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Use of transgenic mice to infer the biological properties of small intestinal stem cells and to examine the lineage relationships of their descendants

(gut stem cells/epithelial differentiation/transgenic mice/enteroendocrine cells)

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ABSTRACT **Transgenes, composed of elements of the 5′ nontranscribed region of the liver fatty acid-binding protein (L-FABP) gene linked to various reporters, have previously been used to explore the cellular, regional, and temporal differentiation of the mouse intestinal epithelium. In this report, we have analyzed a pedigree of L-FABP/human growth hormone (hGH) transgenic mice that display a stable, heritable, mosaic pattern of reporter expression: wholly hGH-positive or hGH-negative populations of differentiating enterocytes arise from hGH-positive or hGH-negative crypts, respectively, and migrate as vertical coherent bands up the villus producing striped (polyclonal) villi. The ability of enteroendocrine cells within a given villus stripe to support hGH expression coincides with the enterocytic reporter phenotype, suggesting that these two terminally differentiated cells arise from a common multipotent stem cell. hGH-negative crypts are nonrandomly distributed around each villus and their frequency increases along the duodenal-to-ileal axis. Statistical analysis of the observed villus striping pattern suggests that transgene expression is not independently determined in individual crypts but rather in multicrypt "patches." The intact endogenous mouse L-FABP gene (*Fabpl*) exhibits a similar striped villus pattern of expression in a portion of the distal small intestine. These studies indicate that *Fabpl* and L-FABP/hGH transgenes represent sensitive markers for exploring the biological properties of gut stem cells and how positional information is encoded in this rapidly and continuously renewing epithelium.**

Much of what is known about the biological properties of stem cells comes from studies of the hematopoietic system, where functional assays of clonal regeneration are available. Stem cells have been characterized as having a high capacity for self-renewal throughout their life (reviewed in ref. 1). Their potential for asymmetric division yields progeny with distinct fates: i.e., one daughter remains a stem cell (ensuring self-maintenance), whereas the other is committed to a differentiation program. Whether differentiation is viewed as an irreversible process of binary decisions or a reversible one requiring continued active regulation (2), it is a relative term that may be operationally defined as the acquisition of new gene products ultimately leading to functional competency (maturation) (1). Stem cells may be uni- or multipotent. The latter are able to produce, by division, another multipotent stem cell as well as descendants (committed progenitors, transit cells) that have features intermediate between those of the stem cell and their own differentiated, mature progeny.

Less is known about the properties of gut stem cells, primarily because of limited success with clonogenic assays (3). The extraordinarily rapid, continuous regeneration of the mouse intestinal epithelium, together with its precise spatial organization (4), make it an attractive and uniquely powerful system for describing stem cell biology. In the adult mouse, each small intestinal villus contains ≈3500 cells representing three of the gut's four principal lineages: absorptive columnar enterocytes, mucus-producing goblet cells, and enteroendocrine cells. The villus' epithelial population is maintained by 6–10 adjacent crypts composed of ≈250 cells each (1). [^{3}H]Thymidine labeling studies indicate that (*i*) ≈150 of these cells, located in the midcrypt region, pass through the cell cycle every 12 hr, resulting in the generation of 300 new cells per day per crypt; (*ii*) ≈12 cells depart each crypt per hr and move upward at a remarkably rapid initial rate of 0.75 cell positions per hr; and (*iii*) differentiation/maturation of enterocytes, goblet cells, and enteroendocrine cells occurs during this migration, which is completed within 3 days, when cells are extruded at, or near, the apex of the villus. The fourth principal gut epithelial lineage consists of lysozyme- and defensin-producing Paneth cells, which differentiate during a downward migration to the crypt base, where they have a residence time of 3 weeks. The process of migration/differentiation/exfoliation is perpetual and rapid—each villus sheds 1400 cells per day, resulting in a total loss of 2×10^8 cells per adult mouse small intestine per day (1). [^{3}H]Thymidine labeling studies suggest that this renewal is sustained by multipotent stem cells functionally anchored in a niche located near the bottom of each crypt above the Paneth cells (1).

