

Intestinal Epithelial Differentiation: New Insights from Chimeric and Transgenic Mice

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THE mammalian intestinal epithelium represents a unique model system for studying cellular differentiation since it undergoes continuous and rapid renewal. Perpetual differentiation results in the generation of its four principal cell types, each with a unique phenotype. The processes of proliferation and cellular differentiation are topologically very well organized. Gradients in gene expression are established and maintained in several spatial dimensions within this epithelium. Recent experiments involving mouse aggregation chimeras and transgenic mice have provided insights about the origins of intestinal stem cells, the migration pathways followed during cellular differentiation as well as the molecular mechanisms which produce complex geographic differences in gut gene expression. The purpose of this mini-review is to emphasize the usefulness of these powerful methods for examining questions related to enteric epithelial biology.

Organization of the Mouse Intestinal Crypt-Villus Axis

The adult mouse intestinal epithelium is a monolayer that contains four principal, terminally differentiated cell types. The enterocyte comprises the bulk of the epithelial population and represents its primary absorptive cell. Its apical brush border contains several well-characterized intramembrane transporters (e.g., the Na⁺/glucose cotransporter; reference 15) as well as membrane-anchored hydrolases (e.g., prepro-sucrase-isomaltase [19] and prepro-lactase-phlorizin hydrolase [27]), which have proved to be useful differentiation markers and valuable models for examining protein targeting within these polarized cells. Enterocytes also contain a well-organized, polarized complex of endosomal compartments (61) and have been used to study transepithelial (apical ↔ basolateral) transport pathways (41). Goblet cells package mucin in granules that are exported through a distinct, regulated, and strictly polarized pathway (31). Enteroendocrine cells (52) synthesize a variety of macromolecules. Serotonin-containing enterochromaffin cells represent the predominant enteroendocrine cell type encountered in the mouse gastrointestinal tract (42). Production of a variety of peptide hormones (glucagon, pancreatic polypeptide, gastrin, vasoactive intestinal peptide, β-endorphin, substance P, neurotensin, secretin, FMRFamide) has also been documented in enteroendocrine cells located in different regions of the gut (42). Paneth cells produce lysozyme and may represent part of the physical/biochemical barrier to bacterial translocation across the gut (9, 10, 44, 45).

The intestinal epithelium is organized into two morpholog-

ically and functionally distinct compartments: the crypts of Lieberkühn and the villi (see Fig. 1). The crypt represents its proliferative unit and contains ~250 cells (40). Tritiated thymidine labeling experiments have shown that ~150 cells, located in a broad band in the middle of the crypt, undergo rapid division (mean cell cycle time [T_c] = 13 h; references 38, 40). Each crypt in the mouse intestine produces ~275 cells/d and distributes them to surrounding villi (39, 51).

Epithelial cell migration in the crypt is a bipolar process. Cells representing three of the four lineages exit the crypt and move in a polarized fashion, without division, from the villus base towards its apex. During the translocation process, they undergo differentiation to enterocytes, goblet, and enteroendocrine cells. The rate of migration is remarkable: estimates from tritiated labeling studies range from 1 to 2 cell positions per hour, resulting in an average epithelial cell residence time on the villus of ~3 d (38). Upon reaching the apical or subapical area of each villus, the resulting fully differentiated cells are extruded into the intestinal lumen. In contrast to these three lineages, Paneth cells differentiate as they migrate towards the crypt base.

Stem cells situated in the crypts provide for the continuous renewal of the gut epithelium. Potten and Hendry (38) have outlined several features which these stem cells must possess to fulfill their function. They must be functionally fixed or anchored¹ at the origin of the migration pathway(s) so that they can persist as long-lived residents of the crypts. They must have a large division potential so that they can replace the gut epithelium throughout the life of the animal. Finally, in addition to their capacity for self regeneration, these immortal stem cells must have a low probability of differentiation. In turn, their daughter cells must, by unknown mechanisms, make the decision to either commit themselves to terminal differentiation or to remain a stem cell.

The precise location of stem cells in the mouse small intestinal crypt is not known. Based on tritiated thymidine labeling experiments, Cheng and Leblond (6, 7) proposed a model of crypt organization that had a single circumferential ring of ~16 stem cells located near its base. These stem cells were viewed as pluripotent, giving rise to descendants that would undergo three to four divisions while migrating through the crypt. The likelihood of commitment/differentiation of these migrating cells to each of the four lineages

1. The term anchorage (38) does not imply physical linkage but rather persistent functional containment of these dividing stem cells to a particular region in the crypt.

