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Temporal and spatial patterns of transgene expression in aging adult mice provide insights about the origins, organization, and differentiation of the intestinal epithelium

(colon/stem cells/progenitor cells/transgenic mice/pattern formation)

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ABSTRACT **We have used liver fatty acid-binding protein/human growth hormone (L-FABP/hGH) fusion genes to explore the temporal and spatial differentiation of intestinal epithelial cells in 1- to 12-month-old transgenic mice. The intact, endogenous L-FABP gene (*Fabpl*) was not expressed in the colon at any time. Young adult transgenic mice containing nucleotides −596 to +21 of the rat L-FABP gene linked to the hGH gene (minus its 5′ nontranscribed domain) demonstrated inappropriate expression of hGH in enterocytes and many enteroendocrine cells of most proximal and mid-colonic crypts (glands). Rare patches of hGH-negative crypts were present. With increasing age, a wave of "extinction" of L-FABP(−596 to +21)/hGH expression occurred, first in the distal colon and then in successively more proximal regions, leaving by 10 months of age only rare hGH-positive multicrypt patches. At no time during this progressive silencing of transgene expression were crypts observed that contained a mixture of hGH-positive and -negative cells at a particular cell stratum. Young (5–7 weeks) mice containing a L-FABP(−4000 to +21)/hGH transgene also demonstrated inappropriate expression of the transgene in most proximal colonic crypts. However, the additional 3.3 kilobases of upstream sequence resulted in much more rapid extinction of reporter expression, leaving by 5 months of age only scattered single crypts with detectable levels of hGH. This age-related extinction of L-FABP/hGH expression did not involve enterocytes and enteroendocrine cells in the (proximal) small intestine. These results indicate that cis-acting elements outside of nucleotides −4000 to +21 are necessary to fully modulate suppression of colonic L-FABP expression. They also define fundamental changes in colonic epithelial cell populations during adult life. Our data suggest that (*i*) a single stem cell gives rise to all cells that populate a given colonic crypt, (*ii*) stem cells represented in several adjacent crypts may be derived from a common progenitor, and (*iii*) such a progenitor cell may repopulate colonic crypts with stem cells during adult life. Since each colonic crypt contains the amplified descendants of its stem cell, transgenes may be powerful tools for characterizing the spatial and biological features of gut stem cells and their progenitors during life.**

The intestinal epithelium has several unique features that make it an attractive model for defining the pathways and mechanisms that regulate epithelial cell differentiation: (*i*) the rapid and continuous renewal of its four principal cell types—all apparently derived from a multipotent stem cell; (*ii*) its remarkable spatial organization, with proliferation and differentiation occurring during migration of the descendants of this stem cell from intestinal crypts; and (*iii*) the fact that the progeny of the functionally anchored stem cell undergo an amplification (involving perhaps four to six divisions) within the proliferative zone of each crypt, producing a monoclonal population with a single genotype (reviewed in ref. 1). This amplification together with its physical confinement provides an opportunity for disclosing subtle changes in either the stem cell (capable of self-renewal without accompanying differentiation) or its progenitor cell that would not be definable in other differentiating epithelia.

