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Transgenic mice containing intestinal fatty acid-binding protein–human growth hormone fusion genes exhibit correct regional and cell-specific expression of the reporter gene in their small intestine

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ABSTRACT **The rat intestinal fatty acid binding protein (I-FABP) gene exhibits cell-specific as well as regional differences in its expression within the continuously regenerating small intestinal epithelium. To investigate the underlying mechanisms, we linked portions of its 5′ nontranscribed domain to the human growth hormone (hGH) gene and analyzed expression of the hGH reporter in transgenic mice by RNA blot, solution hybridization, and immunocytochemical techniques. Sequences located within 277 nucleotides of the start site of I-FABP transcription are sufficient to limit hGH expression to the intestine. Although the absolute levels of hGH mRNA in the duodenum and proximal jejunum of these transgenic mice were similar to those of I-FABP mRNA, steady-state hGH mRNA concentrations were ≈100 times lower in their distal small intestine. Addition of nucleotides −278 to −1178 of the I-FABP gene "restored" hGH mRNA concentrations in the distal jejunum and ileum to levels comparable to murine I-FABP mRNA. Serum hGH levels were 1000 times lower in the "short promoter" transgenic mice compared to animals with the "long promoter" transgene, indicating that efficient distal small intestinal hGH expression is required to produce elevated hGH concentrations in serum. The distribution of hGH in villus-associated enterocytes and goblet cells and its lack of expression in the crypts of Lieberkuhn mimicked that of the endogenous I-FABP gene product in all transgenic pedigrees. However, bands of hGH-negative cells extending from the base to the tips of villi were frequently observed in mice that were heterozygous for the short promoter transgene. This mosaic staining was not observed for I-FABP. These data suggest that (*i*) different cis-acting sequences may be required for complete expression of proximal–distal I-FABP gradients than for recapitulation of its normal crypt–villus tip distribution; (*ii*) differences may exist in the export pathways of secreted proteins within enterocytes located in various regions of the small intestine; and (*iii*) there may be subtle genetic differences among various crypt stem cells that can be detected *in vivo* by observing mosaic patterns of transgene expression along the villus epithelium.**

The mammalian intestine is lined with a continuously regenerating epithelium. In the mouse small intestine, ≈150 rapidly dividing cells located in a crypt of Lieberkuhn provide new cells to surrounding villi (1). Epithelial cells derived from small intestinal crypts migrate up villi in relatively straight bands over a period of 3–5 days (1, 2). During this period they undergo differentiation prior to their shedding at the apical extrusion zone located near the villus tip. The cells that reside in individual crypts are monoclonal—arising from single progenitor cells (3–6). The four principal differentiated cell types represented in the mouse gut epithelium—enterocytes, goblet cells, enteroendocrine cells, and Paneth cells—originate from a common, multipotential stem cell (3, 7).

Regional differences in gene expression are established and maintained in this continuously regenerating epithelium resulting in functional and morphologic differentiation of the gastrointestinal tract. This geographic differentiation takes place in two dimensions—from the proximal to the distal intestine (its "horizontal axis") as well as from the crypt of Lieberkuhn to the villus tip (its "vertical axis"). To investigate the mechanisms responsible for this geographic and cell-specific expression, we have begun a series of studies in which promoter elements from genes expressed in the intestinal epithelium are linked to a suitable reporter and expression of the fusion gene studied in transgenic mice (8). For the present study, we have attached portions of the 5′ nontranscribed region of the rat intestinal fatty acid-binding protein (I-FABP) gene to the human growth hormone (hGH) gene. Regulatory elements from the rat I-FABP gene were selected for several reasons. (*i*) Transcription of the I-FABP gene is limited to the small intestinal epithelium (9). (*ii*) I-FABP mRNA is among the most abundant mRNA species in the rat intestine (10), suggesting that its promoter functions with great efficiency in this epithelial cell population. (*iii*) Distinct gradients in I-FABP expression exist from the proximal to distal intestine and from crypt to villus tip (11). (*iv*) It is a member of a dispersed gene family consisting of 10 known members. Comparative sequence analyses of family members have disclosed a 14-base-pair (bp) element composed of two direct 7-bp repeats located in the 5′ nontranscribed region of those genes that are expressed in intestine (9). The I-FABP gene has three copies of this element (consensus, 5′-TGAACTTTGAACTT-3′).

