Mechanisms underlying generation of gradients in gene expression within the intestine: an analysis using transgenic mice containing fatty acid binding protein–human growth hormone fusion genes

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The intestine is lined by a continuously regenerating epithelium that maintains gradients in 'liver' fatty acid binding protein (L-FABP) gene expression along its horizontal and vertical axes, i.e., from duodenum to colon and from crypt to villus tip. To identify *cis*-acting DNA sequences responsible for these regional differences, we linked portions of the L-FABP gene's 5′ nontranscribed region to the human growth hormone (hGH) gene and examined hGH expression in transgenic mice. Nucleotides −596 to +21 of the rat L-FABP gene correctly directed hGH expression to enterocytes and hepatocytes. However, anomalous expression was observed in small intestinal crypts, colon, and renal proximal tubular epithelial cells. Addition of nucleotides −4000 to −597 of the L-FABP gene, in either orientation, suppressed renal hGH expression and restored a nearly normal horizontal, but not a vertical, hGH gradient in the intestine. Thus, horizontal gradients of gene expression within the intestine can be maintained by orientation-independent, *cis*-acting suppressor elements.

[*Key Words*: Intestinal differentiation; transgenic mice; cell-specific gene expression]

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Gradients in gene expression within the adult mammalian intestinal epithelium are maintained in several dimensions, resulting in regional differences in function and morphology. Along the horizontal axis of the gut, variations in specific absorptive and metabolic capabilities are found among duodenum, jejunum, ileum, and colon. Examples include proximal-to-distal gradients in the activities of certain disaccharidases (sucrase–isomaltase, maltase, lactase–phlorizin hydrolase; Asp et al. 1975; Skovbjerg 1981; Norén et al. 1986), as well as alkaline phosphatase (Young et al. 1981; Shields et al. 1982) and proteases (dipeptidyl peptidase IV, cathepsins B and D; Davies and Messer 1984). Gradients in gene expression also exist in another spatial dimension, namely between the crypts of Lieberkühn and the villus tip (its 'vertical' axis) (Nordström et al. 1968; Simon et al. 1979; Shields et al. 1982, 1986).

This geographic regulation of specific gene expression is established and maintained in an epithelium that is regenerated perpetually. The small intestinal crypt contains ~150 rapidly dividing cells (cycle time is 9–13 hr in the mouse), which provides a steady source of new cells to surrounding villi (Potten et al. 1979). These cells migrate without subsequent division to the villus apex where they are extruded (Schmidt et al. 1985). It is during this translocation that differentiation occurs. In rodents and humans, enterocytes complete their migration, differentiation, and subsequent shedding in 3–5 days (Potten et al. 1979). The net result is replacement of the gut epithelium every few days.

The mechanisms responsible for confining expression of certain genes to intestinal epithelial cells occupying specific positions in the horizontal and/or vertical axes are largely unknown. Several factors, including diet (Ockner and Manning 1974; Smith 1985; Apfelbaum et al. 1987) and hormones (Koldovsky 1979), are capable of influencing the intestinal gradients of certain proteins. In addition, it is thought that luminal factors such as pancreatic enzymes, bile salts, and microorganisms may modulate the expression of genes along the length of the intestine (Lebenthal and Lee 1983). However, several

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groups have reported that when segments of rodent fetal intestine are transplanted into either the subcutaneous tissues or under the renal capsules of syngenic weaning and adult animals, normal regional differences in brush border disaccharidase and alkaline phosphatase activities are expressed in the isografts (Ferguson et al. 1973; Kendall et al. 1979; Jolma et al. 1980). This suggests that the establishment of horizontal gradients in these enzyme activities reflects an intrinsic developmental program within the epithelium that is not dependent on luminal contents.

To date, no *cis*- or *trans*-acting elements have been identified that confer enterocyte-specific gene expression and/or participate in defining the regional nature of intestinal gene expression. The liver fatty acid binding protein (L-FABP) gene represents an attractive model system for such analyses. L-FABP is 127 residues long (Gordon et al. 1983) and is thought to participate in the uptake and/or metabolic compartmentalization of long-chain fatty acids (for review, see Bass 1985; Glatz and Veerkamp 1986; Sweetser et al. 1987a). Synthesis is confined to the absorptive columnar epithelial cells of the small intestine (enterocytes) and hepatocytes (Capron et al. 1979; Shields et al. 1986). The highest levels of intestinal L-FABP mRNA and its primary translation product are found in the duodenum and proximal jejunum with relatively lower amounts in the ileum and colon. Immunocytochemical studies in rat have demonstrated that L-FABP accumulation is first apparent after the enterocytes have emerged from the crypts. Levels progressively increase as epithelial cells move closer to the villus tip (Shields et al. 1986). L-FABP mRNA is very abundant in the small intestinal epithelium, comprising ~2–3% of the total translatable RNA sequences (Gordon et al. 1982). This suggests that L-FABP transcription is regulated by a promoter that can function efficiently within enterocytes. In addition, L-FABP is a member of a gene family containing nine known members, several of which are expressed in enterocytes [i.e., L-FABP, intestinal fatty acid binding protein (I-FABP), and cellular retinol binding protein II (CRBPII)]. Comparative sequence analyses of the promoters of these homologous genes may facilitate the mapping of intestinal specific regulatory sequences (Sweetser et al. 1987b).

We have linked various portions of the 5′ nontranscribed domain of the rat L-FABP gene to the human growth hormone (hGH) gene minus its 5′ regulatory elements. Expression of hGH was subsequently examined in transgenic mice containing these recombinant DNAs using RNA blot and solution hybridizations, as well as immunocytochemical methods. The results indicate that the 5′-proximal promoter region of the L-FABP gene directs high levels of hGH gene expression in the small intestine, colon, liver, and kidney. However, elements located farther upstream are responsible for suppressing expression in the kidney, as well as the cecum and colon. These suppressor elements, which function in either orientation, thus appear responsible for maintaining proximal-to-distal gradients in intestinal L-FABP gene expression.

Results

Preparation and initial characterization of L-FABP–hGH transgenic animals

Three recombinant L-FABP–hGH genes were synthesized (Fig. 1). A 'short promoter' L-FABP–hGH construct contained nucleotides −596 to +21 of the rat L-FABP gene fused to nucleotide +3 of the hGH gene. A 'long promoter' L-FABP–hGH construct contained nucleotides −4000 to +21 of rat L-FABP linked to the hGH gene. A third, 'reverse long promoter' construct contained nucleotides −4000 to −597 in reverse orientation (3′–5′) linked to nucleotides −596 to +21 of the rat L-FABP gene. Four founder transgenic animals containing the short promoter construct were identified along with one founder with the long promoter L-FABP–hGH construct and one founder with the reverse long promoter construct. An additional founder was also identified that contained the long promoter L-FABP–hGH construct plus its pUC vector. These vector sequences had been retained due to an incomplete *Hin*dIII digest of pLFhGH4 DNA (Fig. 1). This was the only mouse that had any vector sequences.

