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Sequential Expression and Differential Localization of I-, L-, and T-Fimbrin During Differentiation of the Mouse Intestine and Yolk Sac

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ABSTRACT **During the differentiation of the intestine epithelium, three cytoskeletal proteins, villin, fimbrin, and myosin I, are sequentially expressed and localized to the apical membrane. Recently, we found that in the adult mouse and human, three fimbrin isoforms are expressed in a cell specific manner. I-fimbrin is expressed by intestine and kidney epithelial cells, L-fimbrin is expressed by leukocytes and many tumors, while T-fimbrin is expressed by various cells and tissues. Because non-intestinal isoforms of fimbrin could be expressed early in development, the expression of fimbrin isoforms during days 10.5 to 16.5 of intestine development was investigated. By immunofluorescence microscopy, T-fimbrin was detected in the early stages of intestinal epithelial cell differentiation until day 14.5 and was localized predominantly at the apical surface. L-fimbrin was also detected during this period but it was localized to the basal surface of the epithelium instead of the apical surface. By day 16.5 no L or T-fimbrin was detected in the epithelium. I-fimbrin was first detected at day 14.5 and a brush border-like apical localization pattern was seen by day 16.5. Unlike the intestinal cells, the visceral endoderm expressed I, L, and T-fimbrin throughout the period examined, with the level of I-fimbrin increasing as time progresses. L-fimbrin was more evident at the earlier stage than at the later stage of the development. Collectively, these results suggest that three fimbrin isoforms play different roles during epithelial cell differentiation. T- and I-fimbrin expression could be critical for the formation and extension of the microvilli whereas L-fimbrin may play a role in controlling cell adhesion.** © 1995 Wiley-Liss, Inc.

Key words: Fimbrin, Plastin, Intestinal epithelium, Visceral endoderm, Microvilli, Adhesion, Actin, Development, Embryogenesis

INTRODUCTION

Assembly of the brush border is characterized by the appearance of a dense carpet of cell surface microvilli, microvillar elongation, and the formation of the terminal web. These changes at the cell surface involve cytoskeletal proteins and previous studies have documented the roles of villin, fimbrin, and brush border (BB) myosin I in brush border assembly (see reviews by Louvard et al., 1992; Heintzelman and Mooseker, 1992). All three proteins display the same behavior during development. Initially, each protein is diffusely localized in the cytoplasm and after a several day lag, the protein becomes localized to the apical surface. Apical localization of villin precedes fimbrin and BB myosin I and is correlated with the appearance of primitive microvilli. BB myosin I localization occurs late in brush border assembly, after microvilli have assembled. Its appearance at the cell surface coincides with the formation of the terminal web which underlies the brush border microvilli. The timing suggest BB myosin I is associated with a final maturation step, perhaps associated with the transport of membranes to the cell surface and related to the membrane-microfilament linkages (Matsudaira and Burgess, 1979). Fimbrin becomes localized at the apical surface of the cell when the density of microvilli covering the surface of the cell increases. This correlation with appearance of microvilli is one piece of evidence that fimbrin controls a critical step in the assembly of the brush border.

The brush border cytoskeleton is a paradigm for understanding how three cytoskeletal proteins, villin, fimbrin, and BB myosin I, organize actin into a bundle and link the bundle to the membrane, but several studies suggest the brush border cytoskeleton is composed of special isoforms of common cytoskeletal protein. For example, a unique actin isoform is found in the brush border (Sawtell et al., 1988) and not other non-intestinal cells. In addition villin is a gelsolin-like protein which bundles actin filaments while one isoform of the BB myosin I family is found only in the brush border of the gut, kidney proximal tubules, and embryonic visceral yolk sac (reviewed in Louvard et al., 1992). In the terminal web of chicken enterocytes, the rootlets of the

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brush border microvilli are cross-linked by a terminal web specific spectrin isoform TW240/260 (Glenney et al., 1983). The presence of brush border-specific isoforms suggests the brush border cytoskeleton is highly evolved for a specialized function. In addition it predicts that during the differentiation of the epithelial cell, like during myogenesis, generally found cytoskeletal proteins will be replaced with brush border-specific isoforms.

Brush border fimbrin plays a major role in the assembly of the brush border microvillus. As an actin cross-linking protein it organizes actin filaments into a bundle which together with villin and BB myosin I supports the finger-like shape of the microvillus. Initially, fimbrin was shown by immunofluorescence microscopy to be present in various non-intestinal cells and cell structures including auditory hair cell stereocilia, podosomes of macrophages, microvilli on the surface of virus-infected cells, and the ruffling membrane and adhesion plaques of cultured cells. Now we know of three fimbrin genes (Lin et al., 1993, 1994) which are expressed in different cells and tissues. Intestinal epithelial cells express a unique fimbrin isoform, I-fimbrin. Most other cells and tissues such as brain, muscle, and skin express T-fimbrin. In cultured cells, T-fimbrin is found at several locations: in ruffling membrane, at focal adhesions, and at tips of microspikes. This pattern of localization suggests T-fimbrin may play a role in membrane ruffling and cell adhesion. In addition to I and T-fimbrin, mouse and human cells express L-fimbrin, an isoform expressed in leukocytes and most transformed cells. Immunofluorescence localizes L-fimbrin with actin filament bundles at the podosome region on the ventral surface of macrophages (Marchisio et al., 1987; Messier et al., 1993). Interestingly, in macrophages, L-fimbrin is phosphorylated at serine residue(s) upon IL-1 and phorbol myristate acetate (PMA) activation but the functional significance of phosphorylation is not known.

Our previous observations using immunofluorescence microscopy showed that microvillar formation in the mouse intestinal epithelium is correlated with the apical localization of fimbrin (Ezzell et al., 1989). Villin expression does not appear to be the critical determinant for the assembly of the brush border because villin becomes apical 2 days prior to the appearance of microvilli. Furthermore, the apical localization of fimbrin at day 10.5 in the visceral endoderm correlates with the appearance of straight microvilli and the organized microfilament bundles visible at this stage. Prior to this, the visceral endoderm is covered by long bulbous projections that are loosely structured when only villin is apically concentrated (Ezzell et al., 1989). However, the fimbrin antibody used in that study cross-reacted with all three isoforms.

Using immunofluorescence microscopy, we have examined various stages of mouse embryos and have discovered not only that all three fimbrin isoforms are differentially expressed, but they localize at opposite surfaces of the intestinal epithelial cells during the morphogenesis of the intestinal epithelium and the visceral endoderm. Whereas T- and I-fimbrin isoforms are at the apical microvilli, L-fimbrin is localized at the basal membrane. Furthermore, our results show that I-fimbrin expression correlates well with the microvillar formation and extension, supporting the role of I-fimbrin as a major determinant in the assembly of the brush border.

