Developmental regulation of villin gene expression in the epithelial cell lineages of mouse digestive and urogenital tracts

ROGER MAUNOURY1, SYLVIE ROBINE2,*, ERIC PRINGAULT2, NADINE LÉONARD3, JEAN ALFRED GAILLARD2 and DANIEL LOUVRARD2

1Institut Cochin de Génétique Moléculaire, 22 rue Méchain, 75014 Paris, France
2Institut Pasteur, Département de Biologie Moléculaire, URA-CNRS 1149, 25 rue du Docteur Roux, 75724 Paris Cédex 15, France and
3Laboratoire d’Anatomie Pathologique, Hôpital Sainte-Anne, 1 rue Cubans, 75674 Paris Cédex 14, France
*Corresponding author

Summary

The expression of villin, an actin-binding protein and major structural component of the brush border of specialized absorptive cells, was studied during mouse embryogenesis. We show that the ontogeny of villin expression is limited to the epithelial cell lineages of the digestive and uro-genital tracts and accounts for the tissue-specific expression observed in adult mice. This spatiotemporal pattern of villin expression is distinctive in sequence, intensity, regional distribution and polarization. During the development of the primitive gut, villin is faintly and discontinuously expressed in the invaginating foregut but it is expressed in every cell bordering the hindgut pocket. Later, villin expression increases along the developing intestine and concentrates in the brush border of the epithelium bordering the villi. In gut derivatives, villin is present in liver and pancreas primordia but only biliary and pancreatic cells maintain a faint villin expression as observed in adults. In the urogenital tract, mesonephric tubules are the first mesodermal derived structures to express villin. This expression is maintained in the ductuli efferentes, paradidymis and epoöphoron. Villin then appears in the proximal metanephric tubules and later increases and concentrates in the brush border of the renal proximal tubular epithelial cells. Thus villin expression can be considered as an early marker of the endodermal cell lineage during the development of the digestive system. Conversely, during the development of the excretory and genital system, villin is only expressed after the mesenchyme/epithelium conversion following the appearance of tubular structures.

These observations emphasize the multiple levels of regulation of villin gene activity that occur during mouse embryogenesis and account for the strict pattern of tissue-specific expression observed in adults. In the future, regulatory elements of the villin gene may be used to target the early expression of oncogenes to the digestive and urogenital tracts of transgenic mice.

Key words: gut, endoderm differentiation, excretory system, cytoskeleton.

Introduction

Most of the tissue-specific proteins involved in highly specialized cellular structures or metabolic functions can be used as markers of cell differentiation. However, the corresponding genes are often switched on only at the onset of terminal differentiation and, consequently, their products are not produced in the immature, determined precursor cells. Concerning the digestive tract, previous studies have shown that, in contrast to intestinal hydrolases, villin, a tissue-specific cytoskeletal protein, is already expressed within the intestinal crypts in the proliferative stem cells (Robine et al., 1985; Boller et al., 1988). This protein was thus shown to be an early marker for committed intestinal cells.

Villin was first isolated from chicken intestinal brush borders (Bretscher and Weber, 1979) and later shown to be expressed in several evolutionarily distant animal species. These observations are the result of numerous investigations involving immunocytochemical (Reggio et al., 1982; Dudouet et al., 1987; Figiel et al., 1987; Moll et al., 1987) and molecular hybridization procedures (Pringault et al., 1986; Boller et al., 1988). Villin is located in the core of actin filaments supporting the microvillus membrane forming the brush border as illustrated by ultrastructural studies (Dudouet et al., 1987). The key function of villin in the assembly of the brush border has been suggested by in vitro experiments showing that villin selfassociates with pre-formed actin filaments and can sever or bundle actin filaments in a calcium-dependent manner (Glenney et al., 1981; Matsudaïra et al., 1985). In the chick embryo, villin localizes
apically and associates with the cytoskeleton of short microvilli at day 8 (Shibayama et al., 1987). Furthermore, a recent study in which villin cDNA was transfected in the CV1 cell line, which does not normally express this protein, provides evidence that villin is involved in the induction of microvillus growth, a phenomenon that is associated with the reorganization of F-actin microfilaments (Friederich et al., 1989). The elucidation of the primary structure of villin has shown that villin belongs to a family of closely related F-actin-severing proteins including gelsolin in higher eucaryotes, and fragmin and severin in lower eucaryotes (Arpin et al., 1988; Bazari et al., 1988). Villin displays a large duplicated domain common to other actin-severing proteins and a specific additional C-terminal domain, conferring the F-actin-bundling activity to the molecule (Bretscher and Weber, 1980; Glenney and Weber, 1981). Elucidation of the villin gene structure (Pringault et al., 1991) has confirmed that this protein has evolved in parallel with other severing proteins, from a common ancestor by duplication and fusion of specific domains.