Mouse aggregation chimeras have been used to describe several aspects of the organization of the gut epithelium (2–6). The intestinal epithelial cell populations of some inbred strains of mice express a receptor for the *Dolichos biflorins agglutinin* (DBA) lectin (i.e., those having the *Dlb-1*^{b} rather than the *Dlb-1*^{a} allele). Analysis of adult aggregation chimeras formed from *Dlb-1*^{b}/*Dlb-1*^{b} and *Dlb-1*^{a}/*Dlb-1*^{a} strains provided proof that each small intestinal crypt is composed of a monoclonal population of cells (crypts were either wholly negative or positive for DBA binding; ref. 3). The number of renewing stem cells within each small intestinal crypt has been examined before or after treatment of mice heterozygous for the *Dlb-1* allele with γ radiation or ethylnitrosourea (EtNU). The (amplified) clonal descendants of a mutated precursor cell can be readily identified by their lack of staining with peroxidase-labeled lectin in the otherwise positively stained gut epithelium of these *Dlb-1*^{b}/*Dlb-1*^{a} mice (7–10). Schmidt *et al.* (10) observed that patches of multiple adjacent DBA-negative crypts/villi were produced when 7- to 11-day-old fetal *Dlb-1*^{b}/*Dlb-1*^{a} mice were treated with EtNU (animals were surveyed as adults). These patches were thought to reflect somatic mutations in embryonal intestinal stem-cell precursors (i.e., progenitor cells). When the same mutagen was given to adult animals, only isolated, wholly negative, small intestinal crypts were seen that persisted (9, 10). Their frequency reached a "steady-state" level of 14 mutations per 10^5 crypts in the small intestine 21 weeks after EtNU treatment (9). The frequency was 0.5 mutation per 10^5 small intestinal crypts per year in untreated mice. These data suggest that the epithelial cell population of individual adult small intestinal crypts is renewed by a single stem cell and that the rate of spontaneous mutation in this cell is extremely low.

Comparable studies have not been performed in the large intestine using the DBA marker system since expression of the lectin binding site is variable within its epithelial cell populations. However, Griffiths *et al.* (11) used the X chromosome-linked enzyme glucose-6-phosphate dehydrogenase (G6PD) to study clonality in the colonic epithelium of adult female mice. In mice with wild-type enzyme, all cells in all colonic crypts (glands) express high levels of activity. Mice

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Abbreviations: L-FABP, liver fatty acid-binding protein; hGH, human growth hormone; DBA, *Dolichos biflorins* agglutinin; G6PD, glucose-6-phosphate dehydrogenase; EtNU, ethylnitrosourea; PYY, peptide tyrosine tyrosine; nt, nucleotide(s).

with G6PD deficiency (GPDX) demonstrate uniformly low levels of activity. Adult mice heterozygous for these two alleles contain patches, encompassing multiple colonic crypts, of high or low activity: individual crypts do not have mixtures of cells with high and low activity. Dimethylhydrazine treatment of mice homozygous for the wild-type allele results in the production of scattered, individual crypts composed of cells with uniformly low enzyme activity. No mixed crypts were noted, leading Griffiths *et al.* (11) to conclude that the enterocytes and goblet-cell populations of each colonic crypt were also maintained by a single stem cell. No spontaneous G6PD mutations were noted when $>10^5$ colonic crypts were surveyed in untreated adult animals.

We have used transgenic mice containing liver fatty acid-binding protein/human growth hormone (L-FABP/hGH) fusion genes to characterize cis-acting elements that establish and maintain cell-specific, region-specific, and developmental stage-specific patterns of gene expression in the intestinal epithelium (12–15). The mouse "liver" FABP gene (*Fabpl*) is an attractive model for exploring these aspects of gut enteric biology: it is activated at the time of initial cytodifferentiation of the fetal gut epithelium (13), it is efficiently expressed in enterocytes and in a small subpopulation of enteroendocrine cells (12, 14), and it exhibits distinct regional differences in transcription along both the crypt-to-villus and duodenal-to-ileal axes of the small bowel (L-FABP is not detectable in colonic epithelium; ref. 12). Functional mapping studies in transgenic mice have allowed us to identify cis-acting sequences within the 5′ nontranscribed domain of the L-FABP gene that control its spatial patterns of expression (12, 14, 15). These transgenes defined subtle differences in the differentiation programs of certain epithelial cell populations that were not discernible when these cells were defined only by their endogenous gene products (14, 15). Remarkable differences in the spatial and cellular patterns of transgene expression within the continuously renewing intestinal epithelium have been noted between late fetal and young adult mice (13), raising the possibility of using transgenes to define fundamental temporal changes in the gut's epithelial cell populations. We now show that the patterns of transgene expression observed in the colonic epithelium of aging mice provide additional insights about its clonal organization, mechanisms of cell renewal, and pathways of cellular differentiation.