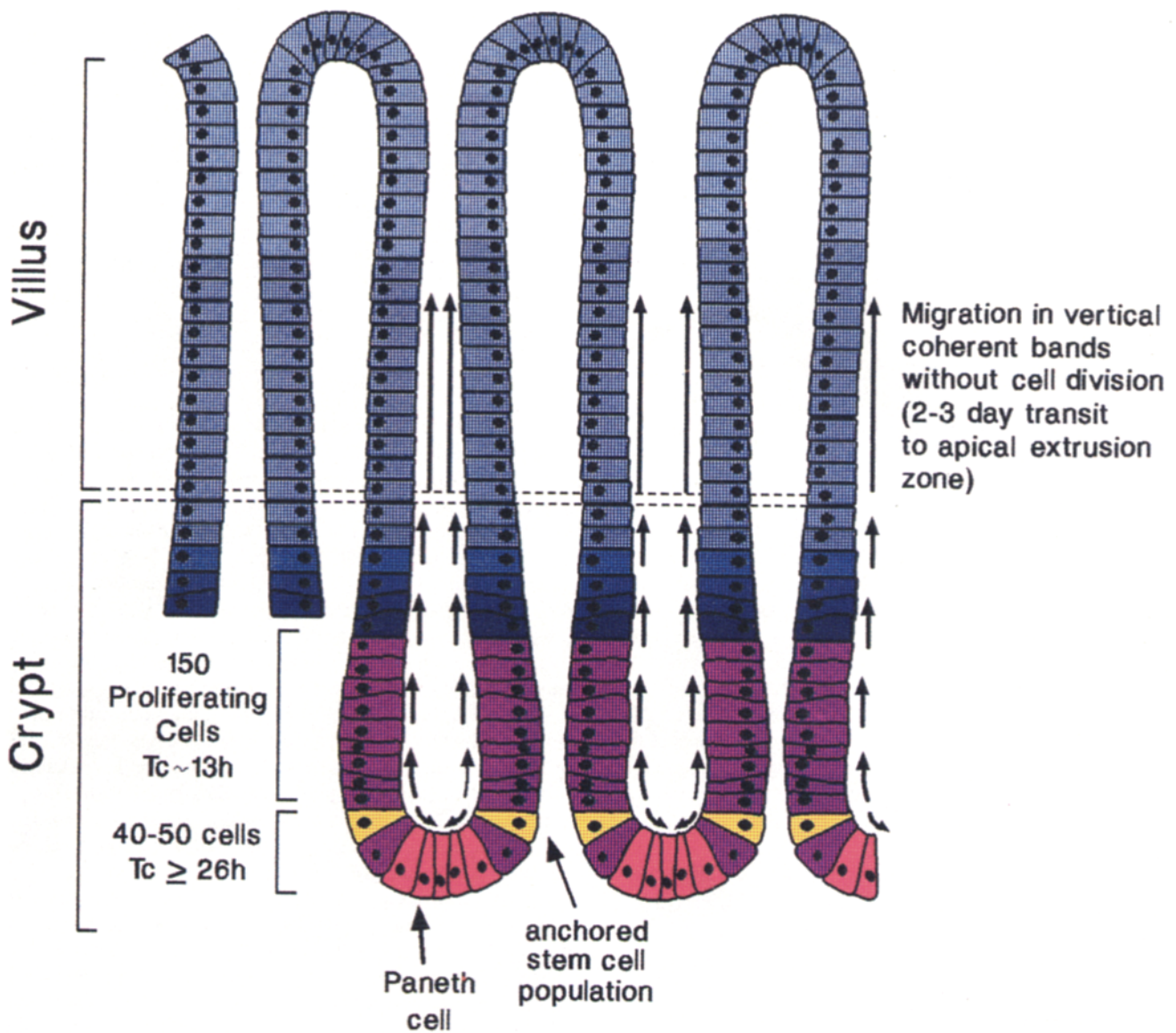


Figure 1. Organization of the crypt-villus axis in the adult mouse small intestine. This figure is based on data summarized in reference 38. The small intestinal crypt contains ~ 250 cells. The lower five cell positions in the crypt contain a total of 40–50 cells which have an average cycle time (T_c) ≥ 26 h. This “cluster” includes 20–30 Paneth cells which reside at the base of the crypt. A group of stem cells possessing high self-maintenance and low differentiation probabilities are believed to be situated at the fifth cell position above the base. Cell migration is bipolar in the crypt: the Paneth cell lineage undergoes a downward translocation from the anchored stem cell zone while the mid position of the crypt is occupied by a band of ~ 150 proliferating (?committed) cells with a T_c of ~ 13 h that are involved in an upward migration process. These latter cells achieve a velocity of 1–2 cell positions per hour at the “apex” of the crypt. 6–14 crypts surround the base of each small intestinal villus, the number depending upon the location of the villus along the duodenal to ileal (or “horizontal”) axis of the gut. Approximately 275 cells are delivered per day to the villus base from each crypt. Cells migrate from the villus base towards its apex in coherent vertical bands (38, 40, 48).

would increase as they moved away from the functionally anchored stem cell layer.

More complex models which invoke a two-tier stem cell population were subsequently advanced (3, 4) in an attempt to explain how the Paneth cell lineage, which appears to arise at approximately the fifth stratum of cells relative to the crypt base, is able to undergo differentiation and translocation to the bottom of the crypt. A tier of stem cells located just below the fifth cell layer would give rise to cells that are exclusively committed to the Paneth cell pathway, while a ring of stem cells located at cell position 5 could produce progeny that

can commit to any of the (other) lineages. While there is no definitive evidence that allows selection of one model of stem cell location over the other, all these models of crypt organization suggest that cell position is an important factor in determining the probabilities for self-maintenance or “removal” to differentiation pathways (38).

Analyses of the Origins of Stem Cells and the Fate of their Progeny

A number of fundamental questions related to the intestinal epithelial cell population have recently been addressed using