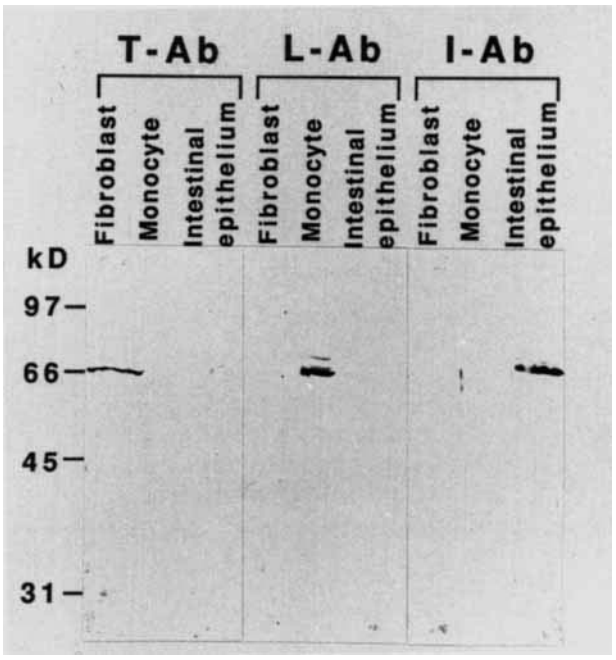

Fig. 1. Specificity of human fimbrin antibodies against mouse fimbrin isoforms. Immunoblots of mouse fibroblasts (**left lanes**), monocytes (**middle lanes**), and intestinal epithelium (**right lanes**) were probed with antibodies specific for the N-terminus of human T-fimbrin (**left panel**), L-fimbrin (**middle panel**), and I-fimbrin (**right panel**). The blots showed that T-fimbrin antibodies recognize a 66,000 MW band only in fibroblasts while L-fimbrin and I-fimbrin antibodies recognized an identically sized band in monocytes and intestinal epithelia, respectively. The nature of the larger MW immunogen reactive with the L-fimbrin antiserum is unknown but could be explained by a post-translational modification, such as phosphorylation.

RESULTS

Specificity of Antibodies to Isoforms of Mouse Fimbrin

Because our rabbit polyclonal antibodies were raised against peptides specific for each human fimbrin isoform, we first established that the antibodies recognized the appropriate mouse fimbrin isoform. Based on Northern blot analysis, mouse monocytes express L-fimbrin, fibroblasts express T-fimbrin, and the intestine expresses I-fimbrin (Lin et al., 1993). On immunoblots of these cells (Fig. 1), the human T-fimbrin antiserum (left panel) detected a polypeptide of the correct molecular weight in lysates of mouse embryonic fibroblasts (left lane) but not in mouse J774 monocytes (middle lane) or adult mouse intestinal epithe-

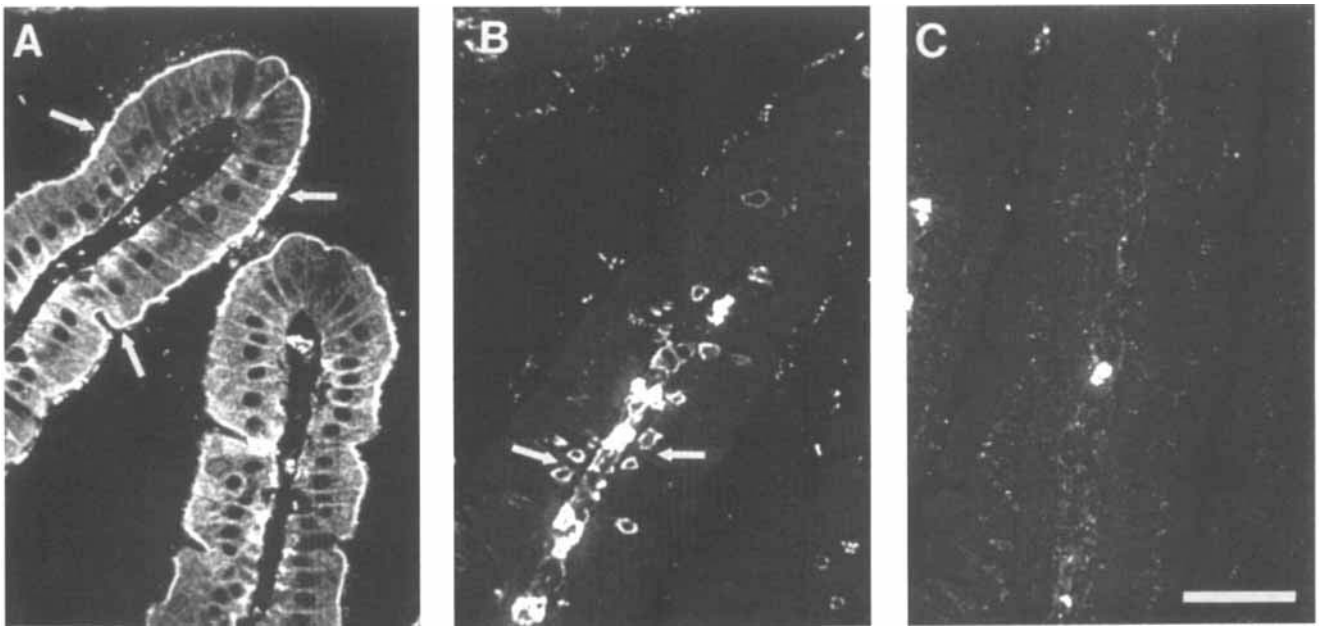

Fig. 2. Localization of I-fimbrin (**A**), L-fimbrin (**B**), and T-fimbrin (**C**) in adult mouse duodenum. I-fimbrin is localized primarily in the microvilli and terminal web in the brush border (arrows in A). Staining is also seen along the basolateral membranes. Small circular outlines of L-fimbrin are detected in some cells, presumably leukocytes, located in the lamina propria and epithelium (arrows in B). No staining of the duodenum was seen in sections that were incubated with human T-fimbrin antibodies (C). The fluorescent patches originate from auto-fluorescent areas of the tissue. Bar = 50 μm.

lial cells (right lane). The human L-fimbrin antiserum (middle panel) cross-reacted a 66,000 MW band in the J774 cell lysate but not in fibroblasts and intestine. Lastly, a 66,000 MW polypeptide in the intestinal cell lysate but not in fibroblasts and monocytes was detected with the human I-fimbrin antiserum (right panel). These results demonstrated that the antibodies against the human fimbrin isoforms cross-reacted with a mouse protein of the expected size and in the appropriate cell type. Based on this criterion, we concluded that the antisera were specific for the corresponding mouse fimbrin homolog.

Localization of Fimbrin Isoforms in Adult Intestinal Epithelium

To confirm the immunoblot analysis we examined the pattern of expression of the three fimbrins in sections of the adult mouse duodenum. Immunofluorescence microscopy showed I-fimbrin was localized only in the epithelium and not to the underlying lamina propria. Within the epithelium, I-fimbrin staining was confined primarily to the brush border and the lateral membranes. Weak diffuse cytoplasmic staining was also detected (Fig. 2A). In contrast, antibodies against L-fimbrin reacted with sparsely distributed cells having a circular profile and located in the lamina propria and epithelium. Based on their few numbers and distribution, these cells are probably leukocytes. No L-fimbrin was detected in the epithelium (Fig. 2B). In contrast to I- and L-fimbrin, the intensity of T-fimbrin staining in the epithelium and lamina propria was diffuse and very weak (Fig. 2C), at a level comparable to that seen in sections incubated with preimmune staining (not shown).