The results reported here indicate that cis-acting elements located within 277 nucleotides (nt) of the start site of transcription of the I-FABP gene are sufficient to direct efficient cell-specific expression of the hGH reporter in the intestine of transgenic mice. They also produce regional differences in hGH distribution that in many respects are similar to those of the endogenous murine I-FABP gene¶ product.

MATERIALS AND METHODS

Construction of I-FABP–hGH Fusion Genes. A *Sac* I–*Pvu* II restriction fragment containing nt −277 to +28 of the rat I-FABP gene (9) was subcloned into *Sac* I/*Sma* I digested pUC13 DNA. A 2.15-kilobase (kb) *Bam*HI fragment of pMThGH111 DNA (12), which encompasses the entire hGH

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Abbreviations: I-FABP, intestinal fatty acid-binding protein; L-FABP, liver fatty acid-binding protein; hGH, human growth hormone; nt, nucleotide(s); LP, long promoter; SP, short promoter.

¶For simplicity the I-FABP abbreviation has also been used to denote the mouse gene, although the proper designation is *Fabpi* (9).

structural gene (beginning at nt +3), was introduced into the *Bam*HI site of the pUC13 recombinant plasmid. This "short promoter" (SP) I-FABP–hGH fusion gene was recovered as a 2.4-kb *Eco*RI fragment and subcloned into pBR325. The resulting DNA was termed pIFhGH1. Construction of a "long promoter" (LP) I-FABP–hGH fusion gene was begun by inserting a 1.2-kb *Eco*RI–*Pvu* II restriction fragment containing nt −1178 to +28 of the rat I-FABP gene into *Eco*RI/*Sma* I-digested pUC13 DNA. The recombinant plasmid produced was linearized by digestion with *Bam*HI and ligated to the 2.15-kb *Bam*HI fragment containing the hGH structural gene, and the correct orientation of this inserted DNA was selected by analytic *Eco*RI digestion. The final step involved purification of a 3.3-kb *Eco*RI fragment and its insertion into the *Eco*RI site of pBR325 producing pIFhGH2.

Production and Characterization of Transgenic Mice. *Pronuclear injections.* Two to four thousand copies of the purified (8) 2.4-kb *Eco*RI fragment from pIFhGH1 or the 3.3-kb *Eco*RI fragment from pIFhGH2 were microinjected into pronuclei of zygotes collected from C57BL/6J or (C57BL/6J × SJL)F_1 [(B6SJL)F_1] females mated to either LT/Sv or (B6SJL)F_1 males. Zygotes were cultured to morulae before transfer to pseudopregnant (C57BL/6J × SJL/J)F_1 hybrid females (13).

Transgene copy number determination. Southern blots of *Sph* I- and *Eco*RI-digested spleen DNA were probed (13) with the ^{32}P-labeled 2.15-kb *Bam*HI fragment from pMThGH111. Southern blots contained reference standards of a 2.7-kb *Eco*RI fragment from pLFhGH2 DNA (8). Copy number was calculated by laser densitometry of autoradiographs.

Measurement of serum hGH levels. A double-antibody radioimmunoassay (8) of each serum sample (obtained at the time of sacrifice) was performed in triplicate and the results were averaged.