mouse aggregation chimeras. These studies have (a) provided insights about whether stem cells in a given crypt are derived from one or more progenitors, (b) examined how and when a stem cell zone is established, (c) delved into the issue of whether stem cells are pluripotent or unipotent, and (d) defined the migration pathways of differentiating cells as they travel from the crypt to villus.

A chimeric mouse, composed of cells of two different genotypes, can be created by "aggregating" four to eight cell embryos, each representing a distinct strain. Methods for producing these mice were described by Mintz (29). Schmidt, Ponder, and their coworkers have used such mice to study several aspects of intestinal epithelial biology (37, 47, 48, 50). In "designing" chimeras, they took advantage of the fact that a single locus on mouse chromosome 11 (*Dlb-1*) regulates expression of certain carbohydrate moieties on the surface of gut epithelial cells (59). These structural groups are recognized by *Dolichos biflorus* agglutinin (DBA)² which binds to terminal nonreducing *N*-acetylgalactosamine residues (11, 35, 47, 59). Histochemical studies of certain inbred mouse strains (e.g., C57BL/6) using DBA-peroxidase conjugates revealed that they express the lectin binding site on intestinal epithelial cell surfaces while other strains (e.g., CBA, SWR, DDK) do not express these DBA binding sites. Therefore, aggregation of four- to eight-cell embryos from DBA⁺ and DBA⁻ strains results in a mouse chimeric for intestinal epithelial DBA reactivity which can then be used to ascertain the genotypes of its crypt and villus cell populations (46, 47, 49). Other strain-specific markers that are expressed in the intestine (e.g., H-2; reference 36) can also be simultaneously analyzed in this chimeric mouse.

Ponder et al. (37) examined adult mice that were chimeric for the DBA and H-2 markers and determined that the epithelial population in each small intestinal or colonic crypt was composed of cells of a single genotype; i.e., they were monoclonal. Based on these findings, they concluded that each epithelial cell in a fully formed crypt must be derived from a single progenitor. This progenitor cell gives rise to the stem cells that provide for cellular renewal within the crypt. Their data are consistent with the unitarian theory of Cheng and Leblond (7) which postulates that the crypt contains pluripotent stem cells which can give "birth" to each of the four terminally differentiated cell lineages.

