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Cellular differentiation in the emerging fetal rat small intestinal epithelium: Mosaic patterns of gene expression

(intestinal differentiation/development/enterocytes)

DEBORAH C. RUBIN*, DAVID E. ONG†, AND JEFFREY I. GORDON*‡§

Departments of *Medicine and ‡Biological Chemistry, Washington University School of Medicine, Saint Louis, MO 63110; and †Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37232

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ABSTRACT **We have examined the pattern of differentiation of the small intestinal epithelium in fetal rats during the 17th through 21st days of gestation. Five genes expressed in late fetal, neonatal, and adult enterocytes were used as markers of differentiation. They encode three homologous small cytoplasmic hydrophobic ligand binding proteins—liver fatty acid binding protein (L-FABP), intestinal fatty acid binding protein (I-FABP), and cellular retinol binding protein II (CRBP II)—and two apolipoproteins—apoAI and apoAIV. RNA blot hybridization studies indicated that gradients in mRNA concentration from the proximal small intestine to colon appear coincident with the initiation of rapid epithelial cell proliferation and villus formation (days 17–19 of the 22-day gestation period). Immunocytochemical studies disclosed a remarkably heterogeneous pattern of cell-specific expression of the three hydrophobic ligand binding proteins that was not apparent with either apoAIV or apoAI. This "mosaic" staining pattern was observed in morphologically similar cells occupying identical topographic positions along nascent villi in 17- to 18-day fetuses. The onset and resolution of this mosaicism varies between I-FABP, L-FABP, and CRBP II in the proximal small bowel, although it completely resolves by the first postnatal day. The distal small intestine exhibits a developmental delay of 1–2 days in the appearance of this heterogeneous pattern of initial gene expression. Double-label immunofluorescent analyses using L-FABP and I-FABP antibodies indicated that on the 18th day of gestation the proximal small intestinal columnar epithelium contains several populations of enterocytes expressing neither, one, or both proteins. The potential significance of this mosaic pattern of intestinal epithelial differentiation is discussed in light of recent studies with transgenic and chimeric mice.**

The adult rodent small intestine is lined with a perpetually regenerating and differentiating epithelium. Anchored stem cells located in the crypts of Lieberkuhn represent the source of this epithelium (1). Studies with mouse aggregation chimeras (2) indicate that the stem cells in each crypt are derived from a single progenitor and give rise to four terminally differentiated cells—polarized columnar epithelial cells (enterocytes), mucin-secreting goblet cells, Paneth cells, and enteroendocrine cells (3). Paneth cells remain in the base of the crypt while the other cells are translocated in near vertical bands over an ≈3-day period from the crypt to the apical extrusion zone located near the tips of small intestinal villi (4). Cellular differentiation commences within the crypts and proceeds during translocation. Regional differences in gene expression also occur from the proximal small intestine to the colon in the rat (e.g., see ref. 5). It is unclear how this spatially complex differentiation is maintained in the face of perpetual cellular proliferation and when geographic differences in intestinal epithelial cell gene expression are established during development.

Recent studies of adult transgenic mice provide clues about potential mechanisms that underlie regional variation in intestinal gene expression. The liver and intestinal fatty acid binding protein (FABP) genes are efficiently expressed in the adult rat gut epithelium where they exhibit regional differences in their expression from duodenum to colon and from crypt to villus tip (6–8). To determine whether cis-acting elements in these homologous FABP genes could direct appropriate geographic and cell-specific expression, portions of their 5′ nontranscribed regions were linked to a reporter [human growth hormone (hGH) minus its regulatory elements] and the pattern of hGH expression was examined in transgenic mice (7, 8). Elements located in the 5′ nontranscribed portions of these genes were sufficient to direct a pattern of cell-specific and regional hGH expression that closely resembled that of the endogenous mouse intestinal (I)- and liver (L)-FABP genes. Curiously, a "mosaic" pattern of hGH accumulation was noted in mice who were obligate heterozygotes for these transgenes. Vertical bands of negatively staining enterocytes extending from the base to tips of villi were occasionally encountered, surrounded by hGH-positive bands. This pattern was never observed for the endogenous gene products. The mosaic patterns of transgene expression suggested that there may be subtle differences in clonally derived epithelial cells in these adult animals.