Analysis of the tissue distribution of I-FABP and hGH mRNAs. Transgenic mice and their normal littermates were sacrificed by cervical dislocation, and total cellular RNA was prepared (14) from brain, lung, heart, skeletal muscle, spleen, kidney, pancreas, gonads, liver, and stomach plus seven regions of the intestinal tract. Regional dissection of the intestine was accomplished as described in ref. 8. Dot blots containing four concentrations (0.5–3 μg) of each tissue RNA sample were constructed (15). Each blot contained hGH and I-FABP complementary RNA standards (0.1–100 pg) prepared by *in vitro* transcription (16) of a 245-bp *Rsa* I–*Sma* I fragment from exon V of the hGH gene (17) that had been subcloned into pGEM-2 and a 416-bp *Pvu* II–*Bst* XI fragment of rat I-FABP cDNA (10) that had also been placed in the same vector. Duplicate blots were probed with a ^{32}P-labeled (18) 150-bp *Bgl* II–*Pvu* II fragment from exon V of the hGH gene or the *Pvu* II–*Bst* XI fragment recovered from pJG19, which contains a full-length rat I-FABP cDNA (10). Stringencies selected for hybridization and washing are outlined in ref. 8. The relative and absolute concentrations of each mRNA were determined by laser densitometric scanning of filter autoradiographs. Absolute levels of hGH and I-FABP mRNAs were also independently measured by solution hybridization assays with ^{32}P-labeled complementary RNA probes (8).

The site of initiation of transcription of the two transgenes was established by primer-extension analyses (9) of poly(A)$^+$ intestinal RNA with a 19-nt oligodeoxynucleotide complementary to nt 70–88 of exon I of the hGH gene (17).

Immunocytochemical studies. Samples from two or three regions of each of the seven intestinal segments were fixed in Bouin's solution and embedded in paraffin, and 5-μm thick sections were prepared (8). The peroxidase-antiperoxidase method (19) together with a monospecific goat anti-hGH serum (20) or a monospecific rabbit anti-rat I-FABP serum (21) was used to examine the cell-specific expression of I-FABP and hGH.

RESULTS

Production of I-FABP–hGH Transgenic Mice Containing I-FABP–hGH Fusion Genes. Nt −277 to +28 of the rat I-FABP gene were fused to nt +3 of the hGH gene to produce a SP construct. This portion of the I-FABP promoter was selected for two reasons. (*i*) Nt −260 to −1 of the rat and human I-FABP genes exhibit a high degree of sequence identity (64%) while a dramatic reduction in sequence conservation occurs immediately upstream from this point and extends for at least an additional kilobase (9). (*ii*) One copy of the 14-bp element that has been identified in members of this and other gene families that are expressed in the intestinal epithelium is contained between nt −90 and −66 (9). A LP I-FABP–hGH construct was also produced by using nt −1178 to +28 of the rat I-FABP gene. Two additional copies of the 14-bp element are located in this segment of the I-FABP gene: from nt −443 to −430 and from nt −599 to −579. Two founder (G_0) transgenic mice with the LP construct ($G_0$1 and $G_0$4) and two founder animals with the SP transgene ($G_0$23 and $G_0$54) were identified. Three F_1 progeny were obtained from the two founders with the SP transgene (Table 1). Southern blot analyses indicated that mice from the different pedigrees contained 20–600 copies of the transgene per haploid genome, all in a tandem head-to-tail arrangement. Each pedigree's transgene was located at a single insertion site. LP transgenics had serum hGH levels of 8–23 μg/ml (normal murine GH levels = 10–100 ng/ml; refs. 12 and 22). These animals grew to two to three times the weight of their normal littermates. F_1 SP transgenic mice derived from $G_0$54 ($F_1$54–157 and $F_1$54–122) had serum hGH levels that were ≈1000 times lower than the LP animals (6–38 ng/ml; Table 1). An F_1 progeny of $G_0$23 ($F_1$23–141) had serum hGH levels <1 ng/ml. None of these F_1 animals exhibited any significant increase in their weight compared to age and gender matched littermates.

