100% ethanol. The sections were rehydrated in a series of ethanol/water (90%, 70%, 50%, and 30% ethanol) for 5 minutes each followed by a final wash in PBS (10 minutes). Blocking was carried out with 1% BSA in PBS for 10 minutes. For detection of adseverin the antibody Ads-1 was applied at a concentration of 6 μg/ml and for detection of gelsolin a polyclonal antibody to murine gelsolin was used at a concentration of 5 μg/ml. Controls were preimmune serum (Ads-1), and non-immune serum (anti-gelsolin) which were applied to the sections at approximately 1 mg/ml. Additional controls were preincubation of the antibodies for 30 minutes either with the peptide (Ads-1) or recombinant murine gelsolin (anti-gelsolin) with a 10- to 100-fold excess of the respective antigen. Primary antibodies were applied for 2 hours at room temperature, followed by a brief rinse in PBS, 2 wash steps of 5 minutes each in high salt PBS (PBS containing 0.4 M NaCl) and a final wash in PBS for 5 minutes. Secondary antibody (goat anti-rabbit, FITC conjugate, Vector laboratories) was applied at a dilution of 1:30 for 60 minutes at room temperature. After incubation with the secondary antibody, sections were washed as described above, and counterstained for 60-90 seconds in 0.01% Evans Blue (Sigma). A coverglass was mounted in a 1:1 mixture of mounting medium (Vectashield, Vector laboratories) and 1.5 M Tris-HCl, pH 8.9, and the sections were inspected immediately.

RESULTS

Characterization of anti-adseverin antibodies

We raised antibodies against a peptide containing the first 15 amino acids of murine adseverin (Ads-1) and against the bacterially expressed full length protein (Ads-3). In protein extracts from murine kidney and small intestine, both antibodies displayed a specific reaction with a protein of approximately 79 kDa (Fig. 1) which comigrated with bacterially expressed recombinant murine adseverin, and no cross-reactive proteins were seen. When the antibodies were preincubated with either the peptide (Ads-1) or recombinant murine gelsolin (anti-gelsolin) with a 10- to 100-fold excess of the respective antigen. Primary antibodies were applied for 2 hours at room temperature, followed by a brief rinse in PBS, 2 wash steps of 5 minutes each in high salt PBS (PBS containing 0.4 M NaCl) and a final wash in PBS for 5 minutes. Secondary antibody (goat anti-rabbit, FITC conjugate, Vector laboratories) was applied at a dilution of 1:30 for 60 minutes at room temperature. After incubation with the secondary antibody, sections were washed as described above, and counterstained for 60-90 seconds in 0.01% Evans Blue (Sigma). A coverglass was mounted in a 1:1 mixture of mounting medium (Vectashield, Vector laboratories) and 1.5 M Tris-HCl, pH 8.9, and the sections were inspected immediately.

On human fetal and human adult tissue sections the signal intensity was amplified by using the Renaissance TSA-direct kit green (NEN, Boston) following the protocol of the manufacturer. Primary antibodies were applied to the sections as follows: Ads-3 at a concentration of 0.5 μg/ml for 1 hour at room temperature and anti-gelsolin at a concentration of 0.25 μg/ml for 1 hour at room temperature. Biotin-labeled secondary antibodies used in this procedure were applied at a dilution of 1:100 for 60 minutes at room temperature.

Fig. 1. Characterization of anti adseverin antibodies. 50 μg of total protein extract from mouse kidney, small intestine, and brain and indicated amounts of purified recombinant murine adseverin were loaded per lane and subjected to western blot analysis after SDS-PAGE, using the affinity purified antibodies Ads-1 (left panel) and Ads-3 (right panel) for detection of murine adseverin.
comigrating with recombinant murine adseverin. Ads-3 was exclusively used for detection of human adseverin.

Expression of adseverin and gelsolin at different stages of mouse development

To examine adseverin and gelsolin expression during mouse development, western blot analysis was performed on tissue extracts during development (E17.5, day of birth, 1 week, 3 weeks, and 4 months). We also examined embryonic brain and head samples beginning at E13.5.