The distribution of this protein in normal adult tissues has been studied in detail demonstrating that villin expression is restricted to some cell types of the digestive and urogenital tracts which perform an absorptive role and/or exhibit well-developed microvilli at their surfaces (Robine et al., 1985; Gröne et al., 1986; Osborn et al., 1988; Coudrier et al., 1988; Elsässer et al., 1991).

In neoplastic tissues, villin expression is retained in most tumors or cell lines derived from villin-positive epithelia of the digestive and excretory tracts but has never been observed in non-epithelial tumors such as sarcoma or lymphoma (Moll et al., 1987; Carboni et al., 1987; West et al., 1988; Bacchi and Gown, 1991). This pattern of villin synthesis in normal adult tissues and carcinomas has practical consequences in the differential diagnosis of human tumors. For instance, as proposed by others for some tissue-specific cytoskeletal proteins, the immunodetection of villin in tumors and metastases can guide the pathologist to the histological origin of carcinomas (Moll et al., 1987; Louvard et al., 1989). Moreover, the detection of villin in serum of patients has been proposed for the diagnosis and monitoring of colorectal cancers (Dudouet et al., 1990).

Studies of the ontogeny of villin gene expression in the developing mouse embryo should allow us to address a number of unsolved questions concerning the molecular and developmental biology of villin. For instance, how is this tissue-specific regulation taking place? When is villin first expressed during gut and kidney histogenesis? What is the sequence of villin expression in the different epithelia that produce it? How is villin regionally distributed and polarized within epithelia when they mature? Does villin expression correlate with defined developmental processes? Is there unexpected transient synthesis of this protein in embryonic tissues?

We have previously reported that, at the early postimplantation stage of mouse embryogenesis, villin is first detectable in the primitive endoderm and persists in the extraembryonic visceral endoderm of the yolk sac, until birth (Maunoury et al., 1988); this result has been confirmed by Ezzell et al., (1989).

The present study extends our investigations to the mouse embryo itself and illustrates villin synthesis during development of the gut and nephros anlagen and their derivatives. In these tracts, villin is never found in non-epithelial cells at any stage of development, a feature that correlates with the pattern of villin expression observed in the corresponding adult tissues (either normal or neoplastic). Furthermore, villin appears as an early marker of the primitive and definitive endodermal cell lineage and as a marker of cells arising after mesenchyme/epithelium conversion in the developing kidney.