To better understand the origins and significance of this cellular heterogeneity, we have investigated the patterns of cell-specific activation of these and several other genes in different regions of the rat gut epithelium during late gestation. This developmental stage was selected because the epithelium undergoes remarkable morphologic differentiation in the 4–5 days prior to birth (9–11). Transcription of each of these marker genes [encoding I-FABP, L-FABP, a homologous cellular retinol binding protein (CRBP II) plus two nonhomologous polypeptides, apolipoproteins (apo) AI and AIV] is induced coincident with the initial expansion of the fetal intestinal epithelium (5, 12–15). Also, all exhibit regional differences in their expression from crypt to villus tip and from proximal to distal intestine in the adult rat (5–8, 16). Our comparison of the cellular patterns of activation of these genes revealed a remarkable heterogeneity in enterocytic differentiation, indicating that the developmental program expressed in the emerging proximal-to-distal and crypt-to-villus axes is more complex than previously appreciated.

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Abbreviations: L-FABP, liver fatty acid binding protein; I-FABP, intestinal fatty acid binding protein; CRBP II, cellular retinol binding protein II; apo, apolipoprotein; hGH, human growth hormone; PAP, peroxidase-antiperoxidase; FITC, fluorescein isothiocyanate; TRITC, tetramethylrhodamine isothiocyanate.

§To whom reprint requests should be addressed.

MATERIALS AND METHODS

Animals. Sprague–Dawley rats were purchased from Sasco (Saint Louis). The day that a vaginal sperm plug was first noted was designated as day 0 of gestation.

RNA Isolation. Total cellular RNA was recovered (5) from the stomach and intestine of 17-day-old fetuses. At 19 days of gestation, the stomach and duodenum were isolated and the remainder of the fetal intestine was divided into two equal length segments. At fetal day 21 and postnatal day 1, the duodenum was isolated from the rest of the small intestine, which was subsequently divided into three equal parts, while the cecum and colon were combined. Since the yield of tissue is low at these early stages, we pooled organs or comparable portions of organs from littermates prior to RNA isolation. Two litters were analyzed at each time point. The integrity of RNA preparations was established by denaturing methyl-mercury-agarose gel electrophoresis (17).

RNA Blot Hybridizations. Dot blots of total cellular RNA were prepared as described (13). The blots were probed with full-length ^{32}P-labeled (18) rat I-FABP (8), L-FABP (7), CRBP II (13), apoAI (5), and apoAIV (5) cDNAs plus a 650-base-pair fragment of human α-tubulin cDNA (kindly provided by A. J. Lusis, University of California, Los Angeles). Hybridization and washing conditions are listed in Demmer *et al.* (19). The relative abundance of each mRNA in the different RNA samples was calculated by scanning laser densitometry of filter autoradiographs (13).

Immunocytochemical Localization Studies. Polyclonal antibodies to rat I-FABP and L-FABP that had been expressed in *Escherichia coli* were raised in rabbits (7, 8). Ouchterlony double immunodiffusion and Western blot analyses established their unique specificities. A polyclonal monospecific rabbit anti-rat CRBP II antiserum has been characterized previously (20). N. O. Davidson (University of Chicago) provided monospecific rabbit anti-rat apoAI and apoAIV antisera (21, 22). Tissues were fixed in Bouin's solution, dehydrated, and embedded in paraffin. Sections (5–8 μm thick) were stained by the peroxidase-antiperoxidase (PAP) method (7). CRBP II was localized by the ABC technique (20). Controls were used to demonstrate the specificity of the immunocytochemical staining: normal rabbit sera produced no staining, omission of the primary antibody resulted in no detectable signals, and when dilutions of each antiserum (1:250–1:500) were absorbed for 48 hr with 10–20 nmol of purified antigen, no specific staining was observed.