Adseverin expression was high in intestine of mouse embryo (E17.5) (Fig. 2A), and seen at lower levels in stomach and kidney. Expression in kidney increased by birth (Fig. 2B), was high by 1 week of age (Fig. 2C), and stayed high through adult life (Fig. 3A and B). Maximal expression in intestine and kidney were ~50 ng/50 mg (0.1%). Smaller amounts of expression were seen in E15.5 head (but not in the E17.5 brain) (Fig. 2A), 3 week and 4 month thymus (Fig. 3A), and faintly in 4 month adrenal gland (Fig. 3B). The positive signals seen in E14.5 and E15.5 head but not in the E17.5 brain are consistent with in situ hybridization results which indicate adseverin expression in growing bone primordia of the mouse embryo (M. Arai and D. J. Kwiatkowski, unpublished results).

In contrast to adseverin, gelsolin was widely expressed at all developmental stages (Figs 2A-C and 3A,B). Some of this signal is likely due to plasma gelsolin contamination of tissue extracts, as gelsolin is present in adult murine plasma at ~200 μg/ml. In the 4 month old animal (Fig. 3B) tissue perfusion with PBS was performed and a more accurate sampling of tissue gelsolin expression obtained. Gelsolin expression was high in multiple tissues but was low in brain, pancreas, and liver, consistent with previous studies (Kwiatkowski et al., 1988a).

Differential expression of adseverin in mouse kidney

Four different levels were grossly dissected from the murine kidney to explore adseverin expression in more detail. A gradient of expression was seen with lowest levels in cortex

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Fig. 2. Expression of adseverin and gelsolin at early stages of mouse development. Western blot analysis of embryonic tissues of E17.5 (A), newborn (B), and 1 week old (C) mice. Primary antibody for adseverin detection was Ads-1.

Fig. 3. Expression of adseverin and gelsolin at later stages of mouse development. Western blot analysis of tissue extracts from 3 week old (A) and 4 month old (B) mice. (C) Western blot analysis of differential expression of adseverin in adult mouse kidney. 50 μg of total protein extract were loaded per lane except for the papilla lane in which 10 μg protein were loaded. For adseverin detection, Ads-1 was used.
Expression of adseverin and gelsolin in kidney and gut

Fig. 4. Expression of adseverin and gelsolin in human tissues. (A) Western blot analysis of adseverin and gelsolin expression in human fetal (21 week) tissues. (B) Western blot analysis of adseverin expression in human adult tissues. For adseverin detection, the antibody Ads-3 and for gelsolin detection, a polyclonal anti-murine gelsolin antibody (Azuma et al., 1998) was used. Note that some samples show evidence of degradation with a faint doublet in the adseverin blot.

Fig. 5. Immunolocalization of adseverin in mouse kidney. (A) Murine kidney cortex. Adseverin was expressed in peritubular cells (arrows) and collecting ducts (CD) only, G, glomerulus. Cells of the Macula Densa did not express adseverin (arrowhead). (B) Transverse section of the mouse kidney inner stripe. Adseverin was expressed in thick ascending limbs (T) and collecting ducts (asterisk). (C) Papillary collecting ducts (asterisks) did not express adseverin but a high concentration of adseverin was found in papillary thin limbs (TL). The red color is due to counterstain with Evans Blue. (D) High power micrograph of a cortical collecting duct (CD). Adseverin was expressed in principal cells (PC) but not in intercalated cells (IC). Proximal tubules (PT) did not express adseverin. Bars: 30 μm (A); 15 μm (B,C); 10 μm (D).
and highest levels in inner stripe of the medulla and papilla (note that less protein was loaded in the papilla lane) (Fig. 3C).