MATERIALS AND METHODS

Animals. Transgenic mice containing nucleotides (nt) −4000 to +21 of the rat L-FABP gene linked to the hGH gene beginning at its nt +3 [L-FABP(−4000 to +21)/hGH] were derived from founder $G_0$46 in ref. 12. Transgenic mice containing nt −596 to +21 of the L-FABP gene linked to nt +3 of hGH [L-FABP(−596 to +21)/hGH] were derived from either $G_0$13 or $G_0$19 (12). Mice were fed a standard chow diet *ad libitum*. Animals heterozygous for the transgenes were killed by cervical dislocation 5–52 weeks after birth. The colon was divided into proximal, middle, and distal thirds. The small intestine was rapidly dissected into four segments. All tissues were fixed in Bouin's solution (12).

Immunocytochemistry. The methods and antisera used for these studies are described elsewhere (12–15). Three to five mice from each pedigree were studied at each age surveyed. The number of hGH-positive (hGH^+) crypts and enteroendocrine cells were noted for each animal, and the data were averaged for animals within each age group. At least two separate sections were examined from each small and large intestinal segment of each mouse. A colonic crypt was scored as positive for transgene expression if there was enterocytic staining with anti-hGH serum in its uppermost strata of cells and in its corresponding surface epithelial cuff (cells migrate up colonic crypts onto the surface epithelium, where they form hexagonal cuffs around the crypt orifice; ref. 4).

RESULTS

Temporal Changes in Transgene Expression in the Colonic Enterocytic Populations of Adult Mice. Analysis of 5-week- to 1-year-old mice indicated that the endogenous gene *Fabpl* was never expressed in any region of the colon. The presence of multiple (>100) copies of either L-FABP/hGH transgene did not perturb this quiescent state.

Inappropriate expression of L-FABP(−596 to +21)/hGH was observed in all colonic segments of 3- to 6-month-old mice (Fig. 1*A*). In proximal colon, hGH was present in most crypts (≈90%, Fig. 1*A*). There were, however, occasional patches of several adjacent crypts with no detectable hGH (Fig. 2 *A* and *B*). Fewer crypts (≈18%) expressed hGH in the more distal colonic segments (Fig. 1*A*). Those that did were also present in patches (Fig. 2*C*).

Within an individual positive crypt (whether in the proximal or distal colon), little immunoreactive hGH was detectable in enterocytes located at the lowest cell strata. Intracellular levels of hGH increased as cells migrated towards the surface epithelium. Within a given stratum, cells had comparable levels of the reporter. All crypts in each hGH^+ patch contained comparable steady-state levels of the reporter. The boundary between positive and negative crypts was remarkably sharp (Fig. 2 *B* and *C*).

The number of crypts expressing hGH declined with increasing age. This extinction of transgene expression progressed from the distal colon to involve progressively more