The pathways of gut epithelial cell renewal and differentiation have been further characterized by studies of mouse aggregation chimeras formed from inbred strains that do or do not express a receptor for the *Dolichos biforins* agglutinin (DBA). This lectin-binding protein is encoded by the *Dlb-1* locus: the *Dlb-1^a* allele is associated with absence of DBA binding in crypt and villus epithelial cells. Mice with the *Dlb-1^b* allele are DBA-positive. Analyses of adult chimeras formed from *Dlb-1^a* and *Dlb-1^b* homozygotes have indicated that (*i*) their crypts are monoclonal, containing either wholly DBA-negative or wholly DBA-positive, but never mixed, populations (5) and (*ii*) enterocytes and goblet cells derived from each crypt migrate in vertical coherent bands up the associated villus, where they appear as uninterrupted stripes of wholly DBA-positive or DBA-negative reactivity (6). An important caveat to these studies was that enteroendocrine cells were not scored for DBA reactivity owing to their low level of lectin binding and their small numbers.

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Abbreviations: L-FABP, liver fatty acid-binding protein; hGH, human growth hormone; DBA, *Dolichos biforins* agglutinin; EtNU, *N*-ethyl-*N*-nitrosourea; CCK, cholecystokinin; GLP, glucagon-like peptide; GIP, gastric inhibitory peptide.

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C57BL/6J × SWR F_1 mice that are *Dlb-1*^{a}/*Dlb-1*^{b} heterozygotes have been used to assess the number of active (renewing) stem cells per crypt (7–10). Such mice will contain an intestinal epithelium that stains positive with peroxidase-labeled DBA. A mutation in the *Dlb-1*^{b} allele of a gut stem cell may result in loss of DBA binding that, in turn, would be evident in all of its descendants. If such F_1 mice are treated as adults with a single dose of the potent mutagen *N*-ethyl-*N*-nitrosourea (EtNU), single wholly negative crypts accumulate for ≈12 weeks—reaching a steady-state level of ≈14 per 10^5 crypts 21 weeks after therapy that persists for at least 20 more weeks (10). This compares to a spontaneous rate of accumulation of 0.37 per 10^5 crypts every 4 weeks in untreated mice. Mixed crypts are also observed after EtNU administration, reaching a peak level of 6 per 10^5 crypts (12-fold over background). Mixed crypts do not persist, presumably undergoing "purification" to a single phenotype. These observations suggest that the small intestinal crypt may ultimately be renewed by a single stem cell.

We have utilized transgenic mice containing fusion genes consisting of different portions of the 5′ nontranscribed domain of the rat liver fatty acid-binding protein (L-FABP) gene linked to the human growth hormone (hGH) gene to characterize cis-acting elements that establish and maintain cell-specific and region-specific transcription in the gut epithelium (11–16). The pattern of L-FABP/hGH transgene expression has allowed us to identify subtle differences in the differentiation programs of certain cellular populations in the gut that were not evident from an analysis of endogenous gene expression (12, 13, 16). The transgenes have also uncovered subtle temporal changes in the characteristics of intestinal epithelial cell populations (15). In this report, we have characterized members of one pedigree of L-FABP/hGH transgenic mice that exhibit an unusual, heritable mosaic pattern of reporter expression. Our studies indicate that this model system provides a sensitive tool for further characterizing the organization of the small intestinal epithelium without the need for mutational probes.

MATERIALS AND METHODS

Animals. Transgenic mice containing nucleotides −596 to +21 of the rat L-FABP gene linked to the hGH gene at its nucleotide +3 were derived as described in ref. 11. Mice were fed a standard chow diet ad libitum. Transgenic animals (identified by Southern blot analysis of tail DNA; ref. 11) and their normal littermates were sacrificed by cervical dislocation 5–10 months after birth. The small intestine was divided as described in ref. 11 and then fixed in Bouin's solution.