**Materials and methods**

Animals, antibodies and immunocytochemical procedures used have been previously described elsewhere (Maunoury et al., 1988). In brief, embryos obtained from 129/Sv mice with their extraembryonic membranes were immediately fixed in a solution of 1% acetic acid in 95% ethanol for 1-2 days at 0°C and embedded in paraffin. The stage of embryonic development as defined by Theiler (1972) was determined in this study after examination of histological sections. Immunostaining on five micron thick sections was performed using the ABC procedure (Hsu et al., 1981) as modified by Maunoury et al., (1988) with the following additional modifications. All incubations and rinses were carried out in phosphate-buffered saline (PBS) for 15 minutes each ; the biotin-labelled secondary antibody and peroxidase-labelled streptavidin were purchased from Biogenex laboratories. The primary anti-villin antibodies were the rabbit anti-pig villin antibodies affinity purified on pig villin used in the previous work ; in addition, a polyclonal anti-mouse villin antibody, affinity purified on mouse villin, has been recently obtained in our laboratory in order to provide a homologous reagent with high affinity to mouse villin. For this purpose, using the method described by Bretscher and Weber (1980) with some modifications, the mucosa from adult mouse intestine were scraped and snap frozen in liquid nitrogen. A fraction of 2 g of mucosa was homogenized in 20 ml of buffer containing 5 mM Tris-HCl (pH 7.5), 75 mM KCl, 4 mM MgCl₂, 1 mM ethylene glycol bis N, N, N', N'-tetraacetic acid (EGTA), 2% Triton X-100, 0.2 mM adenosine 5' triphosphate (ATP) and 0.4 mM diithiothreitol (DTT) in the presence of a cocktail of protease inhibitors. After high-speed centrifugation (100,000 g for 1 hour) over a sucrose gradient, the supernatant fraction was dialyzed overnight against KTC buffer (10 mM Tris-HCl, pH 8, 20 mM KCl, 0.2 mM CaCl₂, 2.5 mM β-mercaptoethanol and 1 mM PMSF) and then applied to a Q-Sepharose chromatography column. The proteins were eluted with a linear gradient of 20-500 mM KCl and dialyzed overnight against 2 mM Tris-HCl, pH 8, 0.2 mM CaCl₂, 0.5 mM β-mercaptoethanol, 0.2 mM ATP and 1 mM PMSF. The dialyzed solution was applied to the immobilized DNAase I column prepared as described by Bretscher (1986). Mouse villin was eluted with the Ca²⁺ chelating buffer (20 mM Tris-HCl, pH 7.8, 150 mM NaCl, 5 mM EGTA and 1 mM PMSF). 100 µg of pure villin could be recovered by this method and used either to immunize rabbits as described by Louvard et al., (1982), or immobilized on activated Ultrogel beads in order to immunopurify the polyclonal antibodies obtained. The specificity of the rabbit anti-mouse villin antibodies has been controlled by immunocytochemistry on mouse adult tissues sections as well as by immunoreplica.

Negative controls were obtained by replacing the specific antisera with normal non-immune sera; no labelling was observed, indicating that the cell procedures and reagents used result in specific labelling.

Comparative controls were made with a rabbit immunopurified anti-frog muscle actin (generous gift of Dr A. M. Hill and Dr E. Karsenti) and anti-Paramecium tubulin previously described (Adoutte et al., 1985).
Results

The earliest expression of villin in the visceral endoderm during mouse embryogenesis has been previously reported (Maunoury et al., 1988) and confirmed by Ezzell et al., (1989). Briefly, villin is initially detectable in the early postimplantation stage (stage 8) in primitive endodermal cells at the periphery of the egg cylinder and persists in the extraembryonic visceral endoderm of the yolk sac until birth. Distal endoderm of the yolk sac is always deprived of villin.

Stage 11

Our present results report yet uncharacterized stages and patterns of villin expression in the embryo itself. Expression begins at the presomite stage corresponding to formation of three separate cavities: the amniotic cavity, the exocoelom and the ectoplacental cleft. In the embryonic area, a faint villin staining was visible in thin or flattened endodermal cells (Fig. 1).

Stage 12

A conspicuous characteristic of stage 12 embryos is the deepening of the anterior intestinal portal region and concomitantly the formation of definitive endoderm. At the beginning of this stage, only squamous endoderm remnant cells remain positive for villin, and stipple unstained definitive endodermal cells (Fig. 2). The specificity of this faint labelling is confirmed in the adjacent section where no staining of these cells is observed with a muscle actin antibody used as a control. This reagent clearly labels the first heart rudiment cells which appear in the mesoderm surrounding the front end of the embryo (Fig. 2, insert). Later in this stage, epicardium develops rapidly with a very strong expression of muscle actin (Fig. 3, insert). Adjacent to this cellular mass, in the invaginating foregut, villin remains in a few very flattened epithelial cells and appears in a few thick cells of definitive embryonic endoderm (Fig. 3). In the last part of stage 12, just before the turning of the embryo, villin is discontinuously expressed both in thick ventral and thin dorsal walls of the foregut (Fig. 4A, B, C).