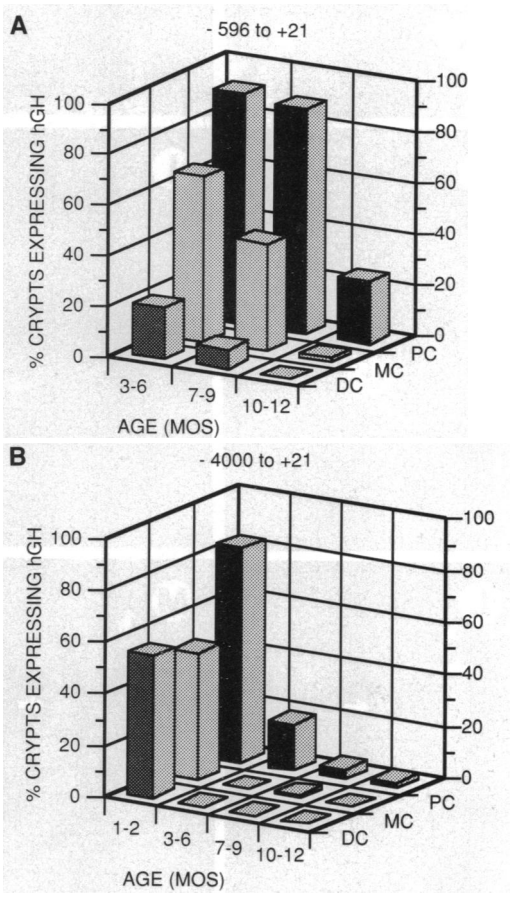

FIG. 1. Regional distribution of transgene expression in crypts as a function of age. Sections of proximal colon (PC), mid-colon (MC), and distal colon (DC) were analyzed for enterocytic expression of hGH in transgenic animals containing either L-FABP(−596 to +21)/hGH (*A*) or L-FABP(−4000 to +21)/hGH (*B*).

proximal segments (Fig. 1*A*). In 10- to 12-month-old mice, transgene expression had been completely extinguished in the distal colonic epithelium and the number of hGH^+ crypts in the proximal colon had declined to ≈22% (Fig. 1*A*).

Throughout this period (3–12 months) we noted that in those colonic segments where transgene-expressing crypts were rare (i.e., <20%), most hGH^+ crypts occurred in patches rather than as isolated individual crypts (Fig. 2*C*). This was not an insertion-site effect: it was observed in mice derived from two different founders. If transgene expression were determined independently at the level of the postulated single crypt stem cell, in segments where the probability of an