Expression of Fimbrin Isoforms in Embryonic Intestinal Epithelium

Using the fimbrin isoform-specific antisera, we studied the distribution of I-, L-, and T-fimbrin in frozen sections of 10.5 to 16.5 day embryos. Based on previous studies, this period of development includes the stages in which the gut is lined by a pseudo-stratified epithelium (10.5 day) which then develops into polarized epithelium possessing short microvilli (12.5 day), and later into a polarized epithelium possessing longer but immature microvilli (16.5 day) (Maunoury et al., 1988; Ezzell et al., 1989).

In sections of the embryonic intestine, T-fimbrin was expressed in both the epithelium and lamina propria at day 10.5 (Fig. 3A). The protein was diffusely localized in the cytoplasm but the intensity of staining was slightly higher in the apical region of the cells. The basolateral membranes of the epithelial cells were also weakly stained. By days 12.5 and 14.5, T-fimbrin was still detected but the fluorescence intensity was weaker than at the earlier stages (Fig. 3B,C). By day 16.5 (Fig. 3D), the overall intensity of the staining was reduced to background levels of fluorescence.

In comparison, L-fimbrin demonstrated a strikingly different pattern of localization throughout develop-

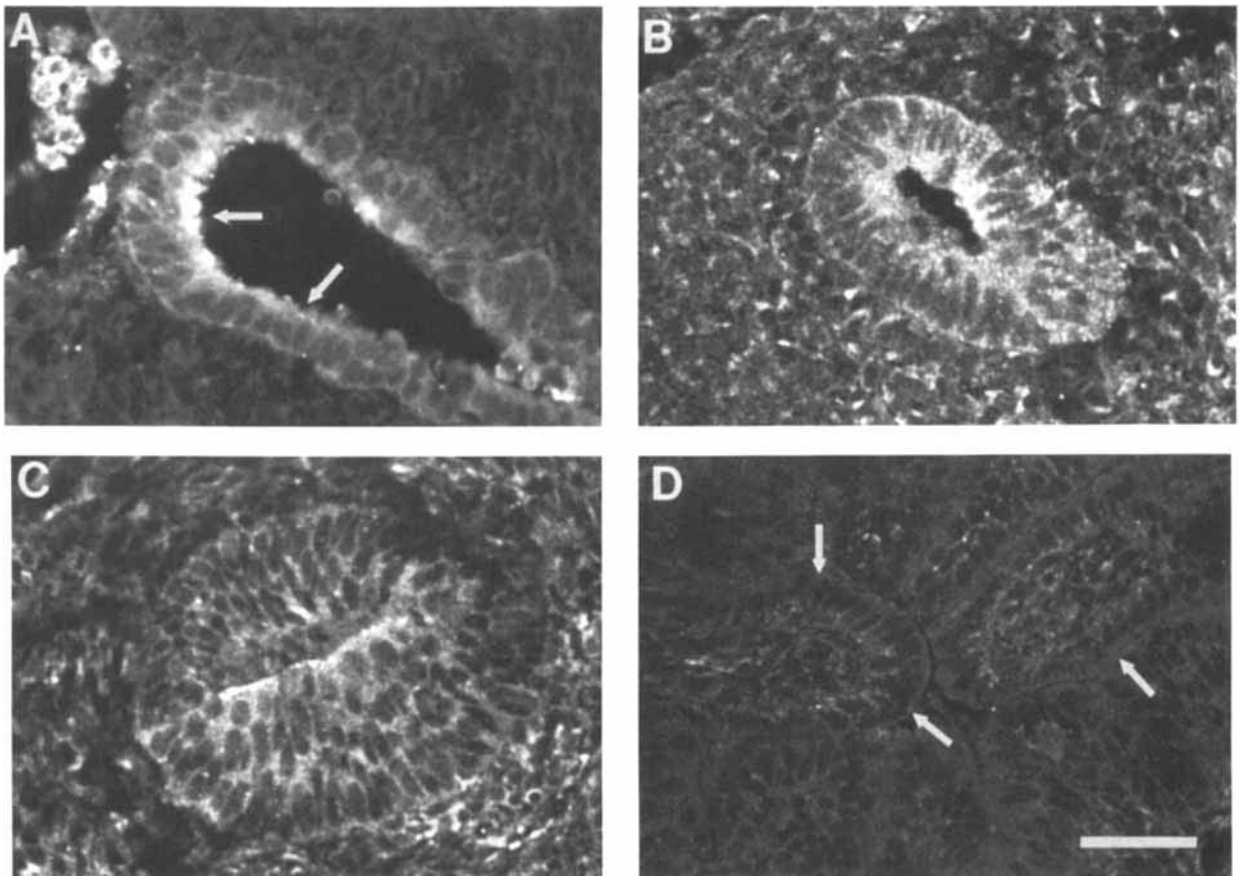

Fig. 3. T-fimbrin localization in developing mouse intestines. In frozen sections through 10.5 (**A**), 12.5 (**B**), 14.5 (**C**), and 16.5 (**D**) day mouse embryos, T-fimbrin-specific staining is detected in the epithelium and the surrounding mesenchyme between days 10.5 and 14.5. Although some staining is concentrated at the lumenal surface (arrows in A), most of the fluorescence is diffuse throughout the cytoplasm. By 16.5 days of development, no T-fimbrin was detected at the apical surface (arrows in D) in the developing intestine. Bar = 50 μm.

ment (Fig. 4). At day 10.5, L-fimbrin was localized, not at the apical or lateral membranes, but instead at the base of the epithelium where it contacts the surrounding mesenchyme. This gives rise to a ring-like staining pattern which traces the epithelial-mesenchymal boundary and is seen in cross sections through the gut as a circle of staining. The same pattern was also seen in day 12.5 and 14.5 embryos. However, the intensity level of fluorescence at the basal membrane was reduced by day 16.5 and was no longer present in the adult. At day 16.5, the L-fimbrin staining reveals the involution of the epithelium as villi begin to form. In 14.5 day embryos stained with antibodies to L-fimbrin and fluorescent phalloidin, a probe for f-actin (Fig. 5), the circular profile of L-fimbrin colocalizes with f-actin at the basal surface of the epithelium. In these cells, the most intense actin-specific fluorescence is localized to the apical surface where no L-fimbrin is detected. The outer ring of actin staining is from smooth muscle in the developing muscularis layer.

I-fimbrin displays a different pattern of expression from L- or T-fimbrin. In the 10.5 day embryonic intestine, I-fimbrin (Fig. 6A) was not detected. By day 12.5 (Fig. 6B) I-fimbrin-specific staining was weak and diffuse in the apical cytoplasm. At day 14.5 (Fig. 6C) I-fimbrin expression increased dramatically. There was strong staining localized to the apical region and less intense staining at the basolateral membranes. Two days later, at the onset of microvillar growth at day 16.5 (Fig. 6D), I-fimbrin displayed the characteristic microvillus staining pattern seen in the adult intestine.