Immunocytochemistry. Five-micron-thick, paraffin-embedded or 10-μm thick cryostat sections were prepared in the horizontal (proximal-to-distal) and vertical (crypt-to-villus) planes. Diluted primary antisera were applied overnight at 4°C and subsequently detected with gold-labeled secondary antibodies and silver enhancement and/or with fluorescent labeled secondary antibodies. The sources of antisera and the methods used to establish their specificities are described in previous papers (12–14).

RESULTS

A Pedigree of L-FABP$^{-596\ to\ +21}$/hGH Transgenic Mice Exhibits a Heritable, Mosaic Pattern of Transgene Expression in the Small Intestinal Epithelium. In the adult mouse, *Fabpl* is "activated" as enterocytes exit the crypt and move to the base of small intestinal villi (11). It is expressed throughout their subsequent upward migration/maturation. Enterocytic levels of L-FABP are highest in the proximal jejunum and fall progressively to the distal ileum (11). *Fabpl* remains silent in the colonic epithelium throughout life. Nucleotides −596 to +21 of the rat L-FABP gene are able to recapitulate this proximal-to-distal gradient of small intestinal enterocytic expression when linked to various reporters (11–16). These 600 base pairs (bp) of the rat *Fabpl* gene also contain the cis-acting element(s) required to establish its normal developmental pattern of activation between the 15th and 16th day of fetal life (14). However, young adult (3- to 6-month-old) transgenic mice exhibit inappropriate expression of the reporter in the upper half of the crypt (11, 17), in various small intestinal enteroendocrine populations (12, 13, 16, 17), and in the colonic epithelium (11, 15). With increasing age, colonic expression is extinguished in a distal to proximal "wave" that produces silencing of reporter synthesis in multiple contiguous crypts (patches). This extinction phenomenon is not an insertion site effect as it occurs in several pedigrees of L-FABP/hGH mice and does not extend to involve the small intestinal enterocyte or enteroendocrine populations (15).

We have now identified one pedigree of L-FABP$^{-596\ to\ +21}$/hGH mice that exhibits a curious pattern of transgene expression; villi appear in horizontal sections to be interrupted by stripes of hGH-negative cells (Fig. 1*A*). Vertical sections reveal that these hGH-negative stripes extend from the apical extrusion zone to wholly hGH-negative crypts (Fig. 1*B*). Multiple serial sections in either plane confirm that these stripes are uninterrupted (coherent) columns of cells highly reminiscent of the DBA bands observed in mouse aggregation chimeras or in mutagen-treated *Dlb-1*^{a}/*Dlb-1*^{b} F_1 heterozygotes. The number and width of hGH-negative stripes per villus can be correlated with the number of wholly negative crypts contributing to that villus. Serial sectioning along the horizontal plane revealed patches of multiple, contiguous, wholly hGH-negative crypts (Fig. 1*C*). Male and female adult (5–10 months old) mice who are obligate heterozygotes for this transgene display this phenomenon. It is heritable from generation to generation. This pedigree had no obvious rearrangements of *Fabpl* or of the transgene, as judged by Southern blot analyses of tissue DNAs. A separate pedigree having comparable transgene copy number does not exhibit villus striping (data not shown).