The in situ DBA marker system present, their adult mouse aggregation chimeras allowed Schmidt et al. to examine the cell migration pathways of differentiating stem cell descendants (48). They noted that cells move in relatively straight lines from the crypt orifice towards the apex of small intestinal villi. These vertical sheets contained cells of a single genotype (i.e., they were DBA⁺ or DBA⁻) and had a width that was generally greater than one quarter of the villus circumference. A significantly higher rate of "sheet interruption" by cells of the opposite genotype was noted in duodenal compared to ileal villi. Interruption was confined to the distal two-thirds of duodenal villi. These observations suggested that in duodenal villi, cellular loss (or extrusion) occurs below the apex, accounting for their tapered appearance, while in the ileum a true "apical extrusion zone" exists (48). Cell exiting colonic crypts in these chimeric mice were confined to a narrow, hexagonal-shaped cuff of surface epithelium.

2. Abbreviations used in this paper: DBA, *Dolichos biflorus* agglutinin; FABP, fatty acid binding protein; hGH, human growth hormone.

Since the colonic epithelium lacks villi, the well-defined nature of surface cuffs indicate that (a) cell migration patterns are also highly ordered in the large intestine and (b) the surface epithelium surrounding colonic crypts represents its villus homolog (48).

Schmidt et al. have extended these studies of mouse aggregation chimeras to the perinatal period (50). During the final few days of the 21–22-d gestation, the mouse (and rat) intestine exhibits remarkable morphologic alterations: its "simple" stratified columnar epithelium is converted to a monolayer which undergoes cytodifferentiation and expansion as nascent villi appear (28, 58). Crypts develop during the early postnatal period from the flat intervillus epithelium (28). Analysis of chimeric mice during the perinatal period revealed fundamental differences between the cellular organization of neonatal and adult crypts. In contrast to their findings in adult chimeras, Schmidt et al. noted (50) that crypts contain cells of mixed genotype during the early postnatal period (i.e., they are polyclonal). However, "purification" to monoclonality occurs by the end of the second postnatal week. This replacement process, which results in population of each crypt with the progeny of only one progenitor, provides several clues about the mechanisms which lead to establishment of the stem cell zone (50). First, generation of monoclonal crypt populations may be intimately related to changes in crypt depth which occur in the immediate postnatal period. As noted above, self renewal probability is highest at lower cell positions in the crypt: cells located at higher positions in a crypt are more likely to be lost. According to this logic, a cell situated at the base of a nascent, shallow neonatal crypt would have the highest probability for self-renewal although functional "anchorage" may only be possible after a critical crypt depth has been achieved (38, 50). Second, the data suggest that "anchorage" of multiple stem cells in each crypt may follow the purification process. If several stem cells of different genotype were fixed in the nascent crypts of these chimeric mice before "purification", it would appear less likely that monoclonality could be achieved since one would have to invoke a selective, permanent suppression of division or a "death program" that affects all but one cellular genotype.

Unfortunately, it is unclear how the purification process occurs. The villus transit time during the first postnatal week in mice is much longer than in adults (11–14 d compared with 2–3 d; reference 1). During the course of their study of neonatal chimeric mice, Schmidt et al. (50) observed that assembly of coherent, vertically oriented sheets of clonally derived villus epithelial cells did not take place until the 14th day after birth. This may reflect the time required to "expel" nonretained stem cells and their progeny from the crypt-villus axis.

Regulation of Regional Differences in Gene Expression along the Proximal to Distal and Crypt to Villus Axes of the Intestine

The studies with mouse aggregation chimeras raise an additional central question concerning gut enteric biology; namely what molecular mechanisms are responsible for producing regional differences in gene expression within this continuously proliferating epithelium. Experimental analysis of the mechanisms that produce cell-specific and geographic expression have been limited by the obvious problem

of reproducing the spatial characteristics and complex differentiation pathways of the intestinal epithelium in a cell culture system.

Transgenic mice represent an alternative experimental system for gaining insight about how intestine-specific, cell-specific, region-specific and even developmental stage-specific gene expression are achieved. Genes encoding two homologous 15-kD polypeptides which are believed to participate in the uptake and metabolic processing of long chain fatty acids (liver and intestinal fatty acid binding proteins [L-FABP and I-FABP]; references 25, 53) were recently selected to begin this type of study because (a) they both are efficiently transcribed in the adult rat and mouse intestinal epithelium, (b) exhibit regional differences in their expression from the crypt to villus tip and from duodenum to colon, and (c) have distinctive patterns of expression among the four terminally differentiated cell types (54, 55). Portions of the 5' nontranscribed regions of these *rat* genes were linked to a reporter; the human growth hormone (hGH) gene minus its regulatory elements. The patterns of hGH expression were subsequently analyzed in a number of pedigrees of adult transgenic mice containing these fusion genes using RNA blot and solution hybridization techniques as well as immunocytochemical methods (54, 55). The hGH reporter was used because its protein product can be readily distinguished from any sequences normally produced by the gut epithelium, and because its pattern of trafficking may provide information about protein sorting in the secretory pathways of enterocytes plus goblet, enteroendocrine and Paneth cells (see below).