Expression of Fimbrin Isoforms in the Yolk Sac

Because the yolk sac of the embryo is the major absorptive organ, we examined whether the pattern of fimbrin expression between days 10.5 and 16.5 was similar to that in developing intestine. During this time course, the endoderm possesses a brush border whose microvilli shorten in length as development progresses (Ezzell et al., 1989). Throughout these developmental stages, immunofluorescence studies detected high levels of staining for both T- and I-fimbrin (Figs. 7 and 8). In the 10.5 day embryo, the staining

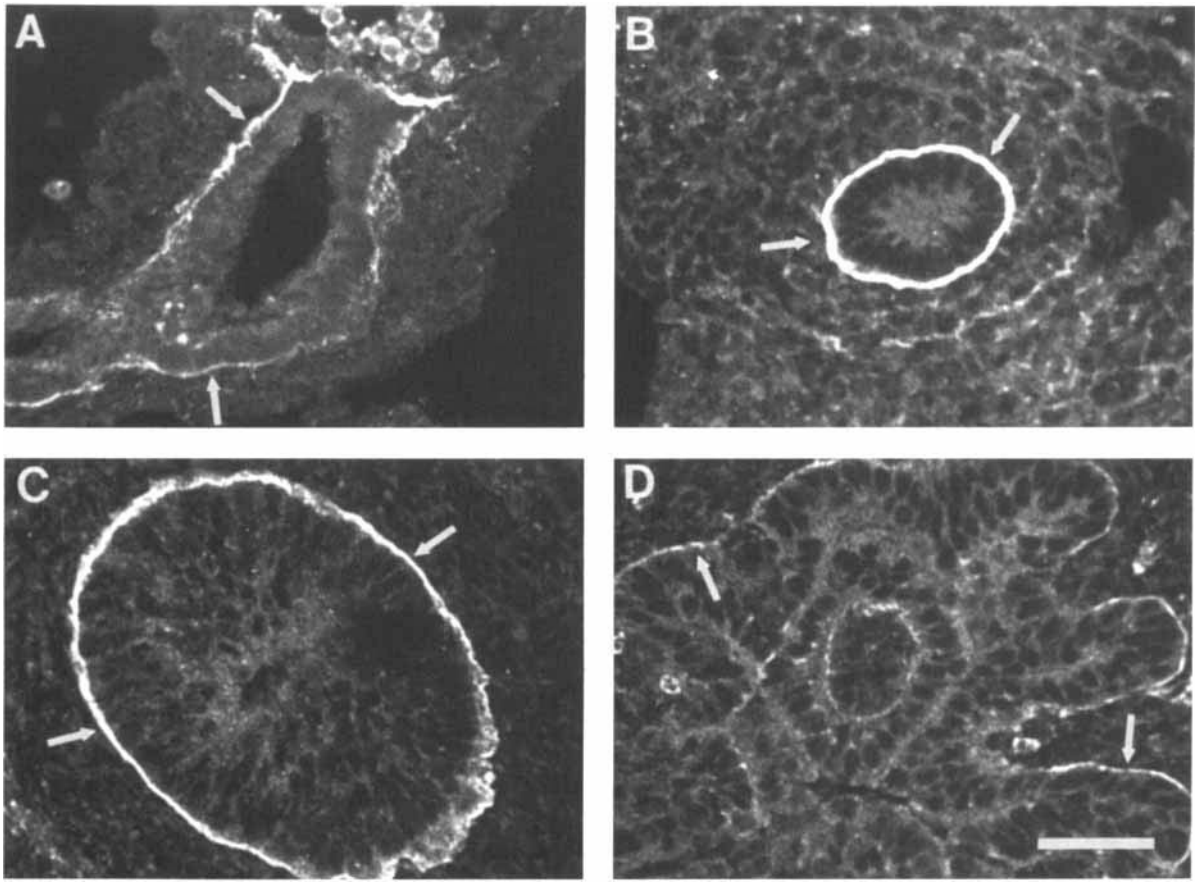

Fig. 4. Localization of L-fimbrin in developing mouse intestines. L-fimbrin was localized along the basal surface (arrows) of the intestinal epithelium at days 10.5 (**A**), 12.5 (**B**), 14.5 (**C**), and 16.5 (**D**) of development. The intensity of staining remained high during this period and becomes reduced at day 16.5. This pattern of localization outlines the basal perimeter of the epithelium. As a result in early development, the epithelium has a circular profile whereas later in development, L-fimbrin traces an involuted circumference because the epithelium folds to generate villi. Bar = 50 μm.

was diffuse for T-fimbrin (Fig. 7A) while I-fimbrin staining outlines the apical and basolateral membranes (Fig. 8A). At days 12.5 and 16.5, T- and I-fimbrin were localized to the apical surface (Fig. 7B–D, 8B–D). During this period, immunoelectron microscopy detected I-fimbrin but not T-fimbrin in the microvilli (data not shown). A major difference between I- and T-fimbrin, is that I-fimbrin was detected only in the visceral endoderm while T-fimbrin was detected in the visceral endoderm and the extra-embryonic mesoderm.

L-fimbrin was also detected in the visceral endoderm and extra-embryonic mesoderm between days 8.5 and 16.5 (Fig. 9). The staining was mainly at the apical cytoplasm.

DISCUSSION

During differentiation of the intestine, the epithelium undergoes a transition from a simple epithelium whose surface is sparsely covered by short, irregular microvilli to a mature epithelium covered by the densely packed microvilli of the brush border. This morphological change is essential for the highly efficient absorptive function of the intestine. Our results provide a more detailed description of the role of one cytoskeletal protein, fimbrin, during this process. Building on previous studies, we now show three different fimbrin isoforms are sequentially expressed by intestine epithelial cells during the major stages of gut development. Because each fimbrin isoform is localized to a different part of the same cell, our observations suggest that each isoform carries out a unique function during the differentiation of the epithelium. As summarized in the timeline shown in Figure 10, the temporal sequence of expression and the intracellular distribution of the fimbrin isoforms are correlated with major changes in the morphology of the epithelium.