Stage 13

This stage is characterized by the turning of the embryo. The rotation begins with the head and tail folds; the mid-trunk region remains initially in its original position, being bound to the yolk sac. In the foregut, villin staining is restricted to a few epithelial cells (Fig. 4D). In contrast, in the newly formed hindgut, villin is expressed in each thick cell lining the pocket in a pattern that delineates the apical borders of the membranes (Fig. 4E).

Stage 14

The turning of the embryo is now complete and the midgut as well as the vitellin duct are constituted (Fig. 5). Foregut and hindgut form a continuous tube. In the ring area between foregut and midgut, liver, gall bladder and pancreas anlagen emerge and express villin in continuity with the caudal hindgut, which elongates actively as far as the level of the posterior neuropore (Fig. 5A-C). In the cranial direction, only a short distance exists between the hepatic diverticulum and the lung rudiment. The stomach has not yet been formed and its presumptive area bounds the cranial limit for villin staining.

For the first time, the mesonephric duct is apparent and is in continuity with the pronephric duct. At this and later stages of embryonic development, pro- and mesonephric ducts are unlabelled for villin.

Stage 15-16

The most conspicuous change at this stage is the formation of the lung anlage; however, villin is not observed in the early lung rudiment (not illustrated) nor later when this organ develops.

Within the hepatoduodenal field, the hepatic cords which are weakly stained for villin start to invade the mesenchymal tissue of the septum transversum (Fig. 6).

Mesonephric tubules are well-formed and present a typical S-shaped pattern; no staining can yet be observed in this tissue (not illustrated).

Stage 17-18

The short oesophagus continues into the large eccentric stomach anlage. More caudally, in the region of the hepatic diverticulum, the evaginations of both the ventral pancreas and the gall bladder primordium are developing.

The mesonephros is now well vascularized. The Wolffian duct has reached the cloacal wall and the ureteric bud begins

Fig. 1. Nominal 8 day embryo, presomite development, stage 11.5. Midfrontal section through cranial end of the notochord (arrow). Two arrowheads limit the visceral extraembryonic thick endoderm (or columnar endoderm) and thin or flattened embryonic endoderm (or squamous endoderm). The tall columnar cells contain the largest amounts of villin concentrated in the apical microvilli, whereas a faint but delineated villin staining is observed in the squamous thin endodermal cells. ac, amniotic cavity; eca, ectoplacental cavity; eco, ectoplacental cone; ex, exocoelom. Bar indicates 200 µm.
to develop. Villin first appears in the mesonephric tubules (Fig. 7) which are more convoluted than at the previous stage.

**Stage 19-20**

At this stage, the gut has elongated and intestinal loops are visible. The stomach is greatly distended and regional differences are visible in its epithelium. The two rudiments of the pancreas are in contact with each other. Within the liver there are megakaryocytes indicating the hematopoietic function of this organ. In the actively developing duodenum, villin is strongly expressed in the thick pseudostratified epithelium in contrast with a weak staining in the liver (Fig. 8A). As soon as the embryo elongates caudally, the hindgut increases in length and terminates in a dilated blind end: the primitive cloaca. Villin is present in endodermal cells lining the cloaca (Fig. 8B) and persists in free cells into the cloacal cavity (Fig. 8B). At this stage, the embryo possesses portions of all three embryonic excretory systems. Mesonephric tubules are highly convoluted and are found adjacent to the gonad primordium. Villin is concentrated in the apical cytoplasm of the monolayered epithelium lining these tubules (Fig. 8C). The metanephros, still negative for villin, is well delineated and has many secondary urethric buds.

**Stage 22**

The gut projects into the wide umbilical hernial sac. All the mucosal cells lining the intestinal tube from the duodenum to the rectum stain strongly for villin (Fig. 9A,B). Although stained with a more moderate intensity, the glandular portion of the stomach marks the cranial boundary of the villin expression in the gut (Fig. 9C). Oesophageus as well as the non-glandular stomach portion are unlabelled for villin. In derivatives from the hepatopancreatic ring, villin expression remains moderate and is polarized to the apical side of the epithelia lining hepatic and pancreatic ducts of various sizes (Fig. 9A,B).