Functional mapping studies of the rat L-FABP gene (54) revealed that nucleotides -596 to +21 are sufficient to produce efficient expression of hGH in the liver and intestinal epithelium. A duodenal to ileal gradient in hGH mRNA and protein concentration is established by these *cis*-acting elements which mimics that of the endogenous murine L-FABP gene products. Moreover, expression of hGH occurs in the appropriate, terminally differentiated cell types. In liver, synthesis is confined to hepatocytes. In intestine, light microscopic studies indicate that high levels of hGH accumulate within enterocytes and even higher levels in enteroendocrine cells (just as is the case for L-FABP). While expression of the reporter is directed to the appropriate terminally differentiated villus epithelial cell populations, inappropriate synthesis occurred in the less differentiated cells of the crypts of Lieberkühn (54). *Cis*-acting elements contained in nucleotides -596 to +21 of the rat L-FABP gene also produced inappropriately high levels of hGH expression in the colonic epithelium and ectopic production of the reporter in proximal renal tubular epithelial cells.

When nucleotides -4,000 to -597 of the rat L-FABP gene were added to the L-FABP/hGH fusion in either orientation and the resulting "long promoter" construct used to create several pedigrees of transgenic mice, anomalous expression of hGH in the kidney was prohibited. A "normal" proximal to distal (or "horizontal") hGH gradient was also restored by lowering the concentration of its mRNA in the colonic epithelium without significantly altering its absolute levels or relative distribution within the small intestine (54). These upstream elements did not, however, block hGH synthesis in the crypts of Lieberkühn and thereby produce an appropriate "vertical" (crypt to villus) gradient of reporter gene expression.

Together these observations indicate that *cis*-acting sequences located in the 5' nontranscribed region of this (L-FABP) gene can direct efficient, cell-specific expression in the small intestinal epithelium and that several different elements are required to generate an appropriate proximal to distal gradient in gene expression: those elements located within the first 0.6 kb to the start site of transcription are sufficient to establish a "horizontal" gradient in the small intestine while upstream, orientation-independent, *cis*-acting suppressor elements are required to prohibit (L-FABP) gene expression in the colon. Moreover, the data suggest that distinct mechanisms may be responsible for generating gradients in L-FABP expression along the horizontal and vertical axes of the gut.

Comparable functional mapping studies were also performed on the 5' nontranscribed region of the homologous rat I-FABP gene (55). Nucleotides -1,178 to +28 limit expression of the hGH reporter to the intestine. In addition, the pattern of cell-specific and geographic expression of hGH perfectly mimicked that of the endogenous murine I-FABP gene in both axes of the gut. Nucleotides -277 to +28 of the rat I-FABP gene also directed appropriate organ-specific, cell-specific, and crypt to villus expression. However, while the levels of hGH mRNA were equivalent to those of I-FABP mRNA in the proximal half of the small intestine, reporter mRNA levels were ~250-fold lower than I-FABP mRNA in its distal half (and ~100-fold lower than the levels of hGH mRNA documented this portion of the small bowel of transgenics with the "long promoter" I-FABP/hGH fusion gene). This inefficient distal small intestinal expression could either reflect an insertion site effect or the loss of critical upstream, *cis*-acting sequences (2, 55).

A remarkable and unexpected observation was made when serum hGH levels were measured in the various I-FABP/hGH transgenic pedigrees (55). Serum hGH levels were 1,000-fold lower in the "short promoter" I-FABP/hGH transgenic mice compared to animals with the "long promoter" transgene, indicating that efficient, distal small intestinal expression may be necessary to produce elevated serum hGH concentrations. This observation raises the possibility that the export pathway of this prototypic secretory protein differs within a given cell type as a function of its location along the proximal to distal axis of the small intestine.