Comparison of the Tissue Distribution of I-FABP and hGH mRNAs in Normal and Transgenic Mice. I-FABP and hGH mRNA levels were measured in 11 tissues harvested from G_0 and F_1 transgenic mice plus normal age and gender-matched littermates. In each case, I-FABP mRNA was only detected in the intestine. The proximal–distal distribution of I-FABP mRNA within the intestine was not affected by the presence or absence of up to 600 copies of the transgene (Fig. 1 *Top*). Highest concentrations were documented in the distal jejunum. Levels in the ileum were 75% of those in distal jejunum and fell to 6, 3, and 0.5% in the cecum and proximal and distal colon, respectively. In addition, the absolute level of this mRNA was similar in the proximal jejunums of normal and

Table 1. Mice from various transgenic pedigrees containing I-FABP–hGH constructs

Transgenic mouse	Transgene copy number in haploid genome	Serum hGH, ng/ml	Weight, g	Age, weeks
LP				
$G_0$1 (F)	600	22,600	69	21
$G_0$4 (F)	100	8,400	52	21
SP				
$F_1$54–157 (F)	60	38	24	12
$F_1$54–122 (M)	60	6	33	22
$F_1$23–141 (F)	20	ND	21	10

Founder animals are designated as G_0. Progeny from a founder that are obligate heterozygotes for the transgene are indicated by F_1 plus the founder number plus their own number. Immunocytochemical studies of the founder transgenic mice suggested that they were not mosaic for the LP transgene. Both LP founders were infertile, producing no offspring. Ninety-nine live-born mice were screened for the presence of the SP I-FABP–hGH transgene by blot hybridization analyses of their tail DNAs. Two positive SP founders ($G_0$23 and $G_0$54) were identified. ND, not detectable.

transgenic animals (from 10 to 50 pg/μg of total cellular RNA or 190–950 copies of I-FABP mRNA per cell).

hGH mRNA was present in the intestines of the two founder transgenic mice with the LP construct. However, hGH mRNA was present in the intestines of heterozygous F_1 mice from only one of the two SP founder animals ($G_0$54). Like I-FABP mRNA, hGH mRNA was not detected in any of the other 10 tissues surveyed. Primer-extension and Northern blot studies of intestinal RNA isolated from mice that expressed either the LP or the SP transgene established that in both cases initiation of transcription occurred at the proper site producing a mature hGH mRNA of 950 nt. Heterozygous F_1 mice from the second SP founder ($G_0$23) did not contain detectable levels of hGH mRNA in any tissue, including intestine, even though Southern blots failed to reveal any structural rearrangements of the transgene.

Regional differences in intestinal hGH mRNA concentration were observed in all animals that transcribed the transgene. Nt −1178 to +21 of the I-FABP gene produced the highest steady-state levels of hGH mRNA in the jejunum. hGH mRNA concentrations fell abruptly from the ileum to the cecum where levels were 1–5% of those in the distal jejunum (Fig. 1 *Middle*). This proximal–distal gradient was virtually identical in two founders with distinct LP transgene insertion sites and mimicked the pattern of accumulation of murine I-FABP mRNA. In contrast, mice expressing the SP I-FABP–hGH transgene had the highest concentrations of hGH mRNA in more proximal portions of their small intestine with a sharp decline occurring between the proximal jejunum and ileum (Fig. 1 *Bottom*). Analysis of proximal jejunal RNAs revealed only modest (2- to 3-fold) differences in the absolute levels of hGH mRNA between animals containing the SP I-FABP–hGH fusion gene and mice with the LP construct (10 pg/μg of total cellular RNA vs. 20–30 pg/μg, respectively). However, up to 100-fold differences were noted in their ileal RNAs (0.3 pg/μg vs. 20–30 pg/μg).