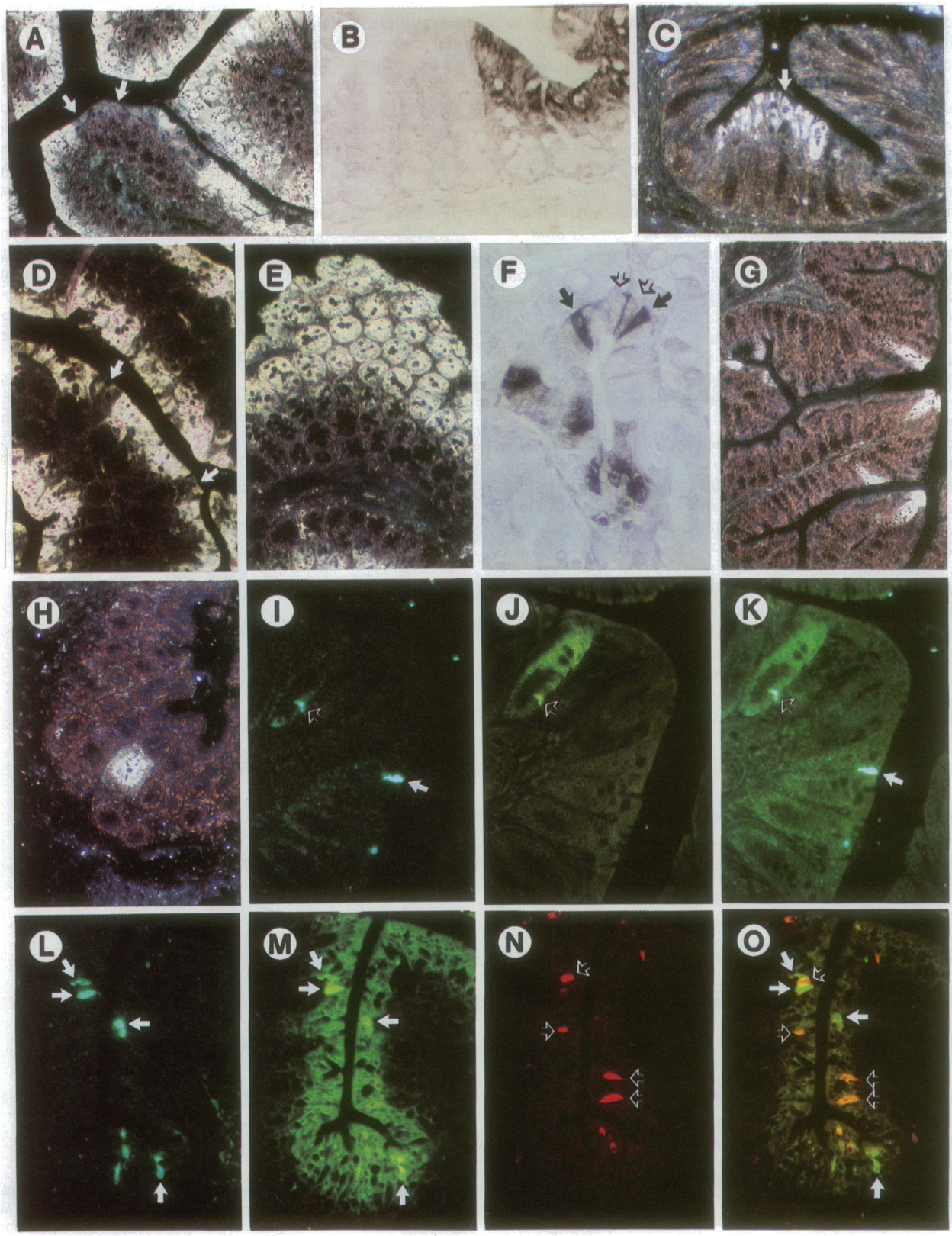

Fig. 2. (*Legend appears at the bottom of the opposite page.*)

individual crypt expressing hGH is small, one would expect positive crypts to occur predominantly in isolation rather than in clusters (patches).¶

Young transgenic mice containing L-FABP(−4000 to +21)/hGH also exhibited inappropriate expression of hGH in most colonic crypts (Fig. 2*D*). About 80% of crypts in the proximal colonic epithelium of 1- to 2-month-old mice contained immunoreactive hGH (Fig. 1*B*). While most crypts showed uniform expression of hGH in enterocytes located at a particular cell stratum (Fig. 2 *D* and *E*), there were some crypts that exhibited considerable variability in the steady-state levels of the reporter within comparably positioned columnar epithelial cells (Fig. 2*F*). Addition of nt −4000 to −597 increased the rate of extinction of colonic hGH expression, compared with L-FABP(−596 to +21)/hGH mice, but did not affect the orderly distal to proximal "wave" of suppression. By 5 months, the number of hGH^+ crypts had declined sharply in all colonic segments (Fig. 1*B*). Crypts showing heterogeneous cellular patterns of transgene expression were no longer apparent. In older L-FABP(−4000 to +21)/hGH mice (5–12 months), isolated, single hGH^+ crypts were found in the proximal colon and rarely in the middle and distal colonic segments (Figs. 1*B*, 2*G*, and 2*H*).

Temporal and Spatial Patterns of Extinction of Transgene Expression in Colonic Enteroendocrine Cell Populations. We have described regional differences in the distribution of enteroendocrine cell populations within the mouse gastrointestinal tract and correlated their position in the crypt-to-villus and duodenal-to-colonic axes with their ability to support L-FABP/hGH expression (14, 15). Serotonin-producing cells represent the most abundant and PYY-immunoreactive cells the next most abundant enteroendocrine subpopulations in the colonic epithelium. We therefore sought to determine whether the temporal and spatial patterns of suppression of transgene expression noted in colonic enterocytes also occurred in enteroendocrine cells. The total number of hGH^+ colonic enteroendocrine cells closely paralleled the total number of crypts that supported transgene expression in all regions of the large intestine at each age surveyed (data not shown). hGH^+ enteroendocrine cells appeared to be confined to crypts that demonstrated enterocytic expression of the reporter.