In the metanephros, villin first appears in the proximal tubule of the more mature nephrons confined in the pericenter of the organ (Fig. 9C). Villin is concentrated in the apical cytoplasm of the monolayered epithelial cells which constitute the proximal convoluted tubules. Other nephron regions such as collecting tubules and glomeruli with well-formed Bowman’s capsules are unlabelled for villin (Fig. 9D).

**Stage 23**

The configuration of the intestinal tract and of the urogenital system shows little change since stage 22, e.g. umbilical hernia is still present. However, histogenetic changes occur both in the small intestine where numerous and relatively thick villi develop, and in the large intestine where crypts are forming. Villin is intensely expressed in the proliferative epithelium of the intestinal loops as far as the rectum.

The urogenital system has become separated from the
Villin in mouse development

rectum during a preceding phase of development and villin is undetectable in epithelia from bladder, ureter or urethra (no shown).

The developing kidneys now contain centrally placed glomeruli, which are more numerous that day 14. From day 14 to 15, the mouse thymus undergoes rapid histogenetic and organogenetic changes, disclosing the structure of the adult thymus and lying close to the pericardial cavity. Each thymic lobe is divided into lobules which are as yet unsegregated into medullary and cortical zones. Faint villin-positive cells appear scattered through the entire organ (Fig. 10A). During the following days until birth, the thymus enlarges and the density of large villin-positive cells decreases, by a “dilution effect” among an increasing lymphoid cell population. Under high magnification, villin-positive cells show epithelial and non-lymphoid characteristics (Fig. 10B). Staining is never very intense but faint and concentrated in, or proximal to, an irregular cytoplasmic membrane. Often some cells are so weakly stained that photographs are unsuccessful. However, this pattern of villin staining correlates with the currently accepted endodermal origin of this epithelial cell population (Rugh, 1968). This observation will be investigated in detail in further studies.

Stage 25
The abdominal cavity has now enlarged, so that the intestinal loops can reposition since the umbilical hernia is reduced.

The small intestine now has longer, but still rather thick villi covered by columnar villin-positive epithelium (Fig. 11). In the liver, blood cell production is increasing and this organ appears to be more hematopoietic than glycogenogenic with numerous sinusoid and blood cells. The pancreas has finely branched, glandular trees, with distinct lumina, and pancreatic islets are budding. A faint villin staining is observed both in expanding exocrine glandular trees and nascent endocrine islets (data not shown).

The kidneys still have a large peripheral metanephric blastema. Near its center, many glomeruli are well developed and the topography of the corresponding proximal tubules is conspicuously defined by their selective and intense staining for villin, which is concentrated in the differentiating brush border.

At this stage, the anatomy of the mouse is essentially complete.

Stage 28: postnatal development
INTESTINE
Up to birth, intestinal cells are able to divide along the whole villus axis. After birth, the crypts are formed and they progressively compartmentalize the predifferentiated cells which actively proliferate and undergo morphological and functional maturation from the depth of the crypt to the villus tip.

Sections of small intestine from the adult mouse cut along
Fig. 5. Nominal 9 day embryo, 13-14 somites, stage 14. Turning of the embryo is complete. Midgut and vitellin duct are well formed. (A) Sagittal section through area junction precisely between foregut and midgut. The ventral endoderm in the anterior intestinal portal region projects into the loose mesenchyme of the septum transversum (st) and forms a diverticulum (arrow) which with the dorsal endoderm constitutes the hepatopancreatic ring. Pseudostratified epithelium of this region express non-polarised villin. (B) Section showing the boundary between the primitive endoderm of the vitellin duct and the definitive endoderm of the midgut (arrows). Notice the distinct villin localization which is polarized to the apex of the vitellin duct epithelial cells while newly formed midgut cells present a more diffuse staining. (C) In hindgut caudal part, the staining for villin extends as far as the level of posterior neuropore. ao, aorta; b, blood island; ca, caudal artery; h, hindgut; star, intestinal portal; m, midgut; nt, neural tube; pn, posterior neuropore; st, septum transversum. Bars : (A, B, C) 100 µm.