These founders and their transgenic offspring exhibited increased growth rates compared to their normal littermates (see Table 1) and had serum hGH levels from 2 μg/ml to over 1 mg/ml. This latter value is ~100,000 times the normal physiologic level of serum mouse growth hormone (mGH) (Sinha et al. 1972) and is higher than any value reported previously in transgenic mice that contained the hGH gene (Palmiter et al. 1983; Morello et al. 1986). Transgenic mice represented in the different pedigrees contained 70–800 copies of the transgene per haploid genome in a tandem head-to-tail arrangement (Table 1).

To determine whether the presence of multiple copies of the transgenes interfered with expression of the endogenous murine L-FABP gene, 12 different tissues from G_0, F_1, and F_2 transgenic mice and their age- and gender-matched normal littermates were surveyed for L-FABP mRNA using dot blot hybridization analyses (see Experimental procedures). L-FABP mRNA was confined to the liver and intestine of all C57BL/6J × LT/Sv mice. The relative distribution of L-FABP mRNA within the intestine was unaltered by the presence of any of the transgenes. Figure 2 shows the distinct intestinal gradient of L-FABP mRNA concentration: Highest levels are in the proximal jejunum with a progressive decline in mRNA concentration occurring distally. Levels in ileum are 2–4% of those in the proximal jejunum, whereas no L-FABP mRNA was detectable in the cecum or colon. In addition, the absolute level of murine L-FABP mRNA in proximal jejunum, as measured by solution hybridization (see Table 2), was similar in all transgenic mice to the levels found normal littermates.

Expression of hGH in animals containing the short promoter L-FABP–hGH construct differs from that of the endogenous murine L-FABP gene in kidney, cecum, and colon

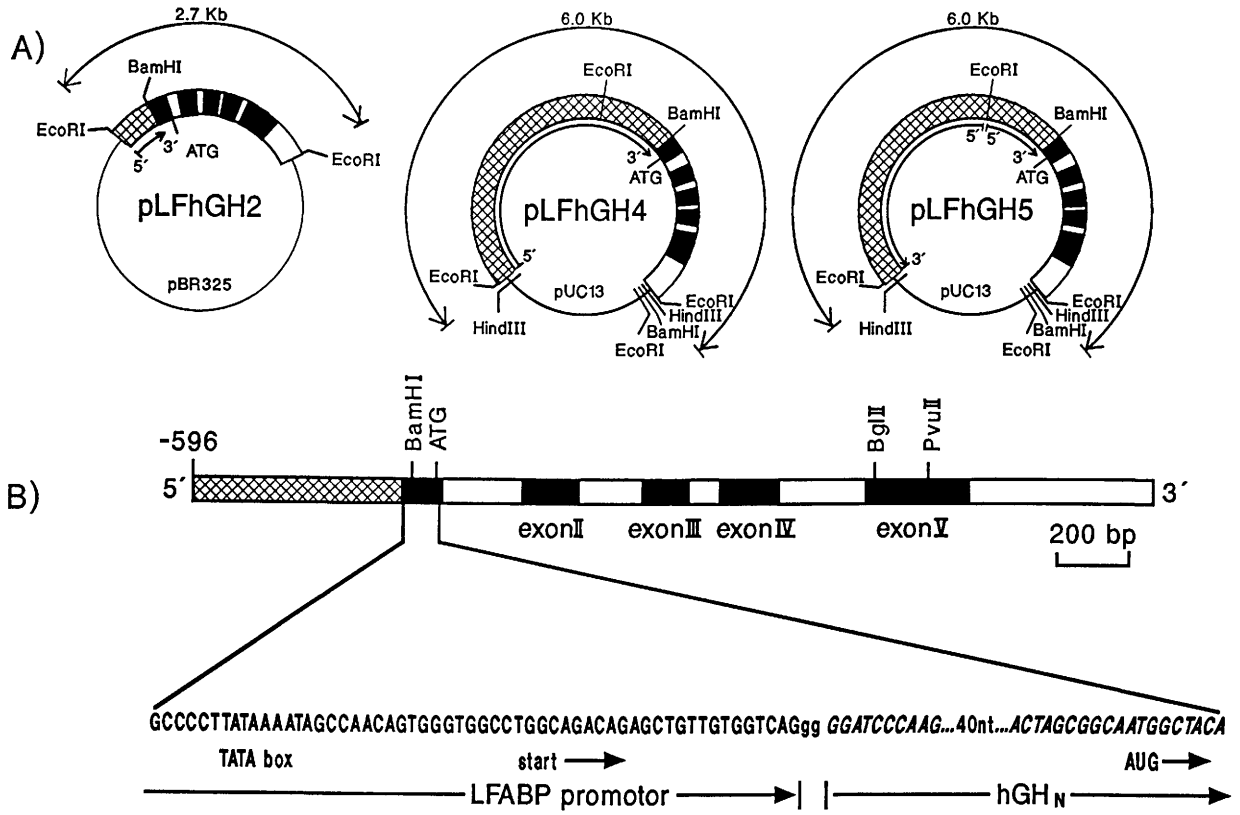

Figure 1. Structures of the rat L-FABP–hGH plasmids. (*A*) Details of plasmid construction are given in Experimental procedures. The thin lines denote vector sequences: pBR325 in the case of pLFhGH2, and pUC13 for both pLFhGH4 and pLFhGH5. Crosshatched areas signify sequences derived from the 5′ nontranscribed region of the rat L-FABP gene, thick solid lines indicate exons of the hGH structural gene, and open boxes indicate hGH introns. Outer arrows indicate the DNA fragments that were isolated and microinjected into the male pronuclei of zygotes harvested from C57BL6/Sv mice. Inner arrows indicate the 5′–3′ orientation of the promoter sequences. The ATG initiator methionine codon in exon I of the hGH gene is shown. (*B*) Schematic illustration of the short promoter L-FABP–hGH fusion gene. The sequence of the L-FABP–hGH junction is given. This fusion gene encodes an mRNA species with an 85-nucleotide-long 5′ nontranslated sequence, the first 21 nucleotides of which are derived from the rat L-FABP gene. The two guanosine residues derived from M13 vector DNA are noted by lowercase letters. A 150-bp *Bgl*II–*Pvu*II fragment from exon V of the hGH gene was used as a probe for RNA blot hybridizations.