Our studies have defined two distinct phases in intestine development. The early stage is characterized by the expression of L- and T-fimbrin in the pseudo stratified or simple cuboidal epithelium. These cells lack the characteristic polarized cell morphology and the plasma membrane at their apical or lumenal surface is covered sparsely with a few irregular microvilli. The second phase in development heralds the assembly

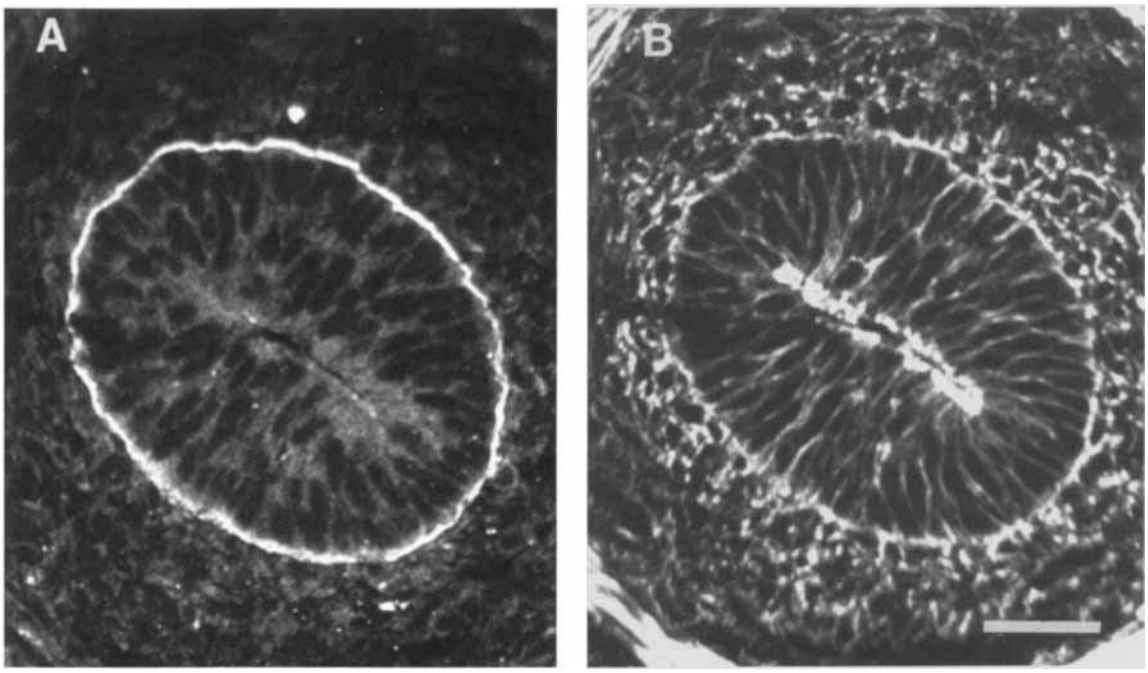

Fig. 5. Co-localization of L-fimbrin and actin in 14.5 day intestine. The circular staining pattern of L-fimbrin (**A**) follows the localization of actin (**B**) at the basal surface of the epithelium. In addition, the fluorescent phalloidin stains actin along the apical and lateral surfaces of the epithelium, the mesenchyme, and the muscle layer. Similar distribution of actin and fimbrin was detected at days 12.5 and 16.5 (not shown). Bar = 50 μm.

of the brush border. The cells become polarized with an increase in number of cell surface microvilli. During this period, new cytoskeletal proteins are expressed, first villin and then followed by I-fimbrin and myosin I. It is unknown whether these microvilli represent a new population assembled de novo or are elongated versions of the microvilli that covered the surface of the primitive epithelial cells. Thus, the battery of cytoskeletal proteins expressed early during differentiation of the intestine is replaced with a new set of intestine-specific cytoskeletal proteins.

I-Fimbrin and Brush Border Assembly

I-fimbrin becomes localized to the apical surface by day 16.5. This stage of intestine differentiation is particularly significant because microvilli first appear in large numbers on the surface of the cell. Prior to this stage, a few microvilli are found even though villin has become localized to the apical region of the cell. The coincidence in the appearance of I-fimbrin and microvilli at the apical surface of the cell suggests that I-fimbrin plays a key role in controlling the assembly of the brush border.

Various models propose that assembly of an actin bundle, held together by villin and fimbrin crosslinks, is a critical early step in brush border formation. An important question concerns the roles of villin and fimbrin in the assembly of cell surface microvilli. Separately, each protein crosslinks actin filaments into a bundle in vitro. Although fimbrin localization is correlated with microvillar assembly in the gut, other studies suggested that villin expression determines microvillar formation. Induction of villin expression in CV-1 cells (Friederich et al., 1989; Franck et al., 1990) caused the elongation of cell surface microvilli. However, these cells, like the early embryonic intestine, also express T-fimbrin and, therefore, the effects of exogenous villin expression could represent the early stages in epithelial cell differentiation. We propose that later in development, I-fimbrin expression triggers the assembly of brush border microvilli.

Localization of L-Fimbrin at the Cell-Matrix Interface

One of the most striking observations in this study is the basal localization of L-fimbrin in epithelia. These epithelial cells also contain I- and T-fimbrin but these proteins are localized to the apical surface. Therefore, there is some mechanism which targets L-fimbrin to the basal surface instead of the apical surface. Three possibilities can be proposed. First, L-fimbrin is functionally different and instead of assembling actin filaments at the cell surface, it assembles actin filaments at the basal surface. This proposal is consistent with the localization of L-fimbrin in podosomes of leukocytes (Marchisio et al., 1987; Zambonin-Zallone et al., 1989; Sato et al., 1990; Messier et al., 1993). These structures mediate the adhesion of leukocytes to the substratum.