Double Immunofluorescence Microscopy to Detect Both the L- and I-FABP Antigens in the Same Tissue Sections. Paraffin was removed from sections of 18-day-old fetal rat intestine by immersion in xylene and 100% isopropanol. They were then treated with 100% methanol, phosphate-buffered saline (PBS), and 100% ethanol, prior to overnight incubation with a solution (1 mg/ml) of sodium borohydride at 4°C. Sections were subsequently rinsed with 100% ethanol/PBS, blocked for 2 hr with 2% bovine serum albumin, and incubated with primary antibody (L-FABP) for 18 hr at 4°C. Tissues were rinsed in PBS, incubated with goat anti-rabbit IgG conjugated to fluorescein isothiocyanate (FITC) for a minimum of 2 hr, and then mounted in 3% *N*-propyl gallate in PBS/glycerol (1:1). Both the L- and I-FABP antibodies are derived from rabbit. Therefore, to detect I-FABP antigen in the same section and avoid "cross-talk" between the L-FABP antibody and the second label [goat anti-rabbit IgG conjugated to tetramethylrhodamine isothiocyanate (TRITC)], the L-FABP antibody was eluted by acid oxidation and subsequent reduction (23). Sections were then rinsed in PBS, reblocked with 2% bovine serum albumin for 1 hr, incubated overnight with antibody to rat I-FABP at 4°C, washed in PBS, incubated with goat anti-rabbit IgG-TRITC for 2 hr, rinsed in PBS, remounted, and photographed. To ensure that the L-FABP antibody had been completely removed prior to double labeling, sections that had been incubated with L-FABP antiserum and labeled with FITC were treated by acid oxidation (23) to elute the antibodies. These sections were subsequently incubated with normal rabbit serum (instead of I-FABP antibody) followed by goat anti-rabbit IgG conjugated to TRITC. No rhodamine immunofluorescence was noted in these sections, whereas characteristic fluorescence patterns were seen in tissue sections simultaneously stained with I-FABP antiserum and goat antirabbit TRITC.

RESULTS

Proximal to Distal Gradients in L-FABP, I-FABP, CRBP II, ApoAI, and ApoAIV mRNA Concentrations Appear Coincident with the First Stages of Rapid Fetal Intestinal Epithelial Cell Proliferation/Differentiation. RNA blot hybridization was used to determine whether the developing fetal intestine manifests regional differences in expression of hydrophobic ligand binding protein and apolipoprotein genes at the time of rapid differentiation of its epithelial cell population. Fig. 1 provides the results of our survey of different regions of the gastrointestinal tracts of 19- and 21-day fetal as well as 1-day-old suckling rats (n = 20–24 rats per time point). By the 19th day of gestation, L-FABP mRNA levels are highest in the proximal small intestine and decrease distally. This distinctive pattern is maintained throughout the remainder of gestation and the first postnatal day. A similar geographic distribution was observed for I-FABP, CRBP II, apoAI, and apoAIV mRNAs in 19-day fetal rat intestine, which persists through the immediate peripartum period. Regional differences in gene expression are not exhibited by all genes in the fetal gut. α-Tubulin mRNA levels do not display any significant regional variation in the intestines of 17- to 21-day fetal or 1-day suckling rats.

Genes Encoding Hydrophobic Ligand Binding Proteins Display a Remarkable Heterogeneity in Their Initial Patterns of Cell-Specific Expression. Immunocytochemical studies were

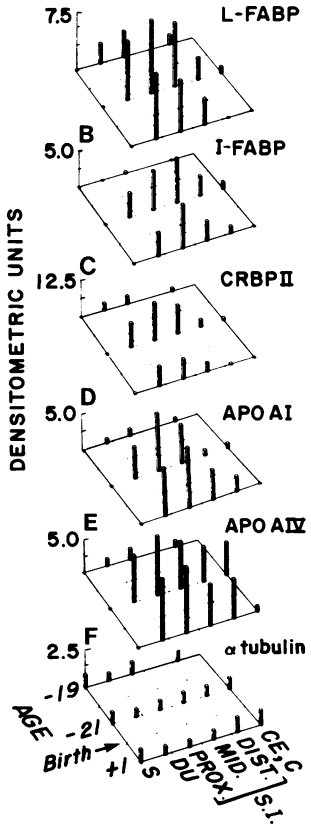

FIG. 1. Developmental changes in L-FABP, I-FABP, CRBP II, apoAI, apoAIV, and α-tubulin mRNA accumulation in the proximal to distal axis of the intestine. The gastrointestinal tract of Sprague–Dawley rats was dissected at various stages of development. Filters containing four concentrations (0.5–3.0 μg) of each RNA sample were probed with ^{32}P-labeled cDNAs. Since the specific activities of the radiolabeled cDNAs were not identical, no conclusions can be made about the relative abundance (expressed in arbitrary densitometric units) of each mRNA in a given tissue RNA sample at a particular stage of development. Filters containing total (small and large) intestinal RNA isolated from 17-day-old fetal rats were also probed with each cDNA revealing very low levels of L-FABP, CRBP II, apoAI, and apoAIV mRNAs, but no detectable I-FABP mRNA. S, stomach; DU, duodenum; S.I., small intestine; CE, cecum; C, colon; PROX., proximal; DIST., distal.