The distribution of hGH and L-FABP mRNA were directly compared by dot blot hybridization analysis in liver, kidney, spleen, lung, heart, skeletal muscle, testes, brain, and pancreas plus eight regions of the gastrointestinal tract. Data from several representative heterozygous short-promoter-containing animals (F_2 #19-61, F_1 #13-1, F_1 #13-96) are given in Table 2. The results indicate that nucleotides −596 to +21 of the rat L-FABP gene correctly direct synthesis of hGH mRNA to the liver and intestine of founders and their heterozygous transgenic offspring. Within the *small intestine*, a gradient similar to that observed for murine L-FABP is seen, although somewhat higher relative levels of hGH are seen in the ileum (cf. Fig. 2 and top panel of Fig. 3).

hGH mRNA was not detected in spleen, lung, heart, skeletal muscle, testes, brain, or pancreatic RNA prepared from any of the short promoter founders or their offspring. However, anomalous expression of the short promoter transgene occurred in cecum, colon, and kidney. Figure 3 shows that in the three representative heterozygous transgenic animals, the concentration of hGH mRNA in the cecum, proximal, and distal halves of the colon averaged 78%, 77%, and 24%, respectively, of that encountered in proximal jejunum. This is in marked contrast to L-FABP mRNA, which was not detectable in these distal regions of the intestinal tracts of short promoter transgenic animals or their normal littermates. Similar results were noted in the four founders where levels of hGH mRNA in total colon RNA ranged from 59% to 180% of that measured in total small intestinal RNA (data not shown). The concentration of renal hGH mRNA varied from 8% to 80% of that found in small intestinal RNA, even though L-FABP mRNA was not detected in their kidneys. Animals from all four short promoter pedigrees demonstrated this 'ectopic' expression of hGH in the kidney, indicating that in all probability it was not caused by host DNA sequences at the site of integration of the transgene.

Table 1. *Quantitation of L-FABP/hGH transgene copy number, serum hGH levels, weight, and age documented at the time of sacrifice*

Transgenic mouse	Transgene copy number[a]	Serum hGH (μg/ml)[b]	Weight (gm)	Age (weeks)
		Short promoter		
G_0#19 (F)	530	170(<0.5 ng/ml)[c]	62.2	25
G_0#20 (F)	340	30(<0.5 ng/ml)	74.5	29
G_0#13 (M)	490	548	68.2	35
G_0#2 (M)	350	210	61.1	39
F_1#19-82 (F)	800	1008	56.0(34.8)	18
F_2#82-61 (F)	800	86.5	55.0	27
F_1#13-1 (F)	510	76(<0.5 ng/ml)	55.3(30.6)	18
F_1#13-96 (M)	510	102(<0.5 ng/ml)	49.0(38.9)	18
		Long promoter		
G_0#46 (M)	33	10.2	72.5	20
F_1#46-56 (M)	370	72	35.5(26.7)	7
F_1#46-8 (M)	370	46.9	55.2	15
F_1#1-25 (F)	66	1.6(<0.5 ng/ml)	42.8(31.5)	16
F_1#1-28 (M)	66	7.8(<0.5 ng/ml)	49.5(32.9)	16
		Reverse long promoter		
G_0#19 (M)	ND[d]	15.3	46.6(19.6)	17
$F_1$19-31 (M)	400	41.7	31.2(19.4)	15

Southern blot analysis detected no gross structural rearrangements of the hGH genes in the genomes of any transgenic animal (results not shown). Each pedigree appeared to have a different site of integration of the foreign gene. In all animals except G_0#13, a single integration site was noted. G_0#13 appeared to have two integration sites. Short promoter founders G_0#19, #20, and #2, long promoter founder G_0#46, as well as reverse long promoter founder G_0#19 were mosaics, as determined by immunocytochemistry and by comparing their transgene copy numbers with those of their offspring. The most extreme mosaic (G_0#46) was estimated to have only 8% of its cells positive for the transgene. The gender of each animal is indicated in parenthesis.

[a] Data are expressed as copy number per haploid genome.

[b] Details of the radioimmunoassays for hGH are provided in Experimental procedures.

[c] Values for age- and sex-matched nontransgenic littermates are given in parentheses, where available.

[d] Not determined.

Nucleotides −4000 to −597 of the rat L-FABP gene suppress expression of hGH in kidney, cecum, and colon

The distribution of hGH mRNA in transgenic animals containing the short promoter L-FABP–hGH fusion gene indicates that *cis*-acting elements present in nucleotides −596 to +21 of the rat L-FABP gene are capable of supporting high levels of hGH expression in the colon, as well as anomalous transcription in the kidney. One implication of these findings was that there are other sequences within or flanking the L-FABP gene that restrict its endogenous expression in colon and kidney. The results obtained with the long promoter L-FABP–hGH construct showed that at least some of these suppressor sequences are located between nucleotides −4000 and −597 of the rat gene. In long promoter transgenics, renal and colonic hGH mRNA levels were reduced markedly compared with levels in short promoter transgenics (cf. top and middle panels of Fig. 3). This results in a duodenal to distal colon gradient in L-FABP mRNA concentration, which closely parallels that of endogenous murine L-FABP mRNA. In the two long promoter transgenic lines, hGH mRNA levels were highest in the proximal small intestine and fell progressively throughout the remainder of the distal small and large intestine, although the decrease was not as profound as that seen with L-FABP mRNA (cf. and contrast top and middle panels of Fig. 3 with Fig. 2). The presence of pUC vector sequences in one of the transgenic lines did not affect this distribution of hGH mRNA along the horizontal axis (see transgenics 25 and 28 in middle panel of Fig. 3). No expression of the long promoter transgene was detected in spleen, lung, heart, skeletal muscle, testes, brain, or pancreas harvested from any of the animals.

We concluded from these results that the L-FABP promoter consists of at least two domains: A proximal region directs high levels of expression in liver, small intestine, cecum, colon, and kidney; a second, more distal region contains elements that suppress or modulate the level of expression in kidney, cecum, and colon.

Nucleotides −4000 to −597 of the L-FABP gene also function in the opposite orientation to suppress renal and large intestinal expression of hGH

We then examined whether the suppressor elements in the distal promoter region of the L-FABP gene could function in a reverse orientation. The bottom panel of Figure 3 shows that the distribution of hGH mRNA