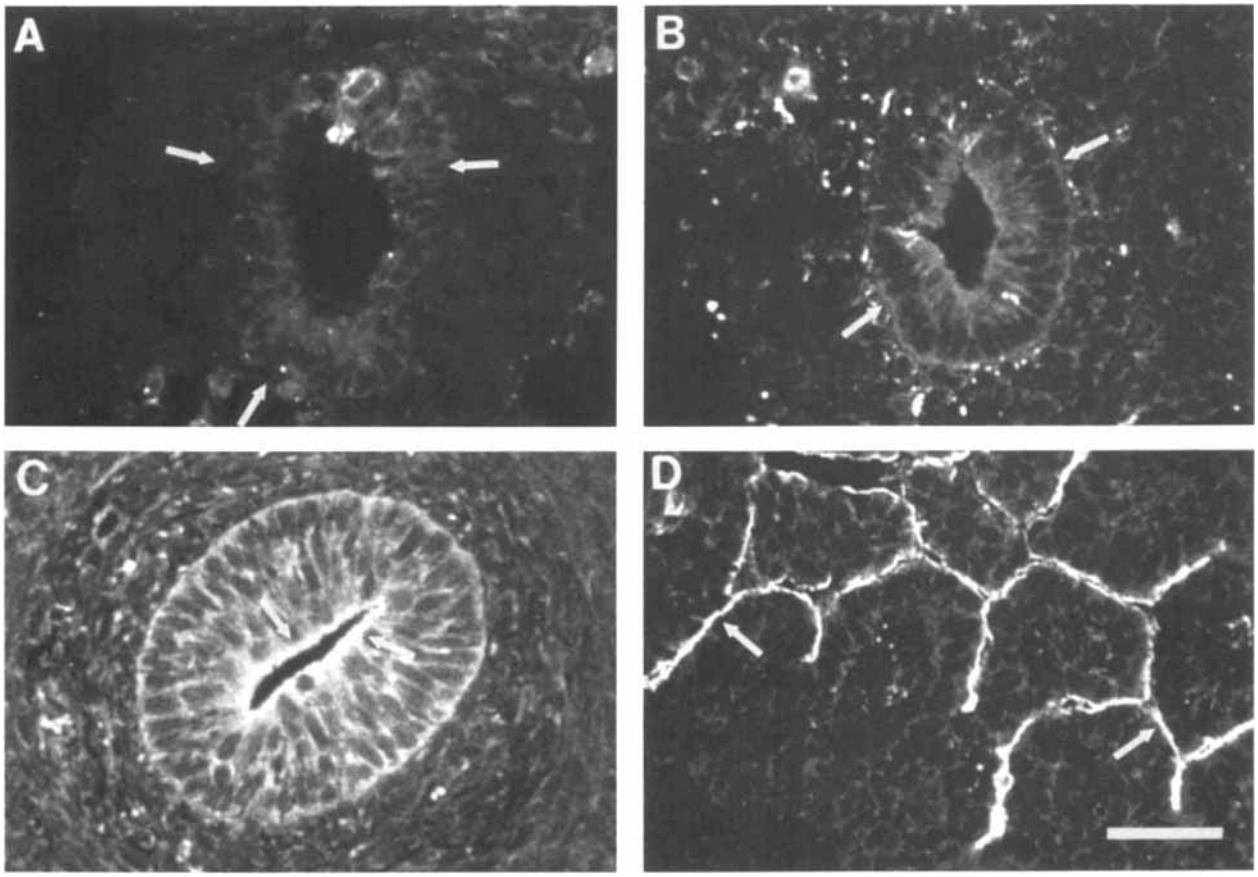

Fig. 6. Distribution of I-fimbrin in the developing mouse intestine. I-fimbrin was not detected in the epithelium at day 10.5 (**A**). By days 12.5 (**B**) and 14.5 (**C**), I-fimbrin staining was detected in the epithelium and localized first to the apical and then later to the basolateral membranes. By day 16.5 (**D**), I-fimbrin has concentrated in the developing microvilli and terminal web region (arrows). Bar = 50 μm.

However, we also showed that L-fimbrin is localized to the apical surface of yolk sac epithelium and thus has the ability to associate with non-basal surface structures. The role of L-fimbrin phosphorylation is not understood but we showed earlier that the phosphorylated form is more tightly associated with the cytoskeleton in macrophages. Perhaps the difference between a basal localization and an apical localization is due to chemical modification of L-fimbrin.

A second possibility is that actin at the basal and apical surfaces are different and that each fimbrin isoform has actin isoform preference. Previous studies have already shown that L-fimbrin binds β-actin more preferentially than α-actin (Namba et al., 1992). A third possibility is that L-fimbrin interacts with other proteins at the cell-matrix adhesion sites in the basal surface of the epithelium. At cell adhesion sites in cultured cells, multiple interactions among cytoskeletal proteins have been documented. For example, α-actinin, an actin cross-linking protein, interacts with vinculin and talin in addition to actin. L-fimbrin could possibly be part of a cell adhesion complex. In immunoprecipitates from macrophages, L-fimbrin and an unidentified 72,000 MW band were detected (Messier et al., 1993).

The basal localization of L-fimbrin suggests that it must be functionally different than L- or T-fimbrin. The most obvious function is that of a cell adhesion molecule. This speculation is supported by studies which show L-fimbrin, actin, and β-integrin are localized to adhesion plaques and podosomes on the cell-matrix interface of adherent cells. Furthermore, the integrins have been shown to be developmentally regulated (Muschler and Horwitz, 1991; Bronner-Fraser et al., 1992) and extracellular matrix proteins, such as laminin, exhibit a pattern of expression that is very similar to L-fimbrin (Klein et al., 1990; Simo et al., 1991). If L-fimbrin is a component of cell-matrix adhesion sites, then during intestine differentiation L-fimbrin may be involved in determining the polarity and architecture of epithelial cells during embryogenesis (for a review, see Adams and Watt, 1993).

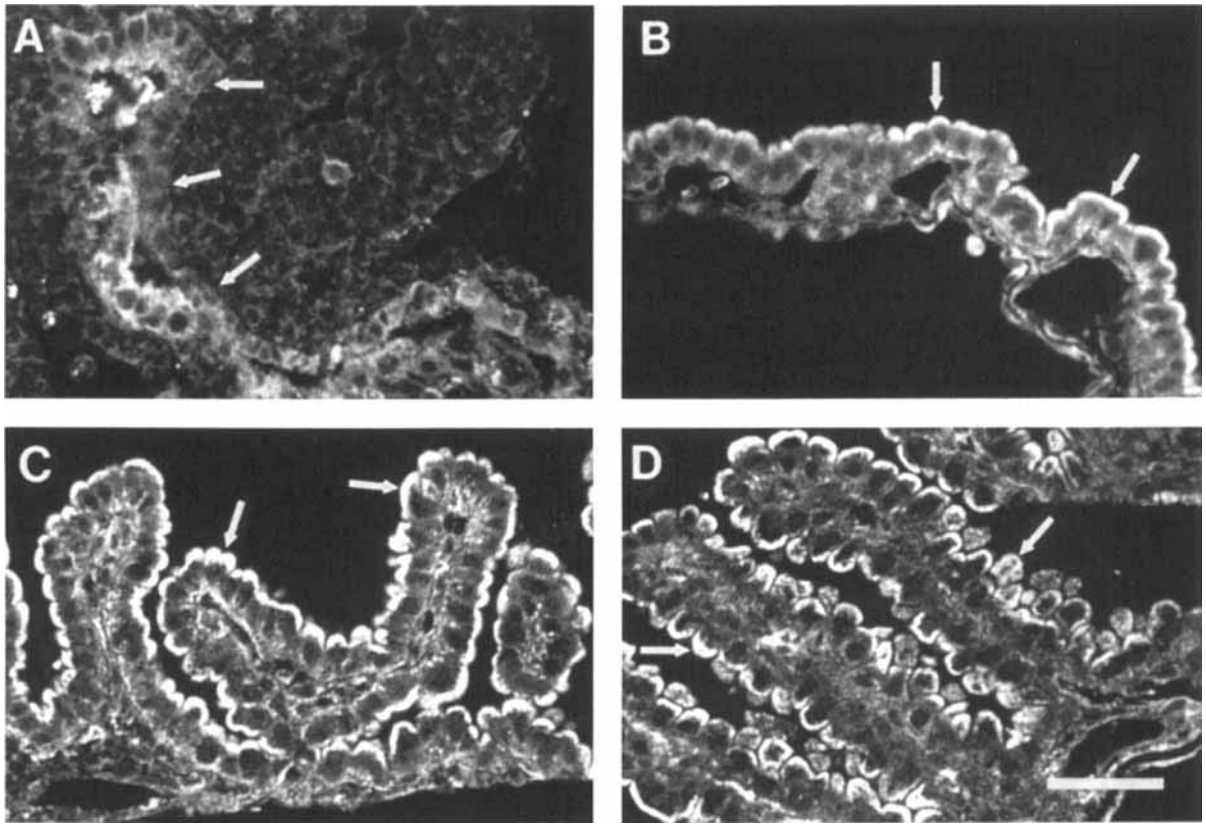

Fig. 7. Distribution of T-fimbrin in the yolk sac. Frozen sections through the developing yolk sac detect T-fimbrin faintly (arrows) at 10.5 days (**A**). By day 12.5 (**B**), T-fimbrin is detected in the visceral yolk sac (arrows) and extraembryonic mesoderm. At 14.5 (**C**) and 16.5 days (**D**), T-fimbrin becomes localized (arrows) to the apical surface. No basolateral localization was evident. Bar = 50 μm.