Fig. 6. Nominal 10 day embryo, stage 16. Sagittal paramedian section through the hepato-pancreatic ring. A slight staining for villin is observed in hepatic cords (arrows) which are sprouting from the cranial region of the hepatic diverticulum and invading the loose mesenchyme of the septum transversum (st). Contrary to the cranial portion, the caudal portion of the hepatic diverticulum (arrowhead) projects no outgrowths in surrounding mesenchyme. Villin is intensely expressed in presumptive stomach (•) and duodenum areae (d) but neither detected in lung bud. Compare with Fig. 5A and note the narrowing of the duodenal portion. Bar : 100 µm.

Fig. 7. Nominal 11 day embryo, stage 18. Section through four nephric units (arrows) in mesonephros showing tubules light-stained for villin. a, aorta; vcp, vein cardinalis posterior; c, coelom. Bar : 100 µm.
Villin in mouse development

the crypt/villus axis show a labelling of epithelial cells but not of the villus core. In the immature cells of the crypts a diffuse labelling was seen within the cytoplasm. In cells maturing along the villi, villin concentrates in the brush border but is absent from mucin granules which appear as clear vesicles in the apex of epithelial cells (not illustrated).

LIVER
The most prominent change during prenatal development of the liver is an increase in volume of the fetal organ, both in number and volume of hepatocytes and in the decrease of the hematopoietic cell compartment. During postnatal weeks, the hepatic cords disappear and the hepatic parenchyma becomes progressively lobulated.

In adult mouse liver, the intrahepatic and extrahepatic bile ducts, and the gall bladder display a faint villin expression in their epithelia, but bile canaliculi were never labelled (not shown).

However, when we used villin antibodies under precisely the same experimental conditions on sections of liver obtained from adult pig, we have observed a clear delineation of bile canaliculi as described by Bacchi et al., (1991) in human normal liver.

PANCREAS
The pancreatic lumen becomes obvious at day 14 and expands progressively by days 15-16. During the following prenatal days, however, the luminal spaces diminish and at birth, as in the adult, the pancreas possesses acini which have a small lumen. In adult mouse pancreas, only occasional acinar cells are weakly labelled and even when strongly concentrated antibodies are used, villin labelling remains uncertain both for exocrine and endocrine cells. This is in contrast to positive cells which line the large collecting ducts (not shown). Those features are reminiscent of the observation reported in human adult pancreas by Elsässer et al., (1991).

METANEPHROS
The renal blastema remains functional during the first two postnatal weeks. During this period, the kidney increases in size and doubles its volume. At 1 month, the kidney attains the complete maturity of the adult animal. Villin is selectively expressed in the proximal convoluted tubules localized in the cortex (Fig. 12A). Under high magnification, the now fully differentiated brush border is associated with a typical strong staining for villin concentrated at the apical pole of the cells (Fig. 12B).

MESONEPHROS
Some mesonephric tubules persist and become part of the duct system of the testis or remain as vestigial structures. Epigenital tubules form the efferent ductules of the testis or the epoophoron in the mesovarium. The paragenital tubules persist as two vestigial structures : the paradidymis and the paraoöphoron, respectively in male and female.

In male mesonephric derived structures, we have observed the villin staining in ductuli efferentes as previously reported (Robine et al., 1985; Horvat et al., 1990) (not illustrated). Furthermore, villin is also present both in apical and basolateral membranes of epithelial cells lining the irregular cavities of the paradidymis (Fig. 12C).

In sections through the mesovarium, the vestigial mesonephros, which constitutes the epoophoron, appears as dispersed villin-positive tubules surrounded by fibrous connective tissue (Fig. 12D). Phase-contrast microscopy or antibodies to Paramecium tubulin show epithelial ciliated cells intermingled with villin-positive cells. The entire oviduct and the uterine epithelium cells did not react with the antibodies to villin (not shown).
adult mice with regressing thymus were not tested.

With increasing age, the thymic lobes increase in size and cellularity. Trabeculae penetrate deeper into the lobes and divide the organ into lobules and the medulla become more branched and extended.

In postnatal and young adult mice, large villin-positive cells are localized in thymic medulla and show the same staining observed in neonatal stage as illustrated in Fig. 10B. The same results were obtained in the young adult rat. Old adult mice with regressing thymus were not tested.