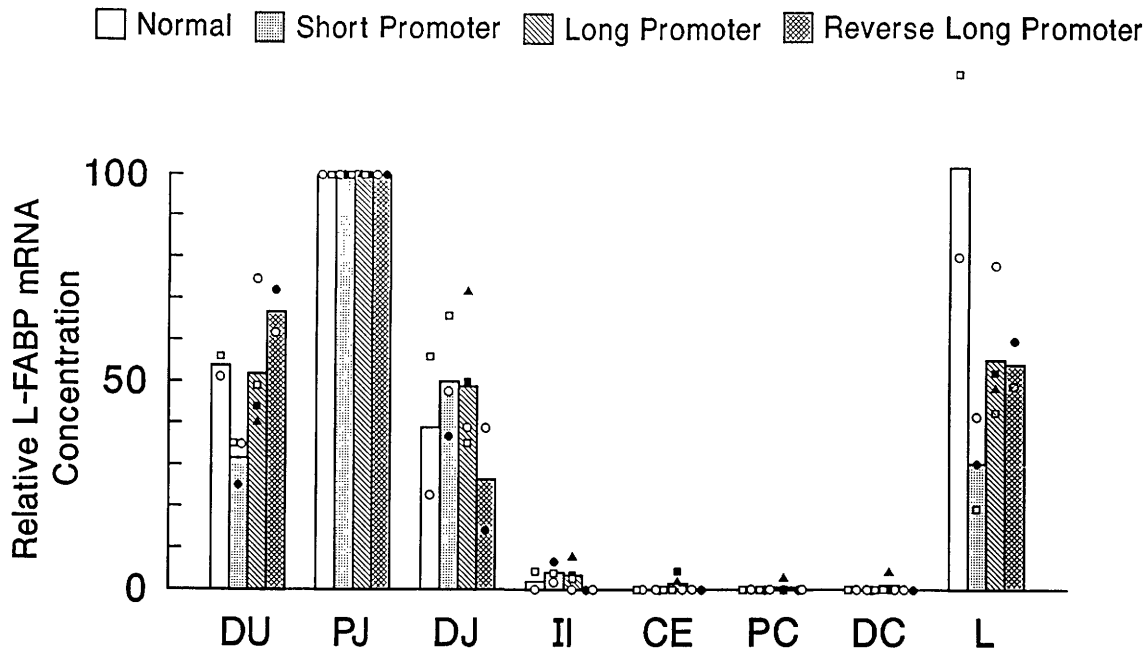

Figure 2. Comparison of the tissue distribution of murine L-FABP mRNA in the intestine and liver of heterozygous transgenic animals from each of the pedigrees and normal littermates. Total cellular RNA was extracted from tissues and blotted onto nitrocellulose filters, as described in Experimental procedures. Filters were probed with a ^{32}P-labeled 335-bp *Pvu*II–*Eco*RI fragment of a rat L-FABP cDNA clone (Gordon et al. 1983). Autoradiographs of the washed blots were scanned by laser densitometry, and the L-FABP mRNA concentrations expressed as a percentage of the levels found in the proximal jejunum of each animal. (DU) Duodenum; (PJ) proximal jejunum; (DJ) distal jejunum; (Il) ileum; (CE) cecum; (PC) proximal colon; (DC) distal colon; (L) liver. The animals used in this study are as follows: normal littermates (F_1s of founder G_o13); #2 (□); #94 (○); short promoter, F_1 #13-96 (□); F_1 #13-1 (○); F_2 #82-61 (●); long promoter, F_1 #46-8 (□); F_1 #46-56 (■); F_1 #1-28 (▲); F_1 #1-25 (○); reverse long promoter, G_o #19 (○); F_1 #19-31 (●).

along the intestinal tract of transgenic animals containing the reverse long promoter L-FABP–hGH construct was very similar to that found in animals containing the long promoter L-FABP–hGH fusion gene. The highest levels of hGH mRNA were, however, encountered in the duodenum rather than in proximal jejunum. By using the proximal jejunum as a reference, the data in middle and bottom panels of Figure 3 demonstrate that the relative degree of suppression in the cecum and colon was similar between transgenic mice containing the two different constructs. In addition, suppression of renal hGH mRNA accumulation was still present when nucleotides −4000 to −597 were in the reverse orientation. No other tissues contained detectable levels of hGH mRNA. The orientation independence of these suppressor elements may indicate that they have some properties similar to those of enhancer elements.

Comparison of hGH and L-FABP mRNA levels in transgenic mice

The RNA dot blot analyses allowed comparison of the relative levels of hGH or L-FABP mRNA in many different tissues. However, they did not indicate whether the rat L-FABP promoter sequences conferred a level of hGH expression that was comparable to that of the endogenous murine L-FABP gene. Solution hybridization analyses were therefore performed to quantitate the

Table 2. *Quantitation of L-FABP and hGH mRNA levels in the proximal jejunum of normal and heterozygote transgenic mice*

	L-FABP mRNA (molecules per cell)[a]	hGH mRNA (molecules per cell)[a]
	Normal littermates	
F_1#13-2	1,500	<2.6
F_1#13-94	1,500	ND[b]
Adult rat	960	ND
	Short promoter	
F_2#61	1,300	970
F_1#13-1	1,200	14,000
F_1#13-96	850	15,000
	Long promoter	
F_1#46-56	1,200	22,000
F_1#46-8	1,600	20,000
F_1#1-25	1,700	2,500
F_1#1-28	1,600	3,500
	Reverse long promoter	
G_o#31	1,300	55,000

[a] The steady-state levels of L-FABP and hGH mRNA in the proximal jejunum of normal and transgenic mice were quantitated by solution hybridization analysis using total cellular RNA and ^{32}P-labeled antisense cRNA probes. To calculate the number of molecules per cell, we used the following values: 6.4 pg of DNA per cell, an RNA : DNA ratio for intestine of 1.5 (Ornitz et al. 1985), L-FABP mRNA length of 750 nucleotides, and a hGH mRNA length of 950 nucleotides.
[b] Value not determined.