Enteroendocrine Cells and Enterocytes Within a Given Villus Stripe Display Identical Reporter Phenotypes. Analysis of the pattern of hGH expression in enterocytes and enteroendocrine cell populations located within a stripe offers an opportunity to assess whether they are affected by common deterministic events and to infer whether they share a common lineage. Previous studies of adult mice have shown that expression of L-FABP$^{-596\ to\ +21}$/hGH varies widely between small intestinal enteroendocrine subpopulations and is influenced by their position along the duodenal-to-ileal and/or crypt-to-villus axes and by luminal factors (12–14, 16). Therefore, we prepared vertical and horizontal sections from duodenal, jejunal, and ileal segments recovered from six mice representing four separate litters of L-FABP$^{-596\ to\ +21}$/hGH transgenics. Individual sections were first stained for serotonin, substance P, secretin, gastrin, cholecystokinin (CCK), glucagon-like peptide (GLP), gastric inhibitory peptide (GIP), or neurotensin and subsequently stained for hGH. Doubly stained sections were then examined for the distribution of immunoreactive enteroendocrine cells within hGH-positive and hGH-negative enterocytic stripes (e.g., see Fig. 1 *D–F*). Within hGH-positive stripes, the percentage of enteroendocrine cells supporting transgene expression varied between the eight populations surveyed: >50% in GIP-, secretin-, gastrin-, CCK-, and GLP-1-immunoreactive cells; <50% in serotonin-, substance P-, and neurotensin-immunoreactive cells. Of the >8000 hGH-positive enteroendocrine cells examined, only 3 cells were found in hGH-negative stripes. This precise overlap is consistent with a common stem cell origin for enteroendocrine cells, enterocytes (and by extrapolation from earlier studies), goblet cells, and Paneth cells. Such a conclusion was not possible for