**Discussion**

From the first steps of mouse development to the term of gestation, we report here villin gene expression in epithelial cell lineages of the digestive and urogenital tracts. The pattern of villin expression is characteristic in sequence, intensity, regional distribution and polarization, and accounts for the tissue-specific expression previously reported in mammalian adult tissues (Robine et al., 1985).

Concerning early ontogenesis, our previous study (Maunoury et al., 1988) has demonstrated that villin is first detected at the early postimplantation stage where it is restricted to the visceral endodermal cells at the periphery of the egg cylinder. In this extraembryonic differentiated tissue, villin is located at the apex of the cells. The present report extends this study to the embryo proper. As soon as the foregut and hindgut invaginate by day 8-9 of gestation, villin is expressed in definitive endodermal cells. Each cell from the hindgut displays a faint and diffuse labelling. In contrast, only a few epithelial cells of the foregut present faint villin staining. This might explain the contradictory result obtained by Ezzell et al. (1989) showing the absence of villin in the developing hindgut and foregut) is characteristic of the cell differentiation stage. This difference allows us to distinguish the primitive from the definitive endoderm. Immediately after complete turning of the embryo, a sharp gradient of villin expression is established along the primitive continuous tube formed by the foregut, midgut and hindgut. As the hepatic diverticulum emerges by 9 days of gestation, the hepatic endodermal cells maintain this villin expression. A similar observation has been found in the pancreas and gall bladder anlagen.

![Fig. 10.](image-url)
During liver formation, a moderate level of villin expression is maintained in cells engaged in duct differentiation of the biliary tree. In contrast to that observed in the hepatic cords, villin appears polarized to the apical pole of the epithelial cells forming the intrahepatic and extrahepatic ductal structures. In cells differentiating into hepatocytes, moderate villin expression persists only as long as hepatic cord structures exist, i.e., until the hematopoietic cell compartment decreases in neonatal life. Villin is undetectable in adult mouse parenchymal hepatocyte. Thus, a differential regulation of villin gene expression arises in these two hepatic epithelia which derive from common precursor cells. A similar observation was found in the developing pancreas: villin gene expression is down-regulated in acinar cells but continues to be active in duct cells.

Multiple levels of villin gene regulation are thus implicated in the histogenesis of the digestive tract: (i) the switch on from the outset of primitive and definitive endodermal cells, (ii) the silencing in the upper part of the primitive gut and during terminal differentiation of liver parenchymal cells and acinar cells of the exocrine pancreas, (iii) the enhancing of expression in differentiated enterocytes. This highlights similar mechanisms of regulation involved in the genes encoding for villin and alpha-feto-protein. For instance, the latter is also expressed in visceral endoderm, fetal gut and fetal liver but restricted in adults to enteroendocrine cells (Tyner et al., 1990). A functional and structural study of the villin promoter is currently underway. The comparison of regulatory elements from genes expressed in the digestive tract such as the human intestinal alkaline phosphatase gene (Millan, 1987; Henthorn et al., 1988), the rat L-pyruvate kinase gene (Cognet et al., 1987), the human apolipoprotein A4 gene (Elshourbagy et al., 1987), the pig neutral amineopeptidase gene (Olsen et al., 1989) and the human fatty acid-binding protein gene (Sweetser et al., 1987) should be useful for understanding the molecular genetic control of the development of the gastrointestinal tract.

In contrast, during urogenital organogenesis, a very different pattern of villin gene regulation is displayed. Villin is not detected in the first stages of nephrogenesis leading to pronephric, mesonephric and metanephric ducts. Villin first appears in mesonephric tubules at stage 17-18. In adults, moderate villin expression has been observed in the ductuli efferentes, paradidymis and epoöphoron, which derive from some persisting mesonephric tubules. The developing metanephros remains negative for villin until stage 22, where it first appears restricted to only the epithelial cells of the proximal tubules from the more mature nephrons. These cells are already engaged in their terminal differentiation and villin is at once concentrated in the apical cytoplasm. Thus, villin is a specific marker of the proximal tubules in the developing nephron.