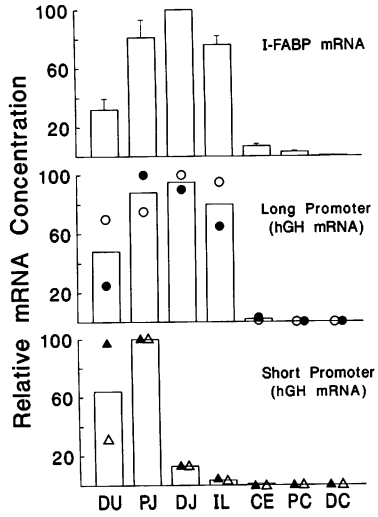

FIG. 1. Distribution of murine I-FABP and hGH mRNAs in the gastrointestinal tracts of normal and transgenic mice. Total cellular RNA was isolated from various regions of the intestinal tract of transgenic animals from each pedigree as well as their normal littermates and analyzed by quantitative dot blot hybridizations. Data for each animal are expressed as a percentage of the intestinal RNA sample which gave the strongest signal. DU, duodenum; PJ, proximal jejunum; DJ, distal jejunum; IL, ileum; CE, cecum; PC, proximal colon; and DC, distal colon. (*Top*) Relative distribution of murine I-FABP mRNA in the transgenic animals listed in Table 1 as well as six normal age- and gender-matched littermates (mean ± SD). (*Middle*) Distribution of hGH mRNA in LP transgenics $G_0$1 (○) and $G_0$4 (●). (*Bottom*) hGH mRNA levels in SP transgenics $F_1$54-122 (△) and $F_1$54-157 (▲).

Comparison of the Cell-Specific Expression of hGH and I-FABP in Transgenic Animals. Immunocytochemical studies of segments of intestine obtained from normal and transgenic mice indicated that the pattern of cell-specific expression of hGH resembled that of the endogenous murine I-FABP gene (Fig. 2). I-FABP exhibits a well-demarcated induction of expression at the gastro-duodenal junction (*A*). Within the small intestine, I-FABP expression is confined to villus-associated epithelial cells (*B–E*). No immunoreactive protein is detectable in the crypt cell population, including Paneth cells located at the crypt base (*D*). Light microscopic analysis of 5-μm-thick sections indicated that enterocytes and goblet cells contain I-FABP, with the highest concentrations occurring in enterocytes (*C*). This pattern of cell-specific expression is encountered in the duodenal, jejunal, ileal, and cecal epithelium where I-FABP appears to be evenly distributed throughout the cytoplasm of enterocytes. Nt −277 to +28 of the rat I-FABP gene are sufficient to produce cellular patterns of hGH staining that closely resemble those of I-FABP: i.e., a well-demarcated zone of expression occurs at the gastro-duodenal junction (*F*); hGH is confined to enterocytes and goblet cells located within small intestines of SP (and LP) transgenic mice (*G–L*); detectable levels of immunoreactive hGH do not appear until epithelial cells emerge from the crypt (*G, I,* and *J*); Paneth cells located at the crypt base do not appear to synthesize hGH (*J*); and the distribution of hGH from the bases to tips of villi is similar to I-FABP (compare *B* and *D* with *G, J,* and *K*). Within small intestinal enterocytes, intense hGH staining is observed in the supranuclear (Golgi) region. This pattern of intracellular staining is seen in enterocytes throughout the small intestine of mice containing the LP transgene (compare *H* and *L*).

Two differences were observed in our immunocytochemical studies of SP and LP transgenic mice. (*i*) As expected from the mRNA distribution data, hGH expression was comparable in their proximal small intestinal epithelium but was quite different in enterocytes located in more distal segments of this organ. Mice containing nt −277 to +28 of the I-FABP gene had less intense hGH staining of their ileal columnar epithelium than mice with nt −1178 to +28. (*ii*) F_1 mice who were obligate heterozygotes for the SP I-FABP–hGH transgene contained islands of columnar epithelial cells with markedly reduced or absent levels of immunoreactive hGH extending from the base of many villi to their apical extrusion zones (Fig. 2 *M–O*). This mosaicism was observed in both male and female animals and in all segments of small intestine surveyed from duodenum to ileum. Islands of negatively staining enterocytes were not observed when I-FABP antiserum was incubated with adjacent sections of intestine (or in segments prepared from normal littermates). In addition, this mosaic hGH staining was only rarely encountered in the small intestinal epithelium of the two LP I-FABP–hGH transgenic animals.