The number of colonic enteroendocrine cells containing immunoreactive serotonin or PYY did not vary with age (data not shown). Most PYY-immunoreactive cells present in crypts containing hGH^+ enterocytes also contained the reporter (Fig. 2 *I–L*). However, none of the PYY cells present in hGH^- crypts/patches supported detectable levels of L-FABP(−596 to +21)/hGH expression. This suggests that PYY cells and enterocytes arise from a common progenitor and/or share common regulatory factors that modulate transgene expression. By contrast, this transgene was rarely expressed in colonic serotonergic cells, even when they were present in hGH^+ crypts/patches (Fig. 2 *M–O*).

Age-Related Silencing of Transgene Expression Does Not Occur in Small Intestinal Enteroendocrine Cells. Serotonergic cells represent the most abundant enteroendocrine subpopulation in the small intestine and exhibit a distinct distribution from duodenum to ileum (14). We detected no changes in their number per cross section in any of the four segments of small bowel surveyed in 3- to 12-month-old normal or L-FABP(−596 to +21)/hGH transgenic mice (data not shown). The total number of hGH^+ enteroendocrine cells per intestinal cross section also exhibited characteristic differences from duodenum to ileum in adult L-FABP(−596 to +21)/hGH mice (the number decreasing progressively from the proximal to the distal segments; data not shown). In contrast to the colon, the number of enteroendocrine cells that supported transgene expression did not significantly change with increasing age in the small intestine (data not shown). These results indicate that temporal extinction of L-FABP(−596 to +21)/hGH expression is limited to enteroendocrine cell (sub)populations located in the colonic epithelium.

Absence of Age-Related Differences in Transgene Expression Within Small Intestinal Enterocytes. Both the L-FABP(−4000 to +21)/hGH and L-FABP(−596 to +21)/hGH transgenes are efficiently expressed in small intestinal enterocytes, with highest steady-state levels of hGH and its mRNA occurring in the proximal small bowel and decreasing distally (12). We therefore examined hGH-stained sections prepared from the proximal small intestine of all animals included in our survey of colonic transgene expression. No comparable age-related

¶The predicted distribution of patch sizes for crypts within a tissue cross-section where transgene expression (in each crypt) is independently determined can be described by $F(n) = 2(1 - P)P^n$, where $F(n)$ equals the fraction of total patches of size n, and P is the probability that a randomly selected crypt in the tissue section is positive. The fraction of total crypts expressing the transgene was used as an approximation of P for this analysis. For colonic segments of L-FABP (−596 to +21)/hGH transgenic mice where the frequency of hGH positive crypts was <20%, the predicted frequency distributions of patch sizes were significantly different from the observed distributions, based on χ^2 analysis.