Our data on the distribution of villin at the apical faces of transporting epithelia show that a high level of villin expression is associated with cells presenting a well-organized brush border while a low level of villin expression is observed in cells that do not display such subcellular structure. In this respect, it is worth recalling the morphogenetic effect of villin when expressed in large amounts inducing the growth of rudimentary microvilli found at the cell surface of transfected fibroblasts (Friederich et al., 1989). This change
of plasma membrane organization is also associated with the recruitment of actin from other microfilament structures, a property that demonstrates the key role of villin in the modulation of actin assembly. The morphogenetic activity of villin also seems to occur during development and account in part for the brush border assembly.

Fig. 12. Villin localization in tissues of the adult mouse. (A-B) Kidney. (A) In a longitudinal section of the entire organ, a strong villin staining delineates the kidney cortex (c). Kidney medulla (m), papilla (p), fat and vessels of the hilus (h) as adrenal gland (a) were negative. (B) High magnification of the same preparation as A. Villin is concentrated in the dense brush border of the proximal convoluted tubules. In the center of the picture, a section through a renal corpuscle shows the urinary pole (arrowhead) with villin-positive prismatic cells in continuity with those of the corresponding proximal tubule. On the opposite side (arrow), the vascular pole elements are negative for villin. (C-D) Evidence of villin in two vestigial mesonephric structures in male and female adult mouse: the paradidymis and the epoophoron. (C) The entire paradidymis appears as a small structure of about 0.2 mm in diameter, surrounded by fibrous connective tissue (ct). In the center, irregular communicating cavities are delimited by a continuous epithelium uniformly stained while the adjacent epididymis duct wall (top right) is negative. (D) Mesonephric remnants so-called the epoophoron persist as dispersed tubular structures surrounded by fibrous connective tissue. Villin immunoreactivity is seen in epithelial cells lining the tubules. Phase contrast (insert) shows that the epithelial ciliated cells (arrows) were negative for villin. Bars: (A) 1 mm, (B, C, D) 50 µm.
A general view of villin gene expression during ontogeny of the mouse is given in Fig. 13. This profile corresponds to that already observed in adult human and has been shown to be maintained during carcinogenic processes (Moll et al., 1987; Carboni et al., 1987; West et al., 1988; Bacchi and Gown, 1991). Only tumors and cell lines derived from villin-positive epithelia express this protein. However, villin has been occasionally detected in tumors derived from tissues or organs devoid of villin in their normal adult state, e.g. endometrium, ovary and lung. As the present study shows that the lung anlage, developing oviducts or endometrium tissue are villin-negative, the unexpected expression in these few adenocarcinomas cannot be explained by an occasional reexpression of a fetal phenotype. However, during human embryogenesis, the time course of development and ontogeny of villin expression could present some divergences with those of the mouse.

Previous studies have suggested that the regulation of the villin synthesis was controlled at the transcriptional level in embryonic primitive and definitive endoderm, and in adult normal and neoplastic tissues (Pringault et al., 1986; Boller et al., 1988; Maunoury et al., 1988).

In consequence, regulatory elements of the villin gene might be used to drive the expression of heterologous genes in immature cells of the definitive endodermal lineage, in the proliferative precursors of the adult intestinal crypts as well as in differentiated cells from the small and large intestine mucosa (Pringault, 1990). Murine models reproducing several steps of the progression of human colorectal cancers could be obtained by targeting the associated oncogenes or mutated tumor suppressor genes to colonocytes. Moreover, new cell lines derived from the digestive tract could be established by targeting the SV40 T antigen to the precursors of intestinal cells mucosa as well as to the biliary cells of the liver and duct cells of the exocrine pancreas.

In this respect, knowledge of the complete profile of villin expression during mouse embryogenesis will help to interpret the transgene expression as well as the occurrence of tumors using mouse models.

We thank Mme J. Regère and Mr J. C. Benichou for the photographic work. We thank Dr R. Kelly for critically reading the manuscript. This study was supported by grants from the Institut National de la Santé et de la Recherche médicale (no. 86-7008), the Association pour la Recherche sur le Cancer (no. 6379), the Ligue Nationale Française contre le Cancer, and the Fondation pour la Recherche Médicale.

References


human carcinomas : A study employing paraffin-embedded tissue. Lab. Invest. 64. 418-424.


(Accepted 14 April 1992.)