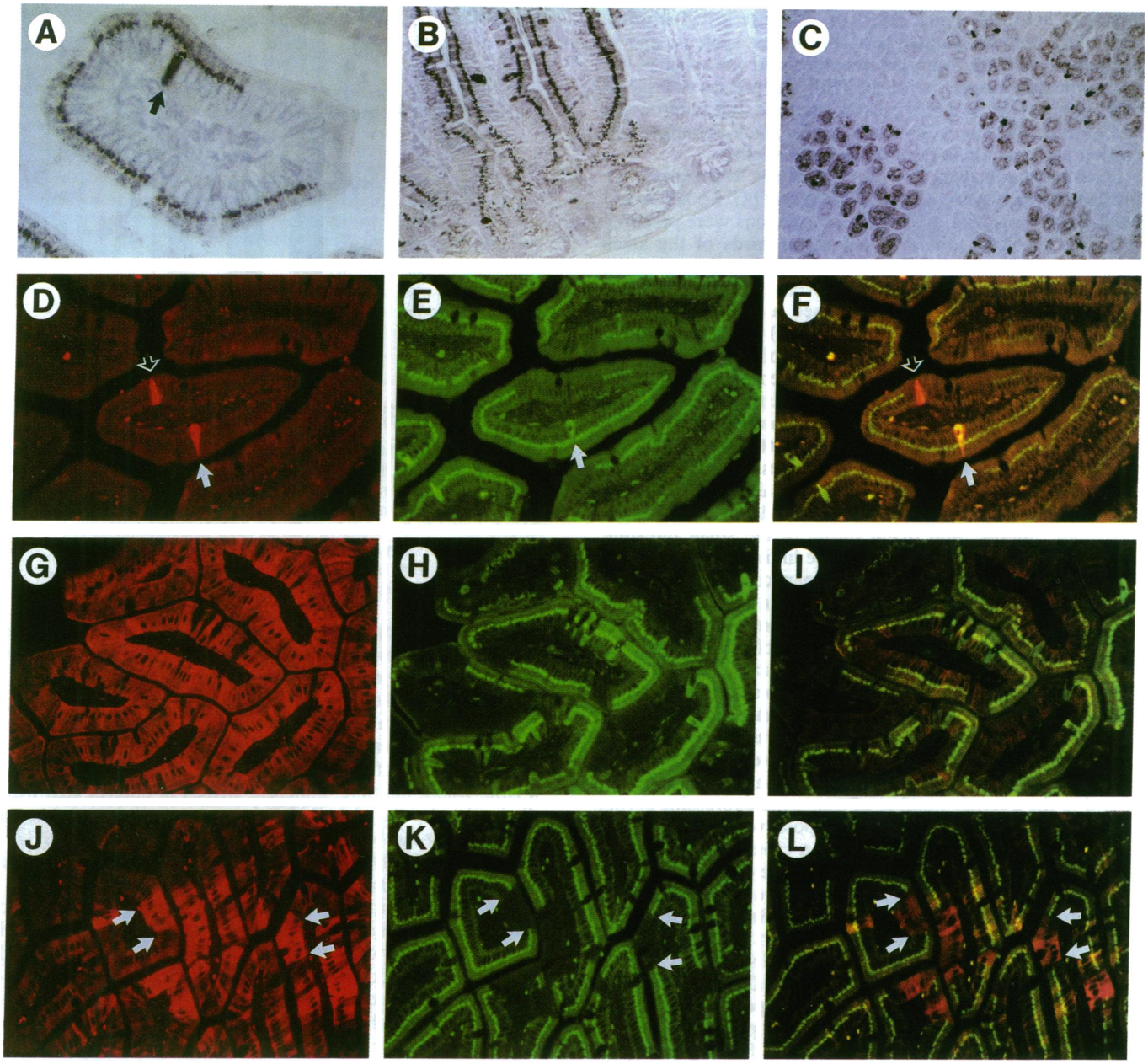

FIG. 1. Immunohistochemical localization of reporter gene expression in the small intestine of L-FABP$^{-596\ to\ +21}$/hGH transgenic mice. (*A–C*) Sections were incubated with rabbit anti-hGH serum and detected with gold-labeled goat anti-rabbit serum; this was followed by silver enhancement and light hematoxylin counterstaining. (*A*) A "horizontal" section of an intestinal villus shows a hGH-immunoreactive enteroendocrine cell (indicated by the arrow) within a band of enterocytes possessing intense supranuclear (Golgi) hGH immunoreactivity. Note the absence of hGH-immunoreactive enteroendocrine cells in the adjacent hGH-negative enterocytic bands. (*B*) A "vertical" section demonstrates hGH expression in the upper crypt zone of *several* contiguous crypts. These cells extend as coherent stripes to the overlying villi. This "patch" of wholly hGH-positive crypts is bordered by patches of hGH-negative crypts (seen on the right of the panel). (*C*) A horizontal section through the upper crypt zone reveals clusters of similar staining crypts. Serial horizontal sections indicate that crypts give rise to bands of like-staining villus-associated cells. (*D–F*) Examination of enteroendocrine cell subpopulations with dual-labeling techniques indicates that hGH expression is restricted to hGH-positive enterocytic bands. A section from the proximal small intestine was coincubated with rabbit anti-CCK and goat anti-hGH sera and visualized with Texas red-labeled donkey anti-rabbit and fluorescein-labeled donkey anti-goat sera. Two CCK-immunoreactive cells are seen in a single villus in *D* and are indicated by arrows. hGH immunoreactivity in the identical section (*E*) shows a band of positive enterocytes that contains a hGH-immunoreactive enteroendocrine cell (indicated by an arrow). Dual exposure (*F*) reveals that the CCK-immunoreactive cell within the hGH-positive enterocytic band supports transgene expression (closed arrow), whereas the one located in the hGH-negative enterocytic band lacks detectable levels of the hGH reporter (open arrow). (*G–I* and *J–L*) Horizontal sections of villi from the proximal (*G–I*) and distal (*J–L*) small intestine of a transgenic mouse coincubated with rabbit anti-L-FABP and goat anti-hGH sera followed by Texas red-labeled donkey anti-rabbit and fluorescein-labeled donkey anti-goat sera. In proximal villi, diffuse L-FABP immunoreactivity is observed in enterocytes with similar steady-state levels occurring in cells located at a particular stratum along the crypt-to-villus axis (*G*). This homogenous pattern contrasts with the "striped" pattern of hGH immunoreactivity seen in the identical villus (*H*). Dual exposure of the section further illustrates these "discrepant" immunostaining patterns (*I*). L-FABP immunoreactivity in a portion of the ileum (*J*) shows bands of villus-associated enterocytes with widely varying staining intensities. The borders of several such stripes are indicated by arrows. hGH immunoreactivity in the same section shows similar stripes of hGH-positive and hGH-negative enterocytes (*K*). Double-labeling indicates that the boundaries of L-FABP and hGH stripes frequently coincide (*L*). (*A*, ×460; *B*, ×180; *C*, ×90; *D–L*, ×180.)