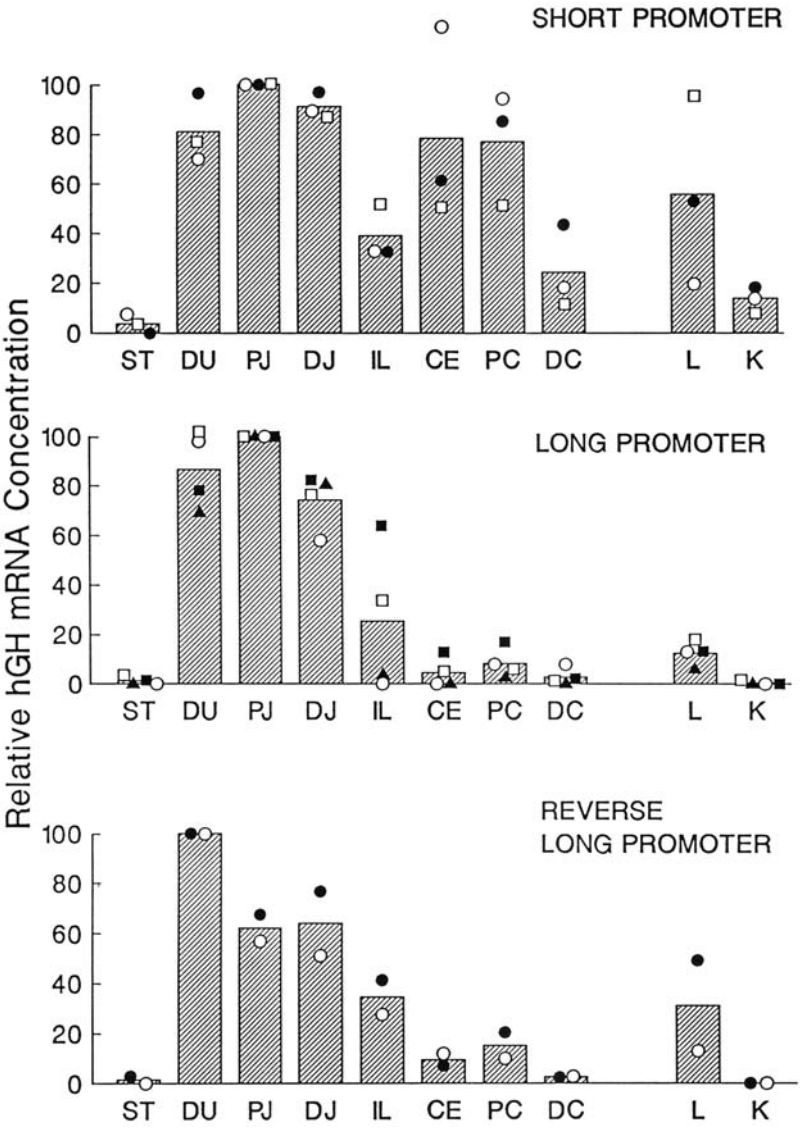

Figure 3. Comparison of the tissue-specific expression of hGH in the stomach, intestine, liver, and kidney of transgenic animals. RNA blots were prepared as described in Experimental procedures and probed with a ^{32}P-labeled 150-bp *Bgl*–*Pvu*II fragment from exon V of the hGH gene and scanned by laser densitometry. The results found with several transgenic animals from each pedigree are displayed. (ST) Stomach; (DU) duodenum; (PJ) proximal jejunum; (DJ) distal jejunum; (Il) ileum; (CE) cecum; (PC) proximal colon; (DC) distal colon; (L) liver; (K) kidney. The animals used in this analysis were: short promoter, F_1 #13-96 (□), F_1 #13-1 (○); F_2 #82-61 (●); long promoter, F_1 #46-8 (□), F_1 #46-56 (■), F_1 #1-28 (▲), F_1 #1-25 (○); reverse long promoter, G_o #19 (●); F_1 #19-31 (○). Primer extension analyses using poly(A)$^+$ RNA derived from the liver, proximal jejunum, colon, and kidney of transgenic mice indicated that the hGH mRNA detected by blot hybridization was initiated at the predicted start site if driven by the rat L-FABP promoter (data not shown).

steady-state levels of L-FABP and hGH mRNAs in the proximal jejunum of heterozygous F_1 and F_2 transgenic animals. The results (Table 2) indicate that 850–1700 L-FABP mRNA molecules accumulate within cells in the jejunum of transgenic animals derived from each pedigree, as well as their normal littermates. These values are similar to that measured in the proximal jejunum of an adult male Sprague–Dawley rat (see Table 2). hGH mRNA was remarkably abundant in this segment of the small intestine of transgenic animals, reaching levels as high as 55,000 molecules per cell (Table 2).

These results indicate that hGH mRNA accumulates to levels several fold higher than murine L-FABP mRNA within the proximal jejunum of nearly all of the transgenic animals. The relative rates of transcription of these genes and the relative stabilities of these two mRNAs were not determined. However, it seems likely that many of the *cis*-acting sequences that are responsible for high levels of L-FABP expression in the proximal intestine are contained within nucleotides −596 to −1.

Immunocytochemical localization of L-FABP and hGH in transgenic mice

Immunocytochemical techniques were employed to examine the cell-specific expression of murine L-FABP and hGH in tissues harvested from normal and transgenic animals. These studies were designed to show whether (1) expression of hGH in liver and intestine was confined to the proper cell types, namely heptocytes and enterocytes, (2) what renal cell type expresses hGH in the short promoter L-FABP–hGH transgenic mice, and (3) whether the elements in the 5′-nontranscribed region of L-FABP that produce a horizontal gradient of hGH mRNA concentration in the intestine also produce a gradient in hGH concentration along the vertical (crypt to villus tip) axis, which mimics that of L-FABP.

The cellular and intralobular distribution of hGH and L-FABP were identical in the livers of animals containing each of the different transgenes

Immunocytochemical studies of the livers of heterozygous animals containing each of the four different transgenes, as well as those of nontransgenic littermates, revealed a pattern of cellular hGH expression identical to that of the endogenous murine L-FABP gene. L-FABP and hGH expression was confined to hepatocytes (Fig. 4). Neither was detected in Küpffer cells, bile ductular epithelial cells, or in vascular smooth muscle and endothelial populations. Considerable variation in staining intensity with both antisera was generally noted between hepatocytes located in different regions of lubules. The weakest staining was noted in hepatocytes located around central veins. The most intense staining was encountered in polyploid and binucleate cells that were situated near the portal triads (Fig. 4A, C, and D), as well as in hepatocytes undergoing mitosis (Fig. 4B). These regional differences in murine L-FABP expression have been noted in the rat by some (Custer and Soroff 1984; Bassuk et al. 1987), but not all, investigators (Capron et al. 1979).

hGH is probably expressed in the proximal renal tubular epithelial cells of animals containing the short promoter L-FABP–hGH transgenes

Sections of kidney were examined from transgenic animals that contained relatively high levels of hGH mRNA (i.e., mice with nucleotides −596 to +21 of the rat L-FABP gene; Fig. 5B) and from those with undetectable levels of this mRNA (selected F_1 and F_2 mice from the long promoter and reverse long promoter pedigrees; Fig. 5C). Animals from *all* the pedigrees exhibited renal staining for hGH. This staining was largely confined to the proximal tubular epithelial cells in the renal cortex. In short promoter L-FABP–hGH animals that possessed high levels of renal hGH mRNA, it is reasonable to assume that these cells are the site of hGH synthesis (Fig. 5C). However, because hGH mRNA was not detectable (by blot or solution hybridization techniques) in kidney RNA prepared from long promoter and reverse long promoter animals, the most likely explanation for the hGH in their renal tubular epithelium was that it had been absorbed from the glomerular filtrate [proteins the size of hGH (22 kD) can be efficiently filtered and reabsorbed in proximal tubules]. Definitive proof of this hypothesis