A curious and unexpected "mosaicism" in hGH expression was encountered within the intestinal epithelium of both male and female adult mice (from multiple pedigrees) who were obligate heterozygotes for each of these various L-FABP/hGH and I-FABP/hGH transgenes (54, 55). In strains containing L-FABP/hGH fusion genes, this "mosaicism" was most notable in the colonic epithelium which normally does not support transcription of the intact, endogenous mouse L-FABP gene (Fig. 2). While transgenic mice containing nucleotides -596 to +21 of the rat L-FABP gene linked to the reporter DNA generally exhibit anomalously high levels of hGH accumulation within most colonic crypts, rare crypts did not express any hGH (see Fig. 2 A). In contrast, rare hGH positive colonic crypts were noted in mice containing nucleotides -4,000 to +21 of the rat L-FABP gene (54). In each case, the pattern of reporter expression indicated that virtually all of the epithelial cells in a single crypt manifested an "aberrant" pattern of transgene expression. Comparable mosaic patterns of hGH expression was observed in the small

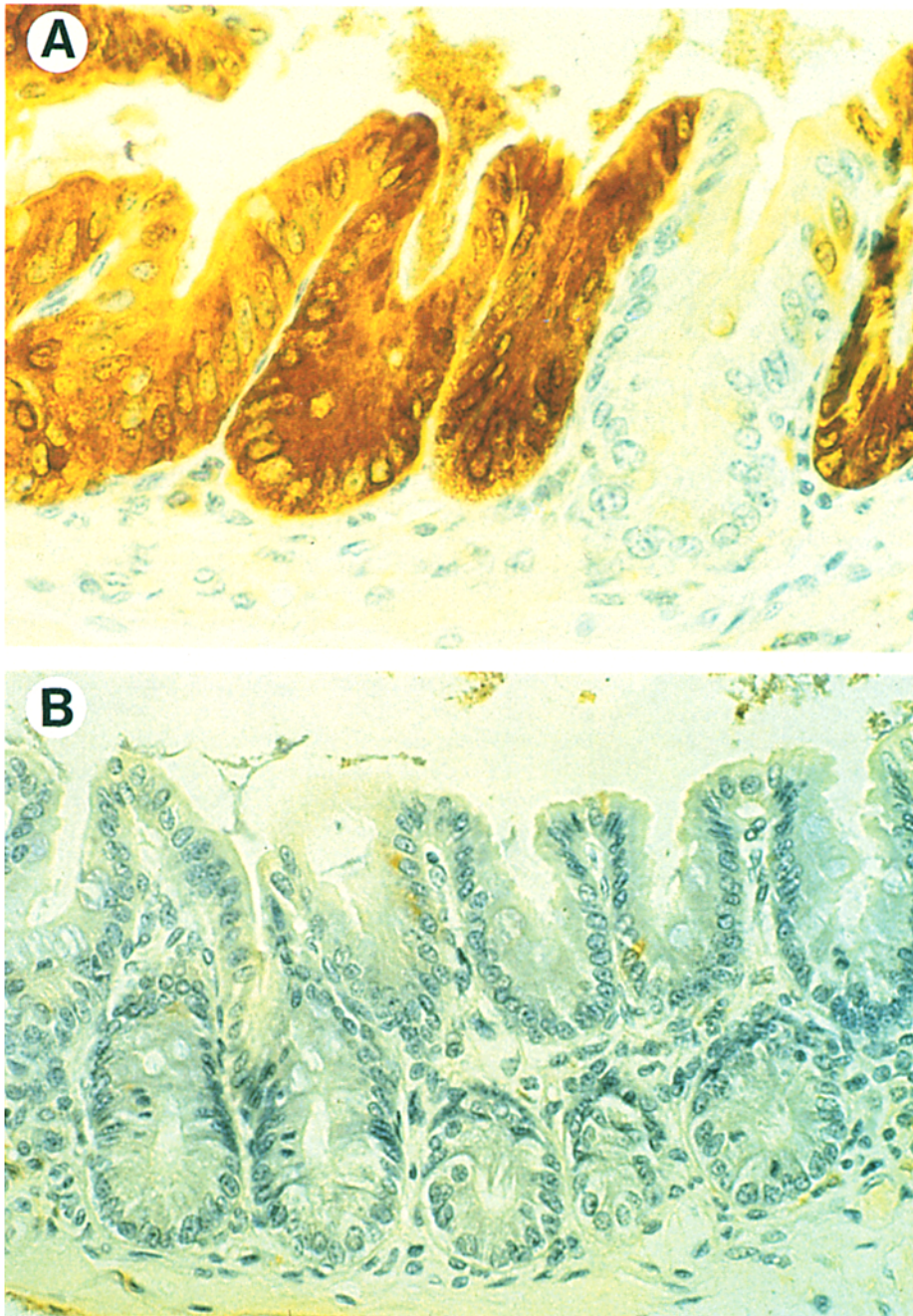


Figure 2. Mosaic patterns of hGH expression in the intestinal epithelium of transgenic mice that are known heterozygotes for L-FABP/hGH and I-FABP/hGH transgenes. (A) Nucleotides -596 to $+21$ of the rat L-FABP gene were fused to the human growth hormone gene (beginning at nucleotide 3 of its first exon; see reference 54). A section of cecum from a male transgenic mouse is shown after staining with a monospecific, polyclonal antibody directed against human growth hormone. The peroxidase antiperoxidase method was used to visualize immunoreactive protein as brown material (54). Sections were counterstained with hematoxylin. Rare crypts contain a monoclonal population of cells that do not express hGH. (B) Section of cecum from the same transgenic mouse incubated with L-FABP antisera.

intestine of male and female transgenic mice who were heterozygous for the short promoter I-FABP/hGH fusion gene (55). This type of mosaic staining pattern was not observed with the endogenous I-FABP or L-FABP gene products (see Fig. 2 B). The mechanisms underlying "mosaic" transgene expression in these mice are not known at present. Possibilities include an unexpectedly high rate of somatic cell mutation or the absence of critical *cis*-acting elements. There is some evidence that favors the latter notion (55). Lack of *cis*-acting elements may make the transgenes more susceptible to differences in the levels of positive or negative *trans*-acting factors. These differences may exist in (anchored) stem cells and/or are manifest during cellular commitment/differentia-

tion. The usefulness of studying regulation of intestinal gene expression in transgenic mice is emphasized by the observation that subtle differences in the "regulatory environments" of cells may not affect endogenous genes at their normal chromosomal location but may be detectable *in vivo* using transgenes as markers.