technical reasons using DBA or other (e.g., glucose-6-phosphate dehydrogenase; ref. 18) marker systems and is compatible with the unitarian hypothesis for the origin of the four principal lineages in the gut (19). The precise overlap of hGH phenotypes in villus stripes suggests that enterocytes and enteroendocrine cells share similar, well-organized migration patterns along the crypt-to-villus axis.

hGH-Positive Crypts Are Not Randomly Distributed. To further describe the spatial relationships of hGH-positive and hGH-negative crypts, multiple cross sections were prepared from the proximal, middle, and distal thirds of the small intestines of these animals and then incubated with rabbit anti-hGH serum. Two parameters were monitored. (*i*) The frequency of positive crypts (defined as possessing hGH-immunoreactive cells in their upper half) was determined in each section. (*ii*) Crypts in the periphery of each cross section were sequentially scored for the presence or absence of immunoreactive hGH and the resultant distribution pattern of positive and negative crypts was recorded. At least 500 crypts from each small intestinal segment from each mouse were examined in this fashion. A progressive decline in the percentage of positive crypts was noted along the duodenal-to-ileal axis (78% ± 3%, 66% ± 3%, and 32% ± 5% in the proximal, middle, and distal thirds of the small intestine, respectively; $\bar{x}$ ± SEM, $n = 4$). The limited variation in the distribution of positive crypts at a particular location along the cephalo-caudal axis of the gut between mice derived from multiple litters and ranging in age from 5 to 10 months suggests that these nonrandom patterns of transgene expression are stably maintained. Our analysis also indicated that transgene expression in *individual* crypts located within a given intestinal segment is not randomly determined. If transgene expression reflected the result of a stochastic process, the distribution of hGH-positive crypts would be expected to follow a Poisson distribution with parameter λ equal to the average number of transgene-expressing crypts. If the process of creating hGH-positive crypts is more complex, then their distribution in a cross section obtained from a given segment might fit a mixture of Poissons significantly better. To test this hypothesis, we used an optimization program based upon the Newton–Rapheson algorithm (20). The results of this analysis for each intestinal segment indicate that a single, random Poisson distribution of hGH-positive crypts can be rejected at $P < 0.001$.

The nonrandom distribution of hGH-positive crypts was independently verified by analyzing the pattern of reporter expression in horizontal sections of villi. If transgene expression is randomly determined in each crypt, the probability that an entire villus' enterocytic population will be wholly hGH-positive is equal to the probability of a given crypt supporting transgene expression raised to the power of the number of crypts supplying cells to that villus. More than 3500 villi were examined and the frequencies of occurrence of wholly positive, wholly negative, and mixed types were noted (Fig. 2). In each segment, χ^2 analysis indicates that the distribution of villus staining patterns is significantly different ($P < 0.001$) from that predicted for contributions from 10 crypts, each of whose probability of supplying hGH-positive cells is randomly determined. Minimum χ^2 estimates of the number of "independently determined crypt units" that contribute cells to a villus range from 2.5 to 3.0 in each of the three intestinal segments analyzed. Since anatomic studies indicate that on average 10 crypts contribute cells to each villus, these results reveal that transgene expression must be coordinately regulated between clusters of crypts.