performed to document the cell-specific patterns of expression of these genes in the emerging fetal rat intestinal epithelium. Fig. 2 shows the pattern of accumulation of L-FABP in epithelial cells located in the proximal and distal fetal small intestine. By the 17th day of gestation, the proximal small intestine contains nascent villi. A surprisingly mosaic pattern of L-FABP accumulation is encountered in its epithelial cell population (Fig. 2*A*). Some cells stain deeply for L-FABP, some show light brown staining, and many do not stain at all. This variable staining affects columnar epithelial cells, which have identical morphology as judged by light microscopic examination. By fetal day 18 (Fig. 2*B*), the proximal small intestinal villi are longer, the epithelium has proliferated, but the mosaicism persists. By fetal day 19, the heterogeneity in cellular L-FABP concentrations has disappeared in the proximal small intestine and all columnar epithelial cells lining elongating villi appear to accumulate this protein to comparable levels (Fig. 2*C*).

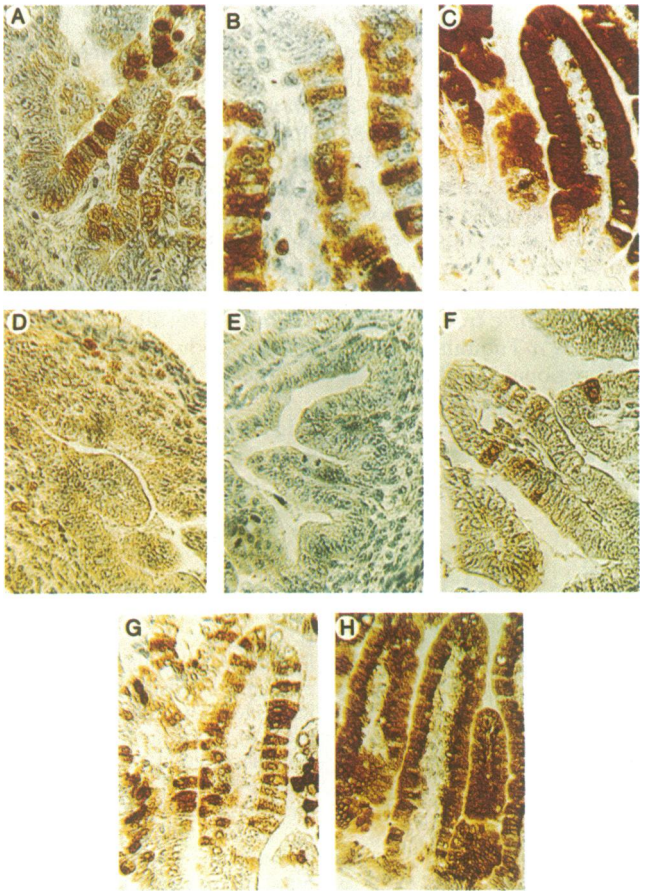

FIG. 2. Immunocytochemical studies of L-FABP expression in emerging villi located in the proximal and distal small intestine. Sections of fetal rat intestine were incubated with polyclonal anti-L-FABP antiserum. Diaminobenzidine and hydrogen peroxide were used as peroxidase substrates to generate a brown stain indicating the presence of immunoreactive protein. (*A*) Proximal small intestine from 17-day fetus demonstrating patchy expression of L-FABP in columnar cells lining a primordial villus. (*B*) Proximal small intestine from 18-day fetus. (*C*) Proximal small intestine from 19-day fetus. All cells except those in the intervillus region contain L-FABP. (*D*) Distal small intestine from 17-day fetus. A stratified epithelium without formed villi is present without detectable L-FABP. (*E*) Distal small intestine from 18-day fetus. (*F*) Distal small intestine from 19-day fetus. (*G*) By 20 days of gestation, distal intestinal villi are longer and many more cells contain L-FABP. (*H*) Distal small intestine from 1-day neonatal rat. Cells are now homogeneously stained for L-FABP. (*A*, ×250; *B*, ×400; *C*, ×190; *D–F*, ×250; *G*, ×190; *H*, ×120.)