**Expression of adseverin and gelsolin in human tissues**

We then explored adseverin and gelsolin expression and distribution in human tissues. Using Ads-3 on human fetal (21 week) tissues, a strong signal for adseverin was found only in kidney (Fig. 4A). Dissection of the human fetal kidney indicated that adseverin expression was highest in cortex and medulla and low/absent in papilla. Very weak signals for adseverin were also detected in brain (cortex and cerebellum) and intestine but nowhere else. Western blot analysis of human adult tissues (Fig. 4B) gave similar results as the fetal tissues with strong expression in kidney and low expression in heart. No adseverin expression was seen in adrenal gland.

Gelsolin in human tissues was widely expressed (Fig. 4A), but again, as in the mouse, recognizing potential plasma gelsolin contamination of these non-perfused extracts. In human fetal kidney gelsolin was expressed most highly in the cortex with little expression in the medulla and papilla.

**Immunolocalization of adseverin and gelsolin in murine kidney**

We examined the expression of adseverin and gelsolin in kidney and intestine in greater detail by analysis of tissue sections. In the mouse the only cells that expressed adseverin in the early nephron were cells at the boundary of Bowman’s capsule and the S1 segment of the proximal tubule (Fig. 5A), known as peripolar cells. In the cortex, adseverin was also expressed in cortical thick ascending limbs and collecting ducts (data not shown and Fig. 5A). In the inner stripe of the medulla adseverin was abundantly expressed in thick ascending limbs and collecting ducts (Fig. 5B). Adseverin was also expressed in the thin descending limb of Henle, extending from the boundary of the S3 segment of the proximal tubule through the entire length of the loop (Fig. 5C). In the collecting duct of cortex, outer and inner stripe, heterogeneous adseverin expression was seen (Fig. 5D). Adseverin was highly expressed in principal cells but was not expressed in intercalated cells. Adseverin was absent from papillary collecting ducts, the ureter and blood vessels (data not shown). In all renal cells where it was expressed, adseverin was diffusely distributed (Fig. 5A-D).

In contrast, gelsolin was more widely expressed in the murine renal cortex than adseverin (Fig. 6a); it was present in glomerular endothelial cells, but not in peripolar cells (data not shown). Gelsolin was also detected in the first two segments (S1 and S2) of the proximal tubule but not in the third (S3) segment. In S1, gelsolin appeared in tiny dot structures localized to the brush border region of the cell whereas in S2 the localization was below the brush border and appeared to be more vesicle-like in location.

Distal convoluted tubule cells stained moderately for gelsolin with a diffuse localization (data not shown). These were the only renal cells expressing both gelsolin and adseverin with identical distribution. In cortical and medullary collecting ducts, gelsolin was highly expressed in intercalated cells which do not contain adseverin (Fig. 6a-c). and was localized to the apical pole in some of these cells but diffusely localized in others (Fig. 6b). Double staining for...
gelsolin/H^+\text{-}ATPase and gelsolin/AE1 (data not shown) indicated that intercalated cells with apical localized gelsolin expression were of the \( \alpha \)-subtype, while intercalated cells with diffuse gelsolin localization were \( \beta \)-type (Brown et al., 1988) In principal cells of cortical and medullary collecting ducts, gelsolin was expressed at low levels and localized at the basolateral pole of the cell, in contrast to adseverin which was highly and diffusely expressed in these cells. Gelsolin was not expressed in thin limbs (data not shown) or thick ascending limbs (Fig. 6c).

Collecting ducts in the terminal 2/3 of the papilla contain principal cells only, which expressed neither adseverin nor gelsolin (data not shown). The pseudostratified epithelium of the mouse ureter expressed a large amount of gelsolin but no adseverin (data not shown).

**Expression of adseverin and gelsolin in human kidney**

In fetal and adult human kidney the expression pattern of both adseverin and gelsolin were similar to that seen in the mouse but with significant differences.