***Fabpl* Shows Mosaic Patterns of Expression in Ileal Villi.** Single- and double-label immunocytochemical studies of sections prepared from duodenal and jejunal segments indicated that enterocytes in hGH-negative stripes exhibit the same steady-state concentrations of L-FABP as enterocytes

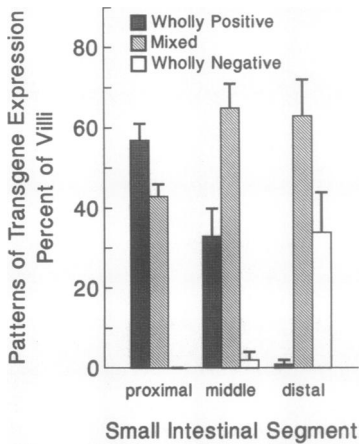

FIG. 2. Spatial patterns of hGH expression in the small intestine of L-FABP$^{-596 \text{ to } +21}$/hGH transgenic mice. Horizontal sections of villi from the proximal, middle, and distal thirds of the small intestine were surveyed for the occurrence of wholly hGH-positive, wholly hGH-negative, and mixed villi ($n = 4$ animals). More than 3500 villi were scored. The mean percentage (+SEM) of wholly positive, mixed, and wholly negative villi in each intestinal segment is presented. In each segment, the villus distribution pattern is significantly different from that predicted if 10 crypts, each with independently determined transgene expression, contributed to each villus.

occupying a comparable position along the crypt-to-villus axis of hGH-positive stripes (Fig. 1 *G–I*). Extensive surveys of duodenal and jejunal sections obtained from adult transgenic mice and their normal littermates failed to reveal any villus stripes when stained with L-FABP antibodies. This was not the case in their ileums. Surveys of this segment revealed a proximal zone of wholly L-FABP-positive villi followed by a zone where multiple, coherent stripes of enterocytes with distinctive levels of L-FABP immunoreactivity appear to extend from the base to the tip of a given villus (Fig. 1*J*). Enterocytes in the distal most segment of the ileum were largely L-FABP-negative.

Double-label studies of the ileal epithelium of transgenic mice indicated that the boundaries of L-FABP and hGH stripes frequently coincide (Fig. 1 *J–L*). Occasionally, a hGH stripe is contained within a broader stripe of L-FABP enterocytes—the reciprocal relationship was also noted. All four possible combinations of L-FABP and hGH levels were observed within these stripes. Thus, *Fabpl* and the L-FABP/hGH transgene represent sensitive markers for differences in the differentiation programs of epithelial cells derived from adjacent monoclonal crypts. Such differences appear to be determined within the crypt and affect the entire enterocytic (and/or enteroendocrine) population during their translocation to the apical extrusion zone.

DISCUSSION

Our analyses of a pedigree of L-FABP/hGH transgenic mice with an unusual mosaic pattern of reporter accumulation in the small intestinal epithelium indicate that (*i*) this transgene is a sensitive lineage marker whose pattern of expression suggests that enterocytes and enteroendocrine cells are derived from a common multipotent stem cell, (*ii*) the 10 crypts that supply epithelial cells to each small intestinal villus do not act as independently regulated entities but in fact exhibit functional coupling definable by the patterns of hGH accumulation in their cellular products, and (*iii*) the position occupied by crypt stem cells along the duodenal-to-ileal axis affects programed differentiation of enterocytes.

Although the striped villi and associated monophenotypic crypts observed in this pedigree of L-FABP$^{-596 \text{ to } +21}$/hGH

transgenic mice are highly reminiscent of the striping that occurs after EtNU treatment of *Dlb-1*^{a}/*Dlb-1*^{b} F_1 mice, the frequency of hGH-negative crypts and stripes is many orders of magnitude greater, providing an independent and more sensitive audit of the biological properties of gut stem cells. The mechanisms that determine whether the transgene is expressed in the descendents of a given crypt's stem cells are unknown. The fact that this is not seen in other pedigrees containing the same transgene suggests the possibility of "position-effect variegation" associated with a spread of heterochromatization—as observed in *Drosophila* (21). Heritable epigenetic modifications (e.g., DNA methylation) may cause or contribute to this phenomenon (22, 23). The extraordinarily well-organized pathways of gut epithelial differentiation along the crypt-to-villus axis, the fact that the progeny of each crypt's multipotent stem cell undergo numerical amplification through four to six rounds of cell division prior to exiting the crypt (24), and the ability to "maintain" (program) a particular quantitative level of transgene expression in all of the enterocytic descendants of a given stem cell during their translocation/differentiation make this type of mosaicism easy to detect in the gut.