The developing distal small intestine exhibits a variation on this theme of L-FABP gene activation. Villus formation in the distal rat fetal small intestine occurs 1–2 days later than in the proximal small intestine (11). At 17 days of gestation, the distal small intestine has no villi. Its epithelium is several cell layers thick and has no detectable L-FABP (Fig. 2*D*). Occasional erythrocytes in the submucosa stain nonspecifically. By the 18th day, villi begin to emerge. However, unlike the proximal small intestine, no immunoreactive L-FABP is demonstrable (Fig. 2*E*). By fetal day 19, more villi have formed but only a few scattered epithelial cells produce this protein (Fig. 2*F*). Again, these positive cells appear to be indistinguishable morphologically from adjacent negative cells. During the 20th day of gestation, the total number of enterocytes producing L-FABP has increased (Fig. 2*G*), yet marked heterogeneity in L-FABP levels between cells located in comparable positions in the emerging crypt–villus axis is observed. This mosaicism disappears in the distal small intestine by the first postnatal day (Fig. 2*H*).

A similar, initially heterogeneous, pattern of intestinal epithelial cell differentiation was documented when I-FABP and CRBP II gene expression was examined. At 17 days, no I-FABP staining could be demonstrated in multiple sections of proximal small intestine but by the 18th day of gestation, a patchy pattern of columnar epithelial cell staining is seen that is remarkably similar to L-FABP (data not shown). Unlike L-FABP expression in the proximal small intestine, all enterocytes do not demonstrate uniform staining for I-FABP until postnatal day 1. The distal small intestine displays the same developmental "delay" in cellular I-FABP activation as was observed for L-FABP; cellular mosaicism for I-FABP is first observed 1–2 days after initiation of its synthesis in proximal small intestinal epithelial cells (data not shown).

Villi from the proximal small intestine of 17-day-old fetal rats were stained with monospecific CRBP II sera by using the ABC method rather than the PAP technique (Fig. 3). This method also revealed a heterogeneous pattern of expression, which begins to resolve by fetal day 20. As in the case of L-FABP and I-FABP, epithelial cells located in the intervillus regions do not contain detectable levels of protein at this or subsequent stages of fetal and early postnatal development.

Based on expression of these marker genes, we can conclude that the initial establishment of proximal to distal gradients in their mRNA concentrations reflected, in large part, proximal to distal differences in the numbers of cells in which they were being transcribed/translated. In addition, the data suggested that differentiation of cells lining primordial villi occurs nonuniformly. However, these studies did not indicate whether cells that "precociously" express one hydrophobic ligand binding protein are also differentiated to the point that they can synthesize other members of the protein family. This issue was examined with double-immunofluorescent labeling techniques (Fig. 4). L-FABP contained in the proximal small intestinal epithelial cells of an 18-day fetus appears as green staining material, while I-FABP appears as bright orange material. All columnar epithelial cells that express I-FABP also express L-FABP. However, many more cells express L-FABP than I-FABP. Some cells do not express either protein at detectable levels. These results were independently confirmed when serial sections were stained for both antigens by the PAP technique (data not shown). Thus, on the same villus, one subpopulation of columnar epithelial cells exists that expresses both proteins (i.e., the most differentiated cells for these marker genes), another subpopulation expresses only L-FABP, while a third subset of cells was operationally defined as the least differentiated based on the fact that it expresses neither protein in detectable amounts at this developmental stage.

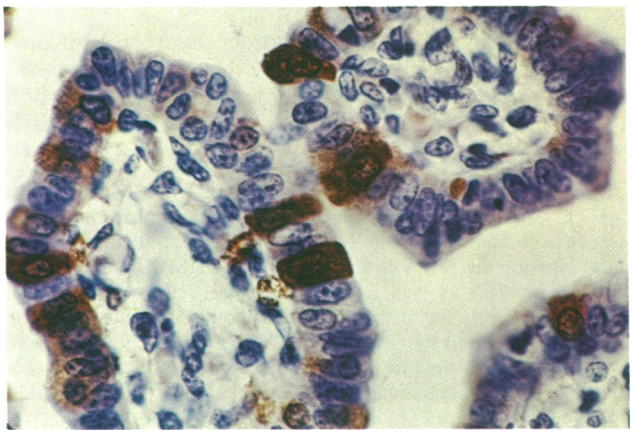

FIG. 3. Immunocytochemical studies of the perinatal expression of CRBP II in the emerging crypt–villus axis. The ABC method was used to stain for CRBP II (20) on a section of proximal small intestine from a sagittally sectioned 17-day fetus. (×550.)