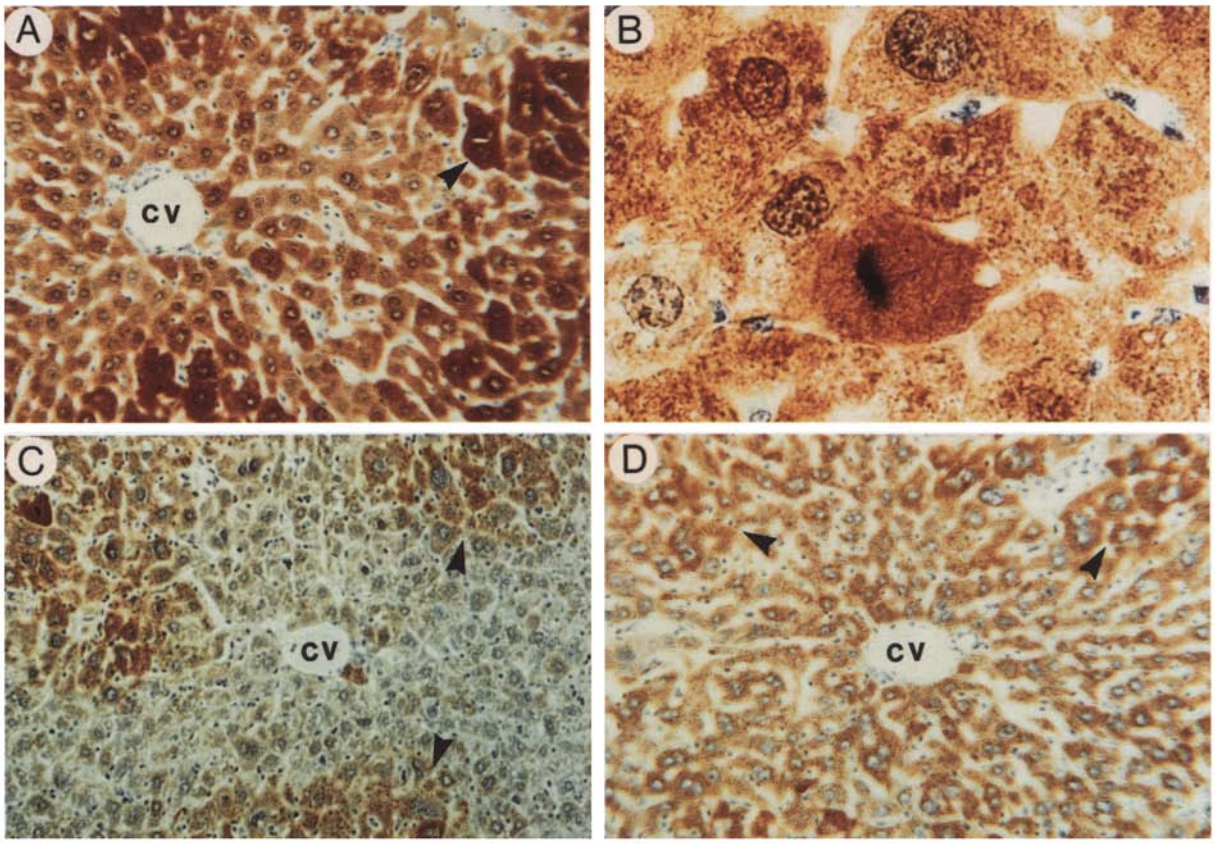

Figure 4. Comparison of the cell-specific expression of hGH and L-FABP in the livers of transgenic animals. Nuclei are counterstained with hematoxylin. The pattern of immunoperoxidase (brown) staining is similar for hGH in long-promoter (*C*, magnification, 70×) and short-promoter-containing animals (*D*, magnification, 170×) to that of L-FABP (*A*, magnification, 170× and *B*, magnification, 700×). Staining is confined to hepatocytes. Bile ductular epithelium, Küpffer cells, and vascular smooth muscle and endothelium are not stained. Centrilobular hepatocytes (surrounding the central vein, CV) are stained less intensely than perilobular hepatocytes (arrowheads). Polyploid cells (evidenced by larger nuclei), binuclear cells, and those rare hepatocytes undergoing mitosis (*B*) generate the most intense signal.

will require in situ hybridization of kidney sections with labeled hGH cDNA (or cRNA) probes.

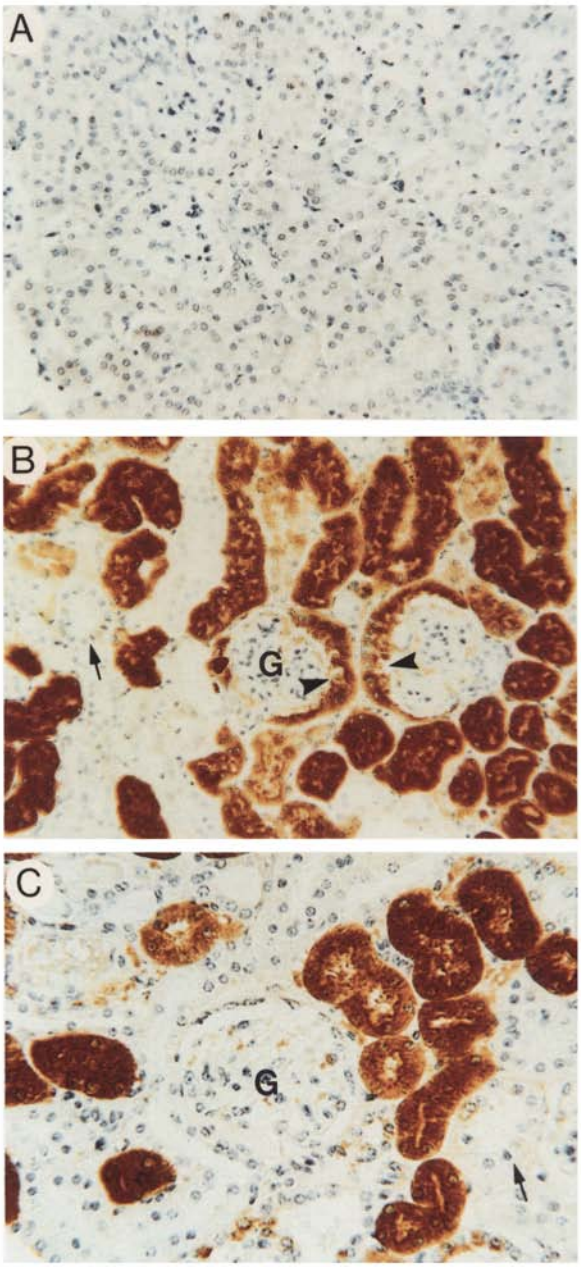

Figure 5. Immunoperoxidase staining for L-FABP and hGH in kidney. No L-FABP staining was evident in normal or transgenic animals (*A*, magnification, 170×). In long-promoter- (*B*, magnification, 170×), and short-promoter-containing animals (*C*, magnification, 270×), hGH staining is largely confined to proximal tubular epithelial cells. Distal tubular epithelial cells (arrows) and glomeruli (G) are negative. In all male transgenic and normal mice, the cells of the parietal layer of Bowman's capsule that surrounds the glomerulus were thickened. These cells are continuous with those of the proximal tubule and also stained for hGH (arrowheads). (For discussion, see text.)

Expression of hGH and L-FABP in the small intestine of transgenic animals is confined to the epithelium, but only hGH is expressed in the crypt

The pattern of *cell-specific* expression of hGH in the intestines of transgenic animals containing each of the recombinant DNAs resembled that of the endogenous murine L-FABP gene. Transgenic animals from each pedigree exhibited the same abrupt increase in hGH expression at the gastroduodenal junction (Fig. 6A) as was seen with the endogenous L-FABP gene (not shown). In all of the animals examined, L-FABP expression was limited to epithelial cells (Fig. 6B). This was also true for hGH in the transgenic animals (Fig. 6E and F). Analysis of 5-μm-thick sections of intestine suggested that immunoreactive hGH and L-FABP were not present in detectable amounts in goblet cells or tuft cells (Fig. 6E). hGH was concentrated in the Golgi apparatus of enterocytes (Fig. 6H), whereas staining for L-FABP was diffusely cytoplasmic (Fig. 6B). Staining for neither of these proteins was apparent in the brush border (Fig. 6B and H). The concentrations of both proteins were greatest in villus-associated enterocytes located in the proximal jejunum. Levels decrease progressively from the proximal jejunum to the distal ileum (Fig. 6, I–K). Scattered among these columnar epithelial cells were cells that stained intensely for both L-FABP and hGH (Fig. 6, D and H). These cells were identified as enteroendocrine cells, based on their morphology, and were found in highest concentration in the proximal duodenum.