FIG. 2. Immunocytochemical studies on hGH expression in colonic epithelium of aging transgenic mice. Distribution of transgene expression in colonic crypts of L-FABP(−596 to +21)/hGH (*A–C*) or L-FABP(−4000 to +21)/hGH (*D–G*) transgenic mice was analyzed with anti-hGH serum and the immunogold silver intensification staining (IGSS) method. (*A*) Proximal colon from a 3-month-old L-FABP(−596 to +21)/hGH mouse derived from $G_0$13, visualized with darkfield microscopy. Note the patches of negatively staining crypts (arrows). (×70.) (*B*) Proximal colon from a 9-month-old L-FABP(−596 to +21)/hGH transgenic descendent of $G_0$19, visualized with brightfield technique. The boundary between patches of hGH^+ and hGH^- crypts is sharp. (×180.) (*C*) Distal colon from a 3-month-old $G_0$13 descendant demonstrating a sharply demarcated patch of hGH^+ crypts (arrow). (Darkfield, ×90.) (*D*) Five-week-old L-FABP(−4000 to +21)/hGH transgenic mouse showing isolated, individual hGH-negative crypts in proximal colon (arrows). (Darkfield, ×70.) (*E*) Tangential section from the proximal colon of a 5-week-old L-FABP(−4000 to +21)/hGH mouse. Enterocytes in cell strata closer to the surface epithelium become uniformly hGH^+. (Darkfield, ×70.) (*F*) Tangential section of a crypt from a 5-week-old L-FABP(−4000 to +21)/hGH mouse. Note the mixture of hGH^+ enterocytes (solid arrows) and hGH^- enterocytes at (open arrows) this cell stratum. (×180.) (*G*) Proximal colon from a 7-month-old L-FABP(−4000 to +21)/hGH mouse demonstrating many isolated, strongly hGH^+ crypts. (Darkfield, ×70.) (*H*) Tangential section through the proximal colon of a 12-month-old L-FABP(−4000 to +21)/hGH mouse. Note the isolated strongly hGH^+ crypt. All enterocytes at this cell stratum were hGH^+. (Darkfield, ×90.) (*I–K*) Relationship of L-FABP(−596 to +21)/hGH transgene expression in peptide tyrosine tyrosine (PYY)-containing enteroendocrine cells. A section of proximal colon from a 7-month-old mouse was immunostained with either rabbit anti-PYY and IGSS (*I*, visualized with reflected-light polarization microscopy) or goat anti-hGH and fluorescein-labeled donkey anti-goat sera (*J*). Two enteroendocrine cells contained PYY (*I*, arrows). hGH colocalized to one of these cells (open arrow) located in an hGH-positive crypt (*I* and *J*). The other PYY-containing cell (solid arrow) does not express the transgene (*J*). Double exposure of the section illustrates these relationships (*K*). (×115.) (*L–O*) Patterns of L-FABP(−596 to +21)/hGH transgene expression in PYY and serotonergic enteroendocrine cell populations in the proximal colon of a 3-month-old mouse. PYY-containing cells (solid arrows) were detected with rabbit anti-PYY serum and IGGS (*L*, visualized with reflected-light polarization microscopy). hGH was visualized in the same section by using goat anti-hGH and fluorescein-labeled donkey anti-goat sera (*M*). Note that the PYY-containing cells (*L*, solid arrows) also are heavily stained for hGH (*M*, solid arrows). Serotonergic cells (open arrows) in the same section were detected using rat anti-serotonin and β-phycoerythrin-labeled donkey anti-rat sera (*N*). Double exposure of *M* and *N* (*O*) demonstrates that the serotonergic cells (open arrows) do not contain significant levels of hGH and are distinct from hGH^+ PYY-immunoreactive cells. (×180.)

changes in the cellular or regional patterns of transgene expression were observed in small intestinal enterocytes. Together with the enteroendocrine data, these observations suggest that time-dependent silencing of expression of both transgenes appears to be unique to colonic epithelial cells.

DISCUSSION

Analysis of the expression of L-FABP/hGH fusion genes in adult transgenic mice has revealed remarkable, age-specific differences in reporter expression within the colonic epithelium. A distinctive, spatially well-organized pattern of extinction of L-FABP(−596 to +21)/hGH transgene expression was observed involving patches of colonic crypts. These results emphasize the importance of considering temporal "gradients" when assessing the appropriateness of transgene expression in the continuously renewing gut epithelium. They suggest that transgenes provide a powerful tool for further defining clonal and lineage relationships of colonic epithelial cell populations during life as well as the mechanisms that regulate their differentiation in space and time.