Columnar Epithelial Cells Overlying Emerging Villi Express ApoAIV and ApoAI in a Homogeneous Rather Than a Mosaic Pattern. A final set of experiments was designed to determine whether this complex epithelial cell differentiation could be further characterized by using two apolipoprotein genes. When the proximal small intestine of a 17-day fetus was stained with monospecific apoAIV antiserum, a homogeneous cellular pattern of very light brown staining was observed in the epithelium (Fig. 5*A*). By the 18th day of gestation, the intensity of staining increases as more protein accumulates (Fig. 5*B*). The pattern remains quite homogeneous; all enterocytes express approximately equal amounts of protein at this location in the proximal to distal axis of the small intestine. No heterogeneity in cell-specific expression of this gene was found in either the mid or distal small intestine (data not shown). These results suggest that the proximal to distal gradient in apoAIV mRNA concentration (Fig. 1) appears to arise in large part from a progressive decrease in the level of gene expression per cell as one proceeds along the gut. A similar homogeneous cellular pattern of staining was also observed in the epithelium after it was stained for apoAI (data not shown). We can conclude from these results that the subset of intestinal epithelial cells that are not differentiated enough to express L-FABP and I-FABP can initiate synthesis of these apolipoproteins.

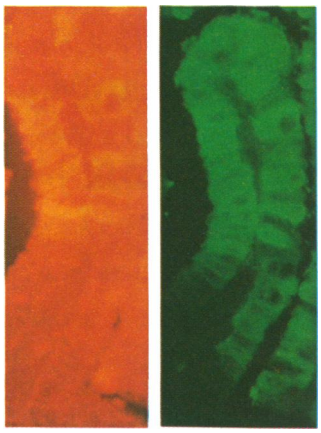

FIG. 4. Double-immunofluorescence labeling of proximal small intestine from 18-day rat fetus to simultaneously detect the presence or absence of I- and L-FABP in the same cell. (*Left*) Bright orange staining cells are positive for I-FABP. (*Right*) The same section from 18-day fetal rat intestine stained for L-FABP. The bright green cells contain L-FABP. Background fluorescence in these sections is identical to fluorescence of control sections incubated with normal rabbit serum and TRITC or FITC. While many cells contain L-FABP, a smaller subset of these also stain for I-FABP. (*Left* = ×300; *Right* = ×300.)

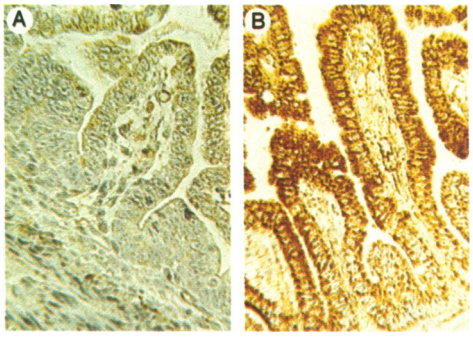

FIG. 5. Immunocytochemical studies of the perinatal expression of apoAIV in the proximal small intestine. (*A*) Proximal small intestine from 17-day fetus. Uniform, very light brown staining is seen in all cells lining the nascent villus. (*B*) Proximal small intestine from 18-day fetus. Homogeneous darker brown staining is found in cells lining the villus. No patchy expression is noted. (*A* = ×180; *B* = ×140.)

DISCUSSION

Our analysis of the differentiation of the rat fetal small intestinal epithelium indicates that (*i*) it is able to establish and maintain marked proximal to distal differences in the expression of several enterocyte-specific genes from the time of initiation of rapid cellular proliferation/differentiation during late gestation; (*ii*) columnar cells overlying nascent villi display a remarkably complex pattern of differentiation as indicated by the great variability in accumulation of hydrophobic ligand binding proteins in morphologically similar cells occupying comparable locations in the emerging crypt–villus axis; and (*iii*) the pattern of gene activation in the distal small intestine resembles that in the proximal gut, but it is delayed by 1–2 days, contributing in large part to the initial establishment of proximal to distal gradients in small intestinal gene expression.

Previous analyses of rat fetal small intestinal epithelial differentiation have included morphologic studies of the evolving cellular populations (9, 11) and measurements of the activities of brush border hydrolases (24, 25) in tissue homogenates. As an example of the latter, segments of 17- and 20-day-old rat fetal intestine were transplanted into the subcutaneous tissue (26) or under the renal capsules (27, 28) of weaning or adult syngeneic rats. Regional differences in brush border disaccharidase or alkaline phosphatase activities were still established in the isografts, implying that "intrinsic" (i.e., nonluminal) factors may play a dominant role in the formation of proximal to distal small intestinal gradients. Cellular patterns of gene activation/expression were not analyzed in these isograft experiments. When Quaroni (29) examined the cell-specific expression of several membrane-associated proteins and brush border enzymes in the 21-day-old fetal intestinal epithelium, no mosaic staining patterns were noted.