DISCUSSION

These studies indicate that cis-acting sequences located within the first 1178 nt 5′ to the start site of transcription of the rat I-FABP gene are sufficient to direct appropriate cell-specific expression of a reporter (hGH) within the small intestinal epithelium of transgenic mice. In addition, these elements can maintain gradients in hGH gene expression from the crypts of Lieberkuhn to the tips of villi and from the proximal to distal small intestine that precisely duplicate the gradients exhibited by the endogenous intact murine I-FABP gene. Quantitative similarities between transgene and endogenous gene expression were also noted as judged by a comparison of the steady-state concentrations of their mRNAs in the proximal through distal small intestine. Nt −277 to +28 of the rat I-FABP gene produced similar results

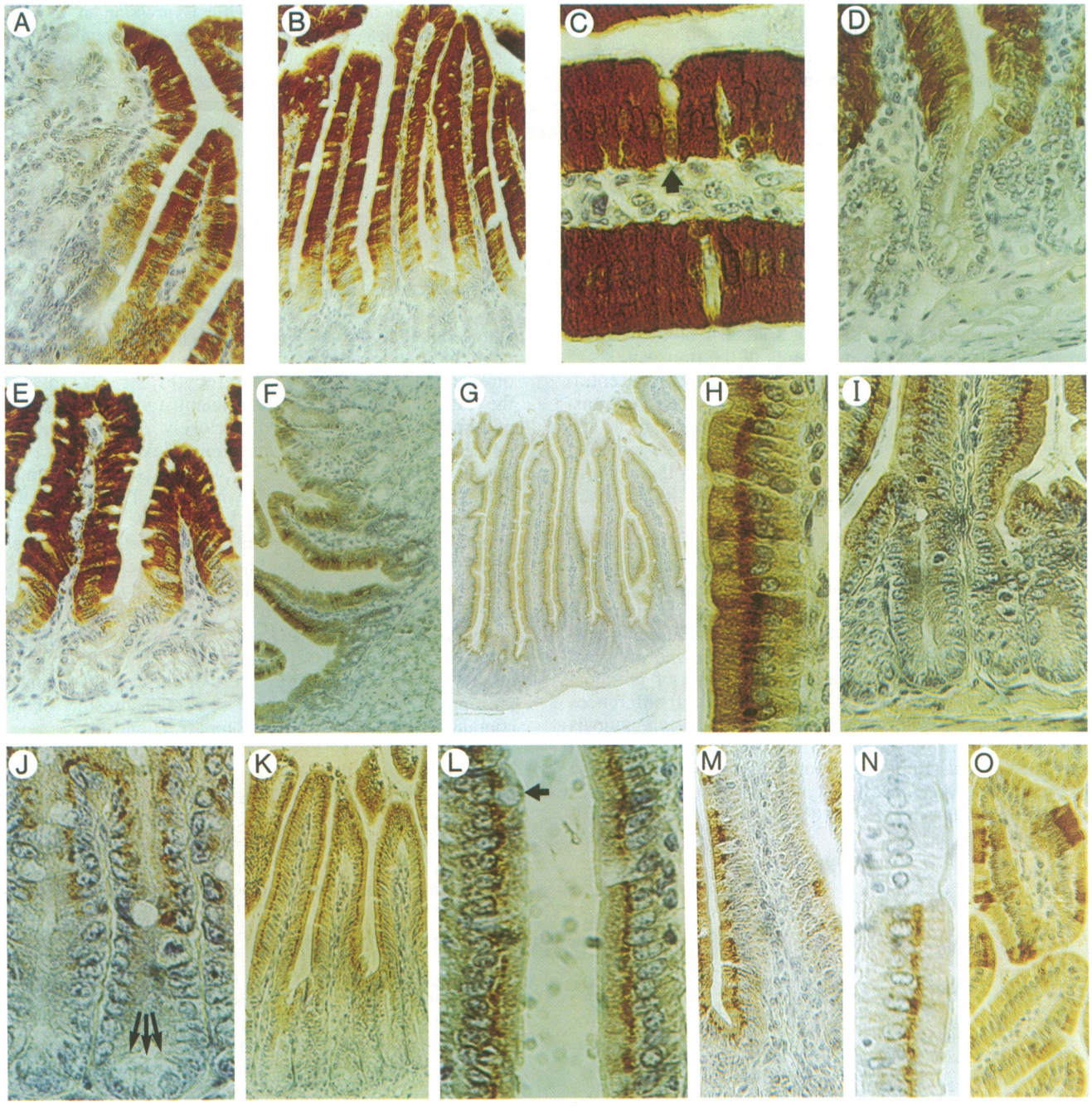

FIG. 2. Comparison of the cell-specific expression of I-FABP and hGH in the intestine of transgenic mice containing SP and LP I-FABP–hGH transgenes. The peroxidase-antiperoxidase method (18) was used to visualize hGH and I-FABP as brown-staining material in the intestines of normal and transgenic mice. Nuclei were counterstained with hematoxylin. (*A*) Induction of I-FABP expression at the gastroduodenal junction of a normal mouse. (*B*) I-FABP distribution in the proximal jejunum of a SP transgenic mouse. (*C*) High magnification of jejunal villus from a SP transgenic mouse demonstrating that I-FABP is confined to enterocytes and goblet cells (arrow). (*D*) High magnification of proximal jejunal crypt stained with anti-I-FABP serum. Similar patterns of I-FABP expression were encountered in normal mice and transgenic animals with the LP fusion gene. (*E*) Expression of I-FABP in the ileum of a LP transgenic mouse. (*F*) hGH distribution at the gastroduodenal junction of a SP transgenic mouse. (*G*) hGH is limited to villus-associated epithelial cells in the jejunum. (*H*) High magnification of villus shown in *G*. Intense supranuclear (Golgi) staining occurs in enterocytes. (*I* and *J*) High magnification of a section of jejunum from the animal shown in *G*, demonstrating the absence of detectable immunoreactive hGH until cells begin to emerge from the crypt and the lack of staining of Paneth cells (arrows) located at the crypt base. (*K*) Ileum from a LP transgenic mouse stained with anti-hGH