In human fetal kidney, gelsolin was expressed in developing podocytes of the glomerulus and was uniform distributed in those cells (Fig. 7A). Gelsolin expression was

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**Fig. 7.** Immunolocalization of adseverin and gelsolin in human fetal kidney. (A) Gelsolin localization in the cortex. Podocytes of developing glomeruli (G) in the inner cortex expressed gelsolin (arrow) whereas it was not expressed in podocytes of glomeruli of the outer cortex. Gelsolin was also expressed in developing limbs of Henle (LOH) where it was localized at the apical pole of the cells. Bar, 30 \(\mu\)m. (B) In human fetal kidney medulla, adseverin was predominantly expressed in collecting ducts (CD), in which it was concentrated at the apical pole but also present at the basolateral membrane. A faint adseverin signal was also detected in some of the developing loops of Henle (LOH). Bar, 15 \(\mu\)m. (C) In the medulla, gelsolin was localized at the apical pole of collecting duct (CD) cells and developing loops of Henle (LOH). Bar, 15 \(\mu\)m.
not seen in glomeruli that were located in the outer cortex, possibly reflecting developmental differences. Gelsolin was also expressed in the thick ascending limbs and the collecting ducts (Fig. 7A,C) where it was sharply localized to the apical pole, indicating a colocalization with actin in these cells.

Adseverin expression in human fetal kidney was seen primarily in developing collecting ducts, isolated interstitial cells, and at much lower level in developing limbs of Henle (Fig. 7B, and data not shown). Adseverin was localized predominantly to the apical pole in the collecting ducts with some expression at the basolateral pole.

In the cortex of human adult kidney, adseverin was expressed only in collecting ducts (Fig. 8A). Some ductal cells expressed significantly more adseverin than others, and the high-expressing cells appear to be intercalated cells.

In human adult kidney cortex, gelsolin was not expressed in Bowman’s capsule, the glomerulus, or proximal tubule (Fig. 8B). Gelsolin was found at high levels in cortical and medullary collecting ducts with an apical localization, identical to that observed in human fetal kidney (Fig. 8B).

In the papilla of the human adult kidney, adseverin was highly expressed in collecting ducts (Fig. 8C) with a uniformly distribution. Gelsolin was expressed in collecting ducts and in thick ascending limbs (Fig. 8D), but was localized to the apical pole in cells of both structures. These observations are in contrast to mouse kidney, indicating that both proteins are expressed in human collecting duct principal and intercalated cells (compare Fig. 8 vs Figs 5 and 6).

Immunolocalization of adseverin and gelsolin in intestine

In mouse small intestine adseverin was expressed only in the upper half of the villus in the epithelial cells (enterocytes) with increasing expression toward the top of the villus (Fig. 9A,C). Adseverin was concentrated in these cells to a region just below the brush border zone (Fig. 9C), as confirmed by double staining for adseverin and F-actin (data not shown). No adseverin was found in the basement membrane, the lamina propria, and the muscle layers of the gut (Fig. 9A, and data not shown).

In goblet cells, adseverin appeared to be localized in dot-like peri-goblet structures. These first appear in goblet cells half way up the villus (Fig. 9A). Goblet cells at the tip of the villus showed an intense adseverin stain on their external surface as well as internally surrounding the goblet (data not shown).

Gelsolin was also expressed by enterocytes of mouse small intestine but at a much lower level than adseverin (Fig. 9B). Similar to adseverin, gelsolin content increased as the cell approached the top of the villus and was localized to the subbrush border zone. In contrast to adseverin numerous cells in the lamina propria also stained strongly for gelsolin (Fig. 9B). These cells appear to be either dendritic cells, macrophages or vascular cells which are all known to express high levels of gelsolin. We also observed gelsolin staining of goblet cells similar to the adseverin stain (data not shown).