The limited number of previous studies documenting patterns of cellular differentiation in fetal rat intestinal epithelium affects our ability to interpret the heterogeneous pattern of expression of the three hydrophobic ligand binding protein genes. Based on the results of apoAIV and apoAI immunolocalizations, it is clear that it is not a phenomenon that affects all genes expressed in fully differentiated enterocytes. We cannot say at the present time whether the mosaicism reflects cellular differences in mRNA transcription, stability, translation, or protein turnover. The patchy

expression of the FABP and CRBP II genes is unlikely to be due to cell-cycle-dependent effects. The zone of cellular proliferation is more diffuse in villus-associated epithelial cells during late gestation than in the early postnatal rat gut, where it is confined to the crypt region (30). However, the heterogeneous pattern of expression of CRBP II and L-FABP resolves by days 19–20 despite the continued proliferative activity of 19- to 21-day fetal columnar epithelial cells. The patchy patterns of differentiation are also unlikely to reflect differences in cellular architecture: the columnar epithelial cells appear morphologically identical in the light microscope whether or not they express these hydrophobic ligand binding proteins and, because they are cytoplasmic proteins, the development of a complex cytoskeletal apparatus or specific organelles would not be expected to affect protein targeting or stability.

The mosaic patterns of expression of the hydrophobic ligand binding protein genes could reflect the presence of several populations of enterocytes in a given nascent villus—each of which has arisen from a different stem cell. A recent developmental study of mouse aggregation chimeras by Schmidt *et al.* (31) indicated that nascent intestinal crypts from 2- and 6-day-old suckling mice contain cells of mixed genotype (i.e., they are polyclonal) and that this mosaicism resolves by the 14th postnatal day when the crypt achieves monoclonality (i.e., all stem cells derived from a common progenitor). The mosaic expression of L-FABP and CRBP II in the proximal small intestine resolves before birth when cells of mixed clonality are presumably still present in the emerging villus. If distinct populations of cells of mixed clonality are present that have slightly different temporal programs for differentiation, it is clear from these studies that the hydrophobic ligand binding protein genes are sensitive markers for this phenomenon, while the apoAI and apoAIV genes are not.

Studies of adult transgenic mice alluded to in the Introduction indicate that obligate heterozygotes for the FABP/hGH transgenes demonstrate mosaic patterns of reporter expression in their small intestine (7, 8), consistent with the notion that there are subtle differences between monoclonally derived epithelial cells: vertical bands of negatively staining cells extending from the base to tips of villi were surrounded by hGH-positive bands. This pattern was never observed for the endogenous genes and perhaps reflected differences in cellular levels of certain trans-acting factors to which the "exogenous" transgene was more sensitive. The finding of a transient mosaicism in the expression of the endogenous rat L-FABP and I-FABP genes during the earliest stages of intestinal epithelial differentiation and its subsequent abolition by the time of birth raises the intriguing possibility that the factor or factors responsible for the mosaic patterns of transgene expression may operate in a more "flagrant" fashion during early intestinal differentiation and thereby affect the intact FABP genes. Having now defined the normal pattern of activation of the rat L-FABP and I-FABP genes, it may be informative to conduct a comparative analysis of developmental patterns of activation of the endogenous genes and the transgenes in heterozygous transgenic mice by double-immunofluorescence staining techniques. Such a study could reveal whether, at a time of polyclonality, the different clonally epithelial cell-derived populations express neither, one, or more of these genes.

Finally, it is interesting to note that the mosaic patterns of differentiation observed during early intestinal development have been "recapitulated" to some degree in a cell culture system. When the human enterocyte-like cell line HT-29 is induced to differentiate in culture by removing glucose as the medium carbon source, virtually all cells develop an apical brush border and tight junctions (32, 33). While all monoclonally derived differentiated HT-29 cells express the colon-specific antigen 517 on their apical surfaces (34), only 20% of these cells express the brush border hydrolase sucrase-isomaltase (33). Such a cell line may therefore prove to be a useful model for isolating and characterizing factors responsible for mosaic patterns of intestinal epithelial differentiation *in vivo*.

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