While the endogenous mouse L-FABP gene (*Fabpl*) remains silent in the colon during adult life, the L-FABP(−596 to +21)/hGH fusion gene appears to be actively and coordinately expressed in groups of multiple adjacent crypts with sharply demarcated boundaries. This inappropriate expression initially involves most colonic crypts. "Extinction" of transgene expression occurs in patches and in a region-specific fashion (from distal colon to proximal colon). The term extinction has to be used cautiously because one of the principal questions raised by this phenomenon is whether it is due to repopulation of the colonic epithelium with cells that have different regulatory environments for which the transgene is a sensitive reporter or whether it reflects the production of some intra- or extracellular factor that coordinately modulates transgene expression within "existing" (differentiating) cells. It is difficult to understand how local production of such a factor(s) could result in sharply demarcated patch boundaries composed of crypts that uniformly express the transgene at a given cell stratum, unless they affect regulatory events occurring in stem cells and/or their progenitors. The patches of transgene expressing crypts are reminiscent of the patches observed in the adult colonic epithelium of mice heterozygous for the G6PD alleles (11). Coordinate regulation of transgene expression in multiple adjacent crypts could arise through determination events occurring in the stem-cell progenitor. Such a model would require that the progenitor supply new stem cells to crypts within a colonic patch in adult mice (without this resupplying of colonic crypts, it would be necessary to invoke more complex models).

As noted in the Introduction, treatment of adult mice heterozygous for the G6PD or *Dlb-1* alleles only resulted in the production of isolated crypts with the mutant phenotype: no patches of multiple crypts were observed. This suggests that the target cell for the somatic mutation was the crypt stem cell rather than its progenitor. There are at least two possible reasons why these marker systems may not be sufficiently sensitive to reveal the postulated active progenitor cell: (*i*) the rates of mutation produced were low and the number of crypt stem-cell progenitors could be very small compared to the number of its progeny or (*ii*) their sensitivities to mutagen-induced allelic change may differ. Transgene expression in the amplified products of the progenitor may represent a way of operationally defining the biological properties of such a cell. It should be possible to test whether coordinate regulation of transgene expression in multiple crypts reflects the influence of the crypt stem-cell progenitor by examining mice containing both the L-FABP(−596 to +21)/hGH transgene and a somatic clonal marker expressed in the colon, such as G6PD. If the regulatory event that determines transgene expression occurs in the crypt stem-cell progenitor, then patches of hGH^+ crypts may not cross clonal boundaries (as defined by histochemical staining for G6PD).

Both the "temporal gradients" and spatial patterns of L-FAPB/hGH expression appeared to be dependent on what portion of the 5′ nontranscribed sequence of the L-FABP gene was represented in the transgene. For instance, the rate of extinction of L-FABP(−4000 to +21)/hGH expression was considerably more rapid than that observed with the L-FABP(−596 to +21)/hGH fusion gene. In contrast to L-FABP(−596 to +21)/hGH, the silencing event(s) in L-FABP(−4000 to +21)/hGH appeared to be primarily manifested in isolated crypts rather than in patches of contiguous crypts. All cells in a given crypt appeared to be ultimately affected by the silencing event (compatible with the notion that extinction involves the single crypt stem cell). However, it is curious that in young L-FABP(−4000 to +21)/hGH transgenic mice, a striking heterogeneity in cellular hGH levels occurred within a given stratum of some colonic crypts. Such heterogeneous crypts were not observed in mice >4 months old and were not detected in L-FABP(−596 to +21)/hGH transgenic mice at any of the ages surveyed. This suggests that the additional 3.3 kilobases of upstream sequence also contains cis-acting elements that detect subtle differences in the regulatory environments of individual colonic enterocytes which are not perceived by nt −596 to +21. These data imply that the mechanism of silencing of *Fabpl* involves regulatory steps that affect multiple levels of the differentiation pathway. Each of these regulatory (determination) events can be operationally described on the basis of the different patterns of expression of L-FABP(−596 to +21)/hGH, L-FAPB(−4000 to +21)/hGH, and *Fabpl*.

In summary, transgenes can be used to characterize subtle changes that occur in the continuously proliferating/differentiating gut epithelium with time—differences that are not currently definable with other approaches. Moreover, these molecular probes hold the promise of providing a way to describe and monitor the properties of stem cells and their progenitors from which the intestine's complex cellular population continuously arise. Such an inferential approach may be allowable because of the unique spatial constraints imposed upon the amplified descendants of these stem cells and their progenitors, permitting the investigator to "march backwards" through the spatial and temporal pathways of (gut) epithelial cell differentiation.

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