Like L-FABP, hGH staining was generally more intense in the lower two-thirds of villus-associated enterocytes compared to those enterocytes located near the villus tip (Fig. 6E). However, unlike L-FABP, which was detectable within enterocytes just after they emerged from the crypts (Fig. 6C), immunoreactive hGH was seen in the crypts of all transgenic lineages (Fig. 6G). Paneth cells at the base of the crypts were generally unstained (Fig. 6G). hGH staining in the crypts was most prominent in the duodenum and proximal jejunum and decreased in the distal jejunum and ileum. Thus, those elements located between nucleotides −597 and −4000 of the rat L-FABP gene that were sufficient to suppress expression of the hGH reporter in the distal intestine were not sufficient to prevent 'precocious' accumulation of hGH in the crypt regions of the vertical axis.

An unexpected mosaic pattern of hGH expression was seen in the large intestines of heterozygous transgenic animals

In contrast to the lack of L-FABP staining in the cecum and colon (Fig. 7, A, D, and G), immunocytochemical studies of F_1 or F_2 transgenic animals containing the short or long promoter L-FABP–hGH constructs revealed a striking degree of mosaicism in hGH staining in their cecal and colonic epithelium. In mice containing nucleotides −596 to +21 of the rat L-FABP gene, most of the cecal and colonic epithelial cells contained high levels of immunoreactive hGH. However, scattered among this epithelium were islands of cells that did not

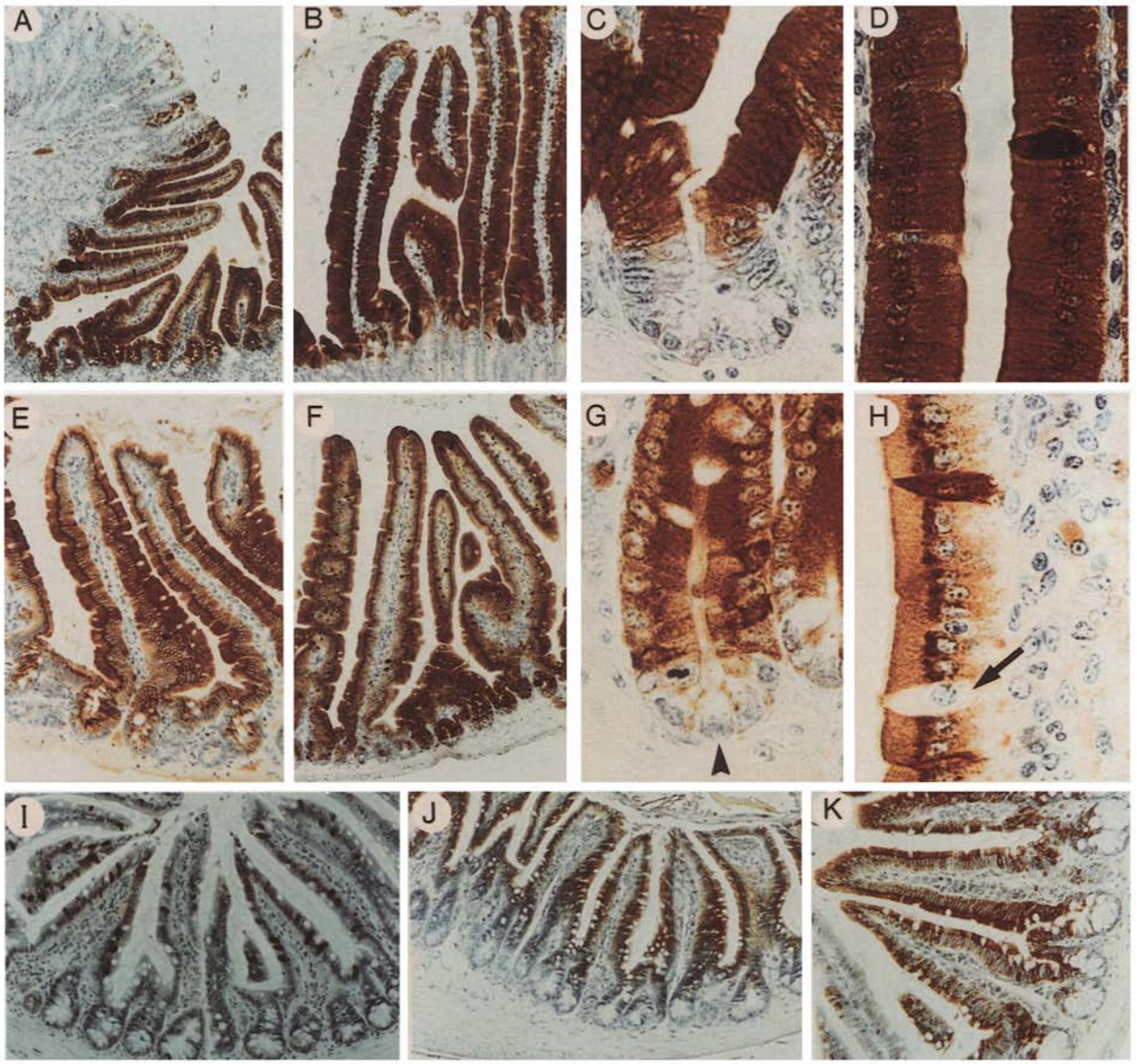

Figure 6. Comparison of the cell-specific expression of L-FABP and hGH in the small intestine of heterozygous F_1 transgenic mice containing short and long promoter constructs. (*A*) hGH staining of the gastroduodenal junction of a long promoter transgenic; magnification, 80×. (*B*) L-FABP distribution in the proximal jejunum of a long promoter animal; magnification, 140×. (*C*) Higher power view showing no staining of L-FABP in crypt-associated epithelial cells; magnification, 680×. (*D*) Section of jejunal villus from long promoter transgenic demonstrating that L-FABP is confined to enterocytes and enteroendocrine cells (which are most intensely stained); magnification, 680×. (*E*) hGH expression in villus and crypt-associated epithelial cells located in the proximal jejunum of a long promoter transgenic; magnification, 170×. (*F*) hGH staining of the proximal jejunum of a short promoter transgenic; magnification, 110×. (*G*) High-power view showing hGH expression in a jejunal crypt of a short promoter animal. Arrowheads point to Paneth cells located at the crypt base, which do not have detectable levels of hGH; magnification, 840×. (*H*) Cell-specific expression of hGH in the villus-associated epithelial cells situated in the jejunum of a long promoter transgenic animal. The arrow indicates a tuft cell (Madera and Trier 1987), which does not appear to express the transgene. Enteroendocrine cells exhibit the same intense staining for hGH as they do for L-FABP. Within enterocytes, the dark supranuclear (Golgi) staining observed for hGH contrasts with the diffuse cytoplasmic staining found with L-FABP, reflecting differences in the intracellular compartmentalization of secreted hGH and cytoplasmic L-FABP; magnification 840×. (*I*) L-FABP expression in the ileum of a long promoter transgenic; magnification, 140×. (*J*) hGH staining in the ileum of the same long promoter animal; magnification, 110×. (*K*) hGH distribution in the ileum of a heterozygous transgenic animal containing the short promoter L-FABP–hGH construct; magnification, 170×.