Gelsolin staining of human adult small was similar to that seen in mouse (data not shown). However, in enterocytes, gelsolin was also localized to a perinuclear region that might reflect an association with the Golgi apparatus. Adseverin, on the other hand, did not appear to be expressed at any significant

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Fig. 8. Immunolocalization of adseverin and gelsolin in human adult kidney cortex (A,B) and papilla (C,D). (A) Adseverin was highly expressed in intercalated cells (arrows) of the collecting duct whereas it was expressed at a lower level in principal cells. (B) Gelsolin was concentrated at the apical membrane of collecting duct cells but was not expressed in the glomerulus, the Bowman’s capsule and the proximal tubule. The interstitial stain for gelsolin is likely due to the presence of plasma gelsolin. CD: collecting duct; G: glomerulus. (C) Adseverin was uniformly highly expressed in papillary collecting ducts (CD) and diffusely distributed in the cytoplasm of these cells. It was not expressed in thick ascending limbs (TAL). (D) Gelsolin was expressed in collecting ducts (CD) as well as in thick ascending limbs (TAL) and localized at the apical pole in both cell types. Bars, 30 µm.
level in human small intestinal columnar epithelial cells (data not shown), consistent with western blot studies (Fig. 4).

In mouse large intestine (colon) adseverin was expressed only in the lining epithelium with highest levels in the apical and basolateral poles (Fig. 10A). As in the small intestine, adseverin was present with a gradient of expression with

Fig. 9. Immunolocalization of adseverin and gelsolin in mouse small intestine. (A) In enterocytes, a gradient of adseverin expression was seen from the crypt to the tip of the villus. Adseverin was also present in goblet cells (arrow). Bar, 30 μm.

(B) Gelsolin was also present in enterocytes but its expression level was lower than that of adseverin. A number of cells in the lamina propria with a macrophage or dendritic cell like appearance expressed a high amount of gelsolin. Bar, 60 μm.

(C) A high power view shows that adseverin is localized just below the brush border in the terminal web of the cells. Bar, 10 μm.

Fig. 10. Immunolocalization of adseverin and gelsolin in adult mouse colon. (A) Adseverin was highly expressed in epithelial cells where it was predominantly localized to the apical and basal poles of the cells. A gradient of adseverin expression was seen along the villus. (B) Gelsolin was not expressed in epithelial cells but was present in the basement membrane (arrows), in the interstitium, and in some cells of the lamina propria. Bars, 30 μm.
highest levels at the crown of the villus. Gelsolin expression could not be detected in colonic epithelial cells but was seen in the basement membrane, blood vessels, and in muscle layers (Fig. 10B, and data not shown).

**DISCUSSION**

Adseverin and gelsolin are closely related (60% amino acid identity) members of a large family of proteins that regulate actin filament structure within cells in response to external stimuli. Previous studies on adseverin expression have been limited due to antibody reagent limitations (Tchakarov et al., 1990; Rodriguez del Castillo, 1992), but adseverin has been reported to be expressed most highly in adrenal medulla, and at much lower levels in a variety of other tissues (Rodriguez del Castillo et al., 1992; Tchakarov et al., 1990; Sakurai et al., 1990). The prominent expression of adseverin in kidney and intestine which we found here by immunoblotting and immunocytochemistry was not previously appreciated. In contrast, gelsolin is known (Kwitkowski et al., 1988a,b) and was shown here to be widely expressed in many organs.

The epithelium lining the nephron is a complex and highly organized specialized structure whose function varies along the pathway of urinary flow. Adseverin and gelsolin were expressed in distinct cells in the murine kidney, apart from collecting duct principal cells where both were expressed but with a distinct subcellular localization. Adseverin was not expressed in the proximal tubule where bulk reabsorption (~70%) of the tubule fluids occurs by constitutive endocytosis and paracellular transport, indicating that it is not required for these functions. Proximal tubules did contain gelsolin, but the appearance of gelsolin in vesicle-like structures and the decline in later segments of the proximal tubule suggest that this staining may be due to reabsorbed plasma gelsolin. Plasma gelsolin (concentration ~200 µg/ml) is likely to be present in the primary filtrate in small amounts, and could be expected to be reabsorbed in the proximal tubule.

Both adseverin and gelsolin were expressed in more distal parts of the nephron. In these tubules the selective reabsorption and secretion of specific urinary components (water and ions) occurs under the control of a complex set of physiological signals, and involves modulation of vesicle trafficking in some cell types (Brown and Stow, 1996). In this respect, adseverin function in the kidney could be similar to that previously suggested for the adrenal gland.