L-Fimbrin—A Marker for a Dedifferentiated State?

Previous studies have shown that L-fimbrin expression in adults is restricted to cells of the hemopoetic lineage and to cells derived from tumors of various tissues. In some cases, the cells express both L- and T-fimbrin. The significance of L-fimbrin expression in tumor-derived cells is currently studied by several groups because L-fimbrin may be a marker for a neoplastic state (Lin et al., 1993). Our studies of the mouse embryo demonstrate that L-fimbrin is expressed during development in the intestine and yolk sac. In the case of the intestine, L-fimbrin expression ceases at a later stage of development. Unpublished studies show high levels of L-fimbrin in other parts of the embryo such as the mouth (Chafel et al., unpublished data). Thus, we suggest L-fimbrin is more generally expressed by embryonic tissue but that expression becomes restricted to the hemopoetic lineage. Expression of L-fimbrin in tumors may reflect a reversion to an embryonic state. Thus, L-fimbrin could be a marker for a de-differentiated cell.

T-Fimbrin and Formation of Primitive Microvilli

T-fimbrin is the isoform that is most generally represented in adult cells and tissues. In the embryo, T-fimbrin displays a localization pattern consistent with a role in organizing actin filaments in cell surface microvilli. Its localization at the apical membrane is somewhat similar to villin. Thus, villin along with T-fimbrin is responsible for rudimental microvilli formation. That fact that villin induces rudimental microvilli formation in CV-1 cells is also in line with our observation. This is because CV-1 cells contain endogenous T-fimbrin, which along with exogenous villin induces rudimental microvilli formation. The localization of T-fimbrin at the membrane also supports this model. However, to form brush border microvilli, I-fimbrin becomes an important molecule.

The visceral endoderm contains a brush border whose microvilli shorten in length as development proceeds. Results from this study show that, in visceral endoderm, T- and I-fimbrin but not L-fimbrin are expressed during the developmental stages examined. In contrast to intestinal development, T- and I-fimbrin are expressed in the same cells and are both localized at the apical surface. The identical pattern of localization and expression suggests both proteins either carry out the same functions or carry out functions which are required at the same time. These differences are of interest considering the structural and functional similarities of the two cell types; both being polarized cells

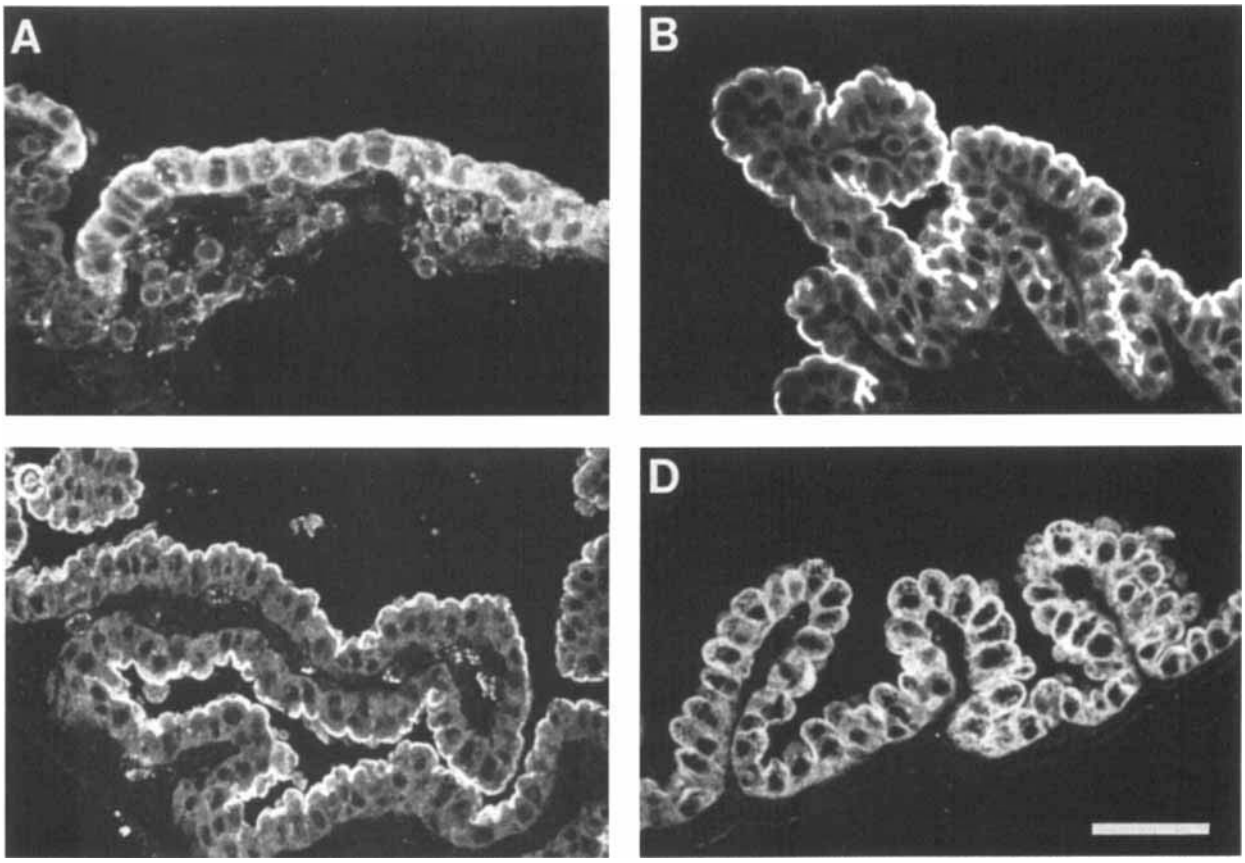

Fig. 8. I-fimbrin in the visceral endoderm. Antibodies against I-fimbrin stain only the visceral endoderm and not the extraembryonic mesoderm in sections through day 10.5 (**A**), 12.5 (**B**), 14.5 (**C**), and 16.5 (**D**) yolk sacs. At 10.5 days, I-fimbrin is localized along the apical and basolateral membranes. By 12.5 days, I-fimbrin has redistributed to the apical membrane. Bar = 50 μm.

that possess a brush border which facilitates absorption.