The distribution of hGH-negative crypts and their derivative stripes around villi and along the proximal-to-distal axis of the small intestine suggests that the level of transgene expression in a given crypt is not randomly determined. The average number of stripes per villus (≈3) contrasts with the number of crypts that "feed" cells to that villus (≈10). This functional coupling between crypts may reflect a number of mechanisms. If the transgene expression is determined at the level of the single crypt stem cell, coordinate levels of hGH accumulation between adjacent crypts could be due to the fact that their stem cells are derived, in turn, from a common progenitor. The existence of embryonic precursor cells that supply crypt stem cells was invoked after observing that 7- to 11-day fetal *Dlb-1*^{a}/*Dlb-1*^{b} F_1 mice exposed to the EtNU *in utero* developed patches of contiguous DBA-negative crypts (8). Such patches were not noted when adult F_1 mice were treated with mutagen (10). The remarkable frequency of crypt "mosaicism" observed in adult members of this L-FABP$^{-596\ to\ +21}$/hGH pedigree may allow detection of such precursors with a level of sensitivity that simply is not achievable by mutagenesis of the *Dlb-1* allele. Our earlier demonstration (15) that L-FABP/hGH expression is extinguished in the colonic epithelium of adult mice over a several-month period by a wave of suppression that affects multicrypt patches also is compatible with the notion that such precursor cells exist and supply contiguous crypts.

Other explanations can be invoked to account for the apparent coupling of transgene expression between adjacent crypts. Locally acting suppressors could contribute to the observed nonrandom distribution of hGH-negative crypts. For example, these have been described in *Drosophila*, where they interact with homeobox genes to regulate spatial differentiation (25). Homeobox genes exhibit distinct gradients in their expression along the proximal-to-distal axis of the mouse intestine (26). A subset of these genes is only expressed in the gut (*Cdx-1, Cdx-2*). The clustering of like-staining crypts could represent the results of (crypt) budding and fission (27). Such a process occurs following ulceration or radiation damage (28, 29) but in this case would have to occur in nondamaged epithelium, in a proximal-to-distal gradient, and at an extraordinarily high rate.

Positional information appears to be programed into gut stem cells prior to, or coincident with, initial cytodifferentiation of its epithelium in late gestation. Studies with normal and transgenic isografts prepared from embryonic day 15 mice indicate that region-specific cellular differentiation can be established and maintained over at least a 6-week period in the absence of luminal contents (16). These results suggest that an additional property of gut stem cells that distinguishes them from hematopoietic stem cells *may* be their capacity to encode spatial memory or retain such information from embryonic progenitors. The challenge ahead is to not only identify markers of position-dependent cellular differentiation and their potential effectors (e.g., homeobox genes) but to also incorporate this feature into functional assays of gut stem/progenitor cells. The regional variations in stripe "formation" observed in this study suggest that *Fabpl* and L-FABP/hGH transgenes represent sensitive markers of this property. Our analyses of ileal villi indicate that although the *decision* to express *Fabpl* or L-FABP$^{-596\ to\ +21}$/hGH at a particular discrete level appears to be made by the multipotent stem cell (or at least coordinated between committed transit cells in adult, monoclonal crypts that give rise to enterocytic and enteroendocrine lineages), different regulatory mechanisms may be operative. While both "genes" appear able to reveal clonality, another independent marker of the stem cell and its descendants may help decipher the level at which the decision is made about the form of their expression. For example, surveys of *Dlb-1*, *Fabpl*, and L-FABP/hGH expression in EtNU-treated, *Dlb-1*^{a}/*Dlb-1*^{b} transgenic mice should be informative.

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