serum. (*L*) High magnification of the section shown in *K* demonstrating supranuclear concentration of hGH in ileal enterocytes and its expression in goblet cells (arrow). (*M–O*) Mosaic staining patterns observed for hGH in a male mouse heterozygous for the SP I-FABP–hGH transgene. (*M*) Single villus one side of which contains a vertical band of hGH-positive columnar epithelial cells. The other side has a band of cells with markedly reduced levels of hGH. (*N*) High magnification of a slightly obliquely sectioned villus with positive and negative cells from adjacent vertical bands. (*O*) Coronally and obliquely sectioned villi emphasize the presence of vertical bands of intensely staining cells (for hGH) surrounded by bands of weakly staining cells.

in most, but not all, respects. Reporter expression was intestine-specific and appropriately restricted to villus-associated enterocytes and goblet cells. However, although the crypt–villus distribution was appropriate, the proximal–distal gradient was disturbed. Steady-state hGH mRNA concentrations were quite similar in the duodenum and proximal jejunum of SP and LP transgenic animals while in their ileums up to 100-fold differences were noted.

The marked reduction in hGH expression in the distal jejunum and ileum of the one SP transgenic strain could reflect several possible mechanisms. Cis-acting elements located upstream from nt −277 of the I-FABP gene may be required for efficient reporter gene transcription in this region of the small intestine. Alternatively, loss of nt −278 to −1178 could make the SP transgene more susceptible to insertion site effects. It is known that transgene expression can be influenced by chromosomal position and that such positional effects are most likely to occur with transgenes that do not contain strong promoters, enhancers, or other sequences that modulate gene expression (23, 24). The potential vulnerability of the SP I-FABP–hGH fusion gene to position effects is suggested by the fact that in the second transgenic strain it was not expressed in any tissue, including intestine. Irrespective of which of these explanations is correct, we can still conclude that SP transgenic mice can accurately regulate hGH gene expression in the vertical or crypt to villus axis of the small intestine. This is achieved even though the horizontal or proximal–distal gradient is altered.