EXPERIMENTAL PROCEDURES

Mice, Embryos, and Cell Culture

Balb/c strain mice were used for this study. The age of an embryo was determined by assigning day 0.5 as the midday when the vaginal plug was discovered. Day 8.5, 10.5, 12.5, 14.5, and 16.5 embryos were used for immunofluorescence studies. For immunoblot analysis, fibroblasts were isolated from 13.5-day-old FVB/N strain embryos (Hogan et al., 1986) and grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and antibiotics. The mouse monocyte-macrophage cell line, J774 (ATCC-TIB67), was cultured in a similar fashion.

Antibody Production and Characterization

Rabbit antiserum, specific for human I-fimbrin (R406.4), L-fimbrin (R325.4), and T-fimbrin (R330.4), were elicited by synthetic peptides to unique protein sequences at the N-terminus (Lin et al., 1994). The specificity of the antiserum for the I-, T-, or L-fimbrin isoform was determined on immunoblots on lysates of adult mouse intestinal epithelium, embryonic mouse fibroblasts, and J774 cells. The immunoblots were performed as described in Ezzell et al. (1989).

Immunofluorescence Microscopy

Intestines from various staged mouse embryos and the duodenum from adult mice were dissected in PBS containing 2 mM EGTA and fixed in 4% paraformaldehyde in PBS-EGTA for 4–6 hr on ice. The samples were rinsed in PBS-EGTA buffer and infused overnight with 0.6 M sucrose in PBS-EGTA buffer at 4°C. The tissues were embedded in OCT compound (Miles, Inc., Naperville, IL) and then quickly frozen in an isopentane-liquid nitrogen bath.

Cryostat sections (5 μm thick) of the frozen samples were collected, extracted for 2 min in ice cold acetone, and rinsed twice in PBS-EGTA. To prvent nonspecific binding, the sections were incubated in 2% BSA in PBS-EGTA for 1 hr. Fimbrin antibodies were applied to the sections and incubated for 1 hr. The unbound antibodies were washed from the sections with 3 changes of PBS-EGTA and then detected with a fluo-

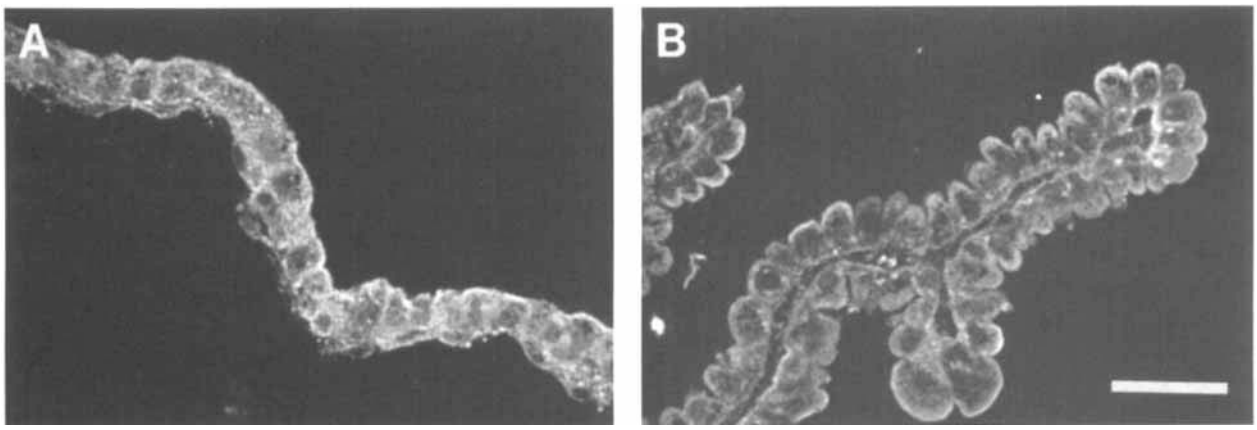

Fig. 9. Sections of 10.5 (**A**) and 16.5 (**B**) day yolk sac stained with L-fimbrin antibodies. L-fimbrin is localized to the visceral endoderm and extra-embryonic mesoderm. Within the endoderm, L-fimbrin is mainly along the apical surface but there is a significant amount of cytoplasmic staining. Bar = 50 μm.

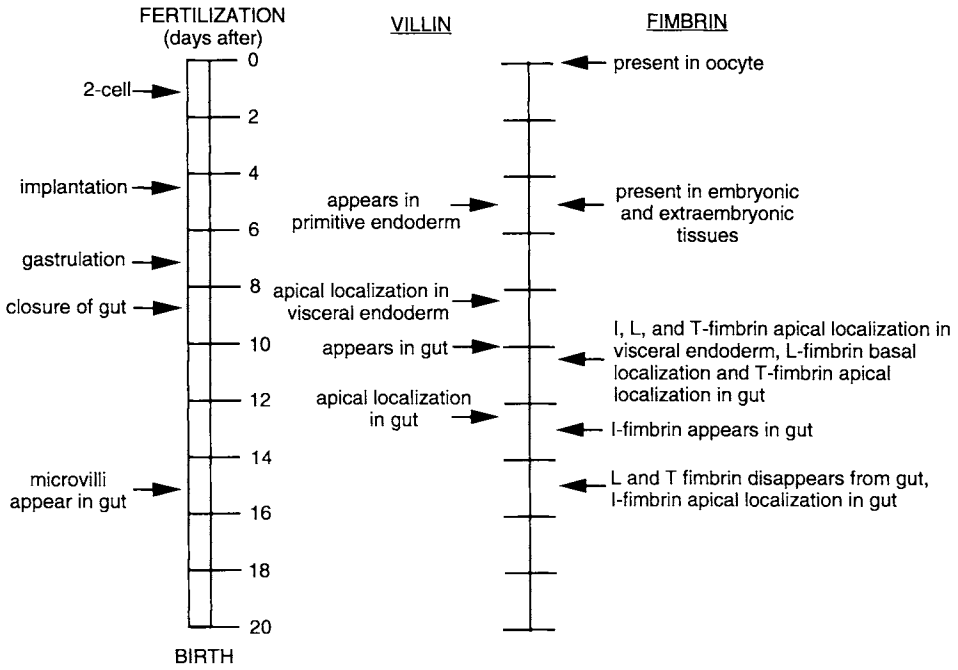

Fig. 10. Timeline of fimbrin and villin expression during mouse development. The timeline shows that intestine development occurs in two stages. The first stage of development coincides with when the intestine is lined by a primitive epithelium which is covered by a few microvilli. The appearance and apical localization of villin and I-fimbrin between days 10.5 and 12.5 mark the maturation stage when the brush border microvilli are assembled.

rescein-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch Lab, Inc., West Grove, PA). All incubations were at 37°C. For co-localization studies of L-fimbrin and actin in embryonic intestine, the sections were treated as above and then incubated in rhodamine-conjugated phalloidin (Molecular Probes Inc., Junction City, OR). The sections were mounted onto a coverslip in a drop of 1 mg/ml of *p*-phenylenediamine. All samples were examined and photographed using a Bio-Rad MRC 600 confocal microscope with a 40× objective (Bio-Rad, Richmond, CA).

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