While these analyses of the mechanisms underlying regional differentiation of the intestinal epithelium have focused on adult transgenic animals, recent work suggests that they should also be conducted during late gestation and during the early postnatal period. Because activation of the L-FABP and I-FABP genes occurs coincident with the initial differentiation of the gut epithelium during late fetal life (43),

they are useful differentiation markers. Immunocytochemical studies have disclosed a remarkably heterogeneous pattern of cell-specific expression of *rat* L-FABP, I-FABP as well as a homologous cellular retinol binding protein (CRBP II; reference 22) during the last 4 d of the 22-d gestation period (43). Some columnar epithelial cells express high levels of a particular hydrophobic ligand binding protein while morphologically similar cells, occupying similar topologic positions along a nascent villus, have low to undetectable levels. The onset and resolution of this heterogeneous expression varies somewhat between I-FABP, L-FABP, and CRBP II in the proximal small intestine although it resolves by the first postnatal day. The same heterogeneity was observed in the distal half of the small intestine but its onset and resolution is delayed by 1–2 d relative to the proximal small bowel, contributing in large part to the initial establishment of proximal to distal gradients in expression of these genes. When double-label immunofluorescent techniques were used (43), it became apparent that the late gestation rat intestine contains several operationally definable populations of enterocytes: some of which express none, one, or more of the proteins. This is not a phenomena that affects all genes that are expressed in fully differentiated enterocytes. No cellular heterogeneity was noted for two other proteins which accumulate in the late fetal rat intestinal epithelium: apolipoproteins AI and AIV (43). The heterogeneous patterns of accumulation of the hydrophobic ligand-binding proteins could reflect the presence of cells of mixed clonality on the emerging villus which have slightly different genetic programs for differentiation. Whatever the underlying mechanism, it appears that the FABP gene products are sensitive indicators of differences in fetal intestinal epithelial cell differentiation and that the timing of this differentiation program is different in the proximal and distal halves of the small intestine. Interestingly, a similar heterogeneous pattern of expression has been noted to affect some, but not all genes in differentiated, enterocyte-like HT-29 cells (18). These cells may therefore provide a model system for identifying specific *trans*-acting factors which contribute to this developmental phenomenon. Understanding such phenomena may also provide critical insights about which factors “allow” cells to activate specific genes as they traverse a particular topologic position in the crypt-villus axis.