The 1000-fold difference in serum hGH levels observed between mice containing the LP and SP transgenes indicates that efficient synthesis of hGH in the distal jejunum and ileum is required to produce elevations in circulating hormone concentration and suggests that there may be regional differences in protein compartmentalization in the small intestine. The site of protein sorting has not been precisely defined in intestinal epithelial cells. These different pedigrees of transgenic mice offer a unique opportunity to examine *in vivo* the intracellular trafficking pattern of a foreign secretory protein that is readily distinguished from endogenous enterocytic (and goblet) cell products. Further examination of foreign protein secretion could provide direct information about whether basolateral delivery represents a default pathway for export from some or all enterocytes and whether comparable pathways exist in goblet cells.

The results obtained with I-FABP–hGH transgenes can be compared to results obtained when regions from the 5′ nontranscribed domains of the homologous rat liver fatty acid-binding protein (L-FABP) gene were linked to hGH and expression of the resulting fusion genes was analyzed in transgenic mice (8). The murine L-FABP gene is normally expressed in the intestine and liver. Like I-FABP, L-FABP is synthesized in enterocytes and this expression exhibits regional differences in both the horizontal and vertical axes. Our analysis of several transgenic pedigrees indicated that proximal–distal gradients in L-FABP gene expression are maintained in part by orientation-independent cis-acting suppressor sequences (8). In transgenic mice containing nt −4000 to +21 of the rat L-FABP gene, small intestinal crypt cells inappropriately expressed hGH even though there was a near normal horizontal hGH (mRNA) gradient (8). As noted above, in SP I-FABP–hGH transgenics there is "appropriate" prohibition of crypt hGH synthesis despite failure to efficiently express hGH in the distal jejunum and ileum. These observations suggest that distinct mechanisms may regulate gene expression in the horizontal and vertical axes of the small intestine. Nonetheless, because L-FABP and I-FABP gene expression in the vertical axis has only been examined by immunocytochemical techniques, we cannot say whether their normal crypt–villus gradients result from transcriptional or translational control mechanisms.

In both this and the previous report (8), we noted a curious unexpected mosaicism in hGH expression within the intestinal epithelium of male and female F_1 mice who were obligate heterozygotes for the transgenes. For L-FABP–hGH transgenic animals, the mosaicism was most obvious in colonic epithelium. The pattern of cellular involvement was consistent with the proposal that all daughter cells from a single crypt progenitor were manifesting aberrant transgene expression (8). An analogous mosaicism was frequently observed in the small intestines of transgenic mice containing nt −277 to +21 of the I-FABP gene. It was rarely seen in mice containing the LP I-FABP–hGH transgene and never observed after staining adjacent sections for the endogenous (I-FABP) gene product. The high levels of mosaicism could result from the absence of critical cis-acting elements or from an unexpectedly high rate of somatic cell mutation (6). As additional support for the former possibility, we noted that when nt −597 to −4000 of the rat L-FABP gene were placed in an opposite orientation, the degree of small intestinal hGH mosaicism increased. The absence of certain cis-acting elements could make the transgenes more susceptible to subtle differences in the levels of positive or negative trans-acting factors. These differences may exist in the small intestinal stem cell population and/or be expressed during cellular commitment/differentiation. Although these differences do not affect endogenous genes at their normal chromosomal location, they can be detected *in vivo* by using transgenes as markers. This emphasizes the potential power and utility of transgenic mice in unraveling the subtle and complex mechanisms that underlie regulation of gene expression in the continuously proliferating intestinal epithelium.

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