Collecting duct principal cells are involved in urine concentration by reabsorption of water, a process controlled by vasopressin, an antidiuretic peptide hormone (Brown 1989, 1991; Brown and Stow, 1996). The cellular response to vasopressin stimulation is the insertion of the water channel aquaporin2 (AQP2) into the apical plasma membrane by regulated exocytosis followed by continued AQP2 recycling. It has been previously shown that modulation of the actin cytoskeleton accompanies vasopressin action in these cells (Hays et al., 1993), and it is possible that adseverin plays a role in this process. However, it has been previously reported that gelsolin is localized in principal cells of rabbit papillary collecting duct (Hartwig et al., 1990), suggesting that while actin remodeling is important, the protein(s) involved may differ among species.

Gelsolin on the other hand was highly expressed in both A and B intercalated cells. In A-intercalated cells, it was localized in the terminal web whereas in B-intercalated cells gelsolin was diffusely distributed. One of the major activities of A-intercalated cells is the acidification of the urine by proton secretion whereas B-intercalated cells secrete bicarbonate into the tubular lumen (Brown et al., 1988; Brown and Stow, 1996). These activities are under physiological control and changes in intracellular Ca$^{2+}$ are involved in the H$^+$ secretory response (Van Adelsberg and Al-Awqati, 1986). By analogy to principal cells, vesicular recycling of H$^+$-ATPase occurs at the apical pole of A-cells, the area where gelsolin is localized. In contrast, B-intercalated cells showed a diffuse cytoplasmic localization of gelsolin. These cells can insert H$^+$-ATPase into either the apical or the basolateral pole of the cell (Brown et al., 1988). The polarity of H$^+$ ATPase in B cells may be regulated by a transcytotic mechanism and the diffuse distribution of gelsolin may reflect its role in this process. Similarly to principal and intercalated cells, cells of the thick ascending limb have an active endo-/exocytotic activity that may require actin remodeling. The role of adseverin and gelsolin in other cell types of the nephron remains unclear. Of interest is the localization of adseverin in a few cells at the neck of Bowman’s capsule. In some species, specialized secretory cells known as peritubular cells are present at this location (Ryan et al., 1979, 1982; Gall et al., 1986) but the role of these cells in mouse kidney is unknown.

Although broadly concordant, adseverin and gelsolin expression in mouse and human kidney were not in perfect agreement. For example, adseverin was expressed in mouse in the limbs of Henle through the medullary collecting duct principal cells. In humans on the other hand, adseverin was present only in collecting duct cells, and was minimal/absent in the limbs of Henle. The subcellular localization also varied. These differences are consistent with other differences in protein expression in the mouse vs. human kidney, and may reflect the distinct physiology of the two kidneys. For example, as an adaption to dehydrating conditions, the rodent kidney is capable of extreme concentration of urine, and it is possible that the adseverin expression seen more broadly in the murine kidney may be required for this concentrating process.

The simultaneous expression of gelsolin and adseverin in some cell types also raises the question of whether these proteins might be regulated by different signaling pathways. In cultured chromaffin cells which express both adseverin and gelsolin, adseverin but not gelsolin is activated when exocytosis is triggered with nicotine or K$^+$ via an unknown pathway (Vitale et al., 1991). A similar effect can be observed by stimulation of protein kinase C by the phorbol ester PMA (Vitale et al., 1992). This may be an indication that adseverin and gelsolin activity could be regulated by entirely different signaling pathways. A major advantage of differential regulation would be the possibility for selective activation or deactivation of these proteins in cells where both proteins are simultaneously expressed. Alternatively, in epithelial cells the two proteins may have identical functions.

Although we can only speculate regarding the precise function of adseverin and gelsolin in resorptive epithelial cells, the work presented here provides novel information on the expression of these two proteins in murine and human tissues that suggest function beyond those previously considered for
adseverin. They also provide a rationale for future functional studies of these proteins and in renal physiology and pathophysiology.

REFERENCES