Future Directions

Transgenic mice will continue to provide a necessary assay system for identifying *cis*-acting elements that produce appropriate geographic expression within the gut epithelium. Preparation of nuclear extracts from different regions of the horizontal and vertical axes of the gut may provide a strategy for defining candidate *cis*-acting sequences that mediate its regional differentiation. Transgenic mice should also be employed to “audit” the results of promoter mapping experiments that use currently available intestinal cell lines since these lines may not precisely mimic the genetic environment of one or more of the terminally differentiated cell types present in the gut epithelium.

While functional mapping studies of promoters using transgenic mice have led to the conclusion that *cis*-acting sequences can produce regional variations in gene expression, they have raised additional questions about the nature of the *trans*-acting factors which help define geographic location in the

gut. The relative contributions of intracellular and extracellular (e.g., luminal) factors could be surveyed using intestinal isografts. This technique involves isolation of intestinal segments from fetuses and their subsequent placement under the subcutaneous tissue or renal capsules of syngenic adult animals (12, 20, 21, 30). Examination of the synthesis of reporter and endogenous gene products in the horizontal and vertical axes of (fetal) transgenic mouse isografts after their implantation into normal animals may provide information about whether luminally derived factors are required to achieve regional differences in transcriptional regulation by particular *cis*-acting sequences.

Analysis of the temporal and regional patterns of transgene expression in the late gestation fetus may be a useful way of (a) mapping *cis*-acting “chronotropic” signals, (b) determining if *cis*-acting elements which modulate geographic differences in the horizontal and vertical axes of the adult gut also operate during this critical period of gut development, and (c) unmasking and examining subtle differences which may exist in the differentiation programs of the polyclonal crypt population. Finally, more detailed studies of chimeric (and possibly transgenic) mice are needed to better understand the mechanisms underlying crypt purification. This should lead to additional insights concerning the process(es) by which stem cells are anchored.

Three cell lines derived from human colon carcinomas (CaCO-2, HT-29, and T-84) are capable of acquiring phenotypes that resemble those of differentiated enterocytes and goblet cells (8, 26, 34). HT-29 cells appear to be multipotent, capable of giving rise to absorptive and mucus-secreting subclones (18, 33). The parental line, together with these subclones, may be appropriate models for identifying factors that regulate the processes of commitment and terminal differentiation. An experimental strategy conceived by Palmiter et al. (32) may also prove useful for directly noting the pathways by which various lineages emanate from the multipotent murine intestinal stem cell. These workers deleted specific (pancreatic) cell lineages in transgenic mice by linking a cell-specific enhancer/promoter to a reporter gene that encodes a toxic gene product; the diptheria toxin A polypeptide.

The ability to deliver foreign gene products to the various cell types in the gut epithelium will also provide an important tool for examining protein sorting *in vivo* as a function of cell type, geographic location, and degree of differentiation. Experiments using foreign proteins known to be constitutively secreted or proteins that are anchored to specific membranes (e.g., via phosphatidylinositol-linkages; references 16, 17, 24, 56) may help define default pathways for export in the various cell types and the mechanisms that underlie polarized targeting (23).

The capacity to deliver foreign proteins to the continuously proliferating gut epithelium of transgenic mice may also allow analysis the mechanisms underlying regional differences in its susceptibility to neoplastic transformation (such transformation is much more frequent in the colon compared to the small intestine). Recent studies of colorectal tumors have noted high incidence of point mutations in the *ras* protooncogene (e.g., 5, 13, 60). Gut-specific and region-specific promoters would allow one to determine the effects of introducing these oncoproteins into cells located in different areas of the proximal to distal (as well as crypt to villus)

axes of the intestine. Similar experiments with SV-40 T antigen (14) may be helpful in generating transformed intestinal epithelial cell lines which are more differentiated than those currently available.

In summary, the ability to produce chimeric and transgenic mice has provided powerful experimental tools which should continue to prove extremely useful in deciphering the answers to a number of fundamental biologic questions related to the well-organized, continuously proliferating, regionally differentiated gut epithelium.

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