stain (see Fig. 7, C, F, and I). This staining pattern is the opposite of that seen in mice containing nucleotides −4000 to +21 of the rat L-FABP gene. In the cecum and colon of these animals, the majority of epithelial cells are negative for hGH (Fig. 7, B, E, and H). Within the *small* intestine of both long- and short-promoter-containing heterozygote animals, rare hGH negative bands of cells extending from the crypt to the apical regions of

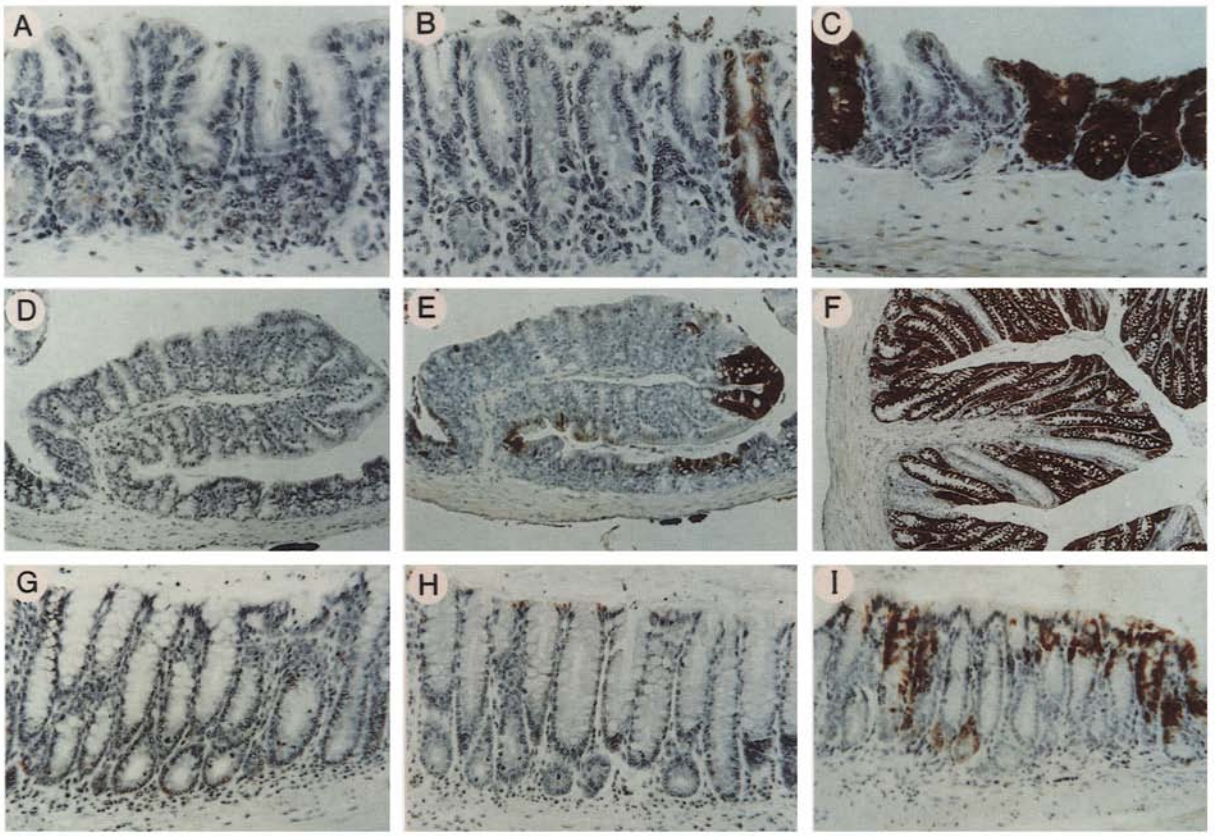

Figure 7. Comparison of the expression of L-FABP and hGH in the cecum and colon of heterozygous F_1 transgenic animals. Panels (*A*) (magnification, 270×), (*D*) (magnification, 110×), (*G*) (magnification, 170×) show that no L-FABP staining is apparent in the cecum, proximal colon, and distal colon of a long promoter transgenic animal. Panels (*B*) (magnification, 270×), (*E*) (magnification, 110×), (*H*) (magnification, 170×) display the results obtained when adjacent sections of these tissues were stained with hGH antisera. Although most of the enterocytes do not stain for hGH, scattered groups of intensely staining cells are present. Panels (*C*) (magnification, 270×), (*F*) (magnification, 80×), (*I*) (magnification, 170×) indicate the patterns of cell-specific hGH expression obtained in the cecum, proximal, and distal colon of an animal containing the short promoter L-FABP–hGH transgene. In contrast to the staining pattern seen with the long promoter animals, the majority of cells stain intensely for hGH. Sharply delineated groups of cells without immunoreactive hGH are scattered in the cecum and colon of these mice. All cells within a given crypt are either negative or positive for hGH staining.

villi were also seen (data not shown).

Some points are worth noting concerning this mosaic staining. (1) The mosaic pattern was not observed with the endogenous L-FABP gene in animals from either pedigree. (2) All of these animals were heterozygous for the transgene (i.e., they were F_1s and F_2s), and mosaic staining was not noted in their livers (see Fig. 4, C and D). (3) Each crypt appeared to produce either positive or negative hGH staining cells—never both. This staining pattern, therefore, likely reflects an event that affected crypt progenitor cells and resulted in cells exhibiting altered expression of the transgene. (4) Within a given pedigree, a similar pattern was present in the colonic epithelium of male as well as female transgenic mice, indicating that it was not associated with random inactivation of the X chromosome. Finally, (5), the mosaic pattern of hGH expression seen in the small intestine and colon of genetically mosaic founder animals also revealed that each crypt is composed entirely of either hGH-positive or hGH-negative cells (data not shown). Our data support the conclusion made from previous studies with mouse aggregation chimeras that epithelial cells in a given intestinal crypt are derived from a single progenitor cell (Ponder et al. 1985; Schmidt et al. 1985).

Discussion

Our analysis of the expression of recombinant rat L-FABP–hGH DNAs in transgenic mice provides insights about the mechanisms involved in establishing gradients in gene expression within the intestine and liver.

In the liver, nucleotides −596 to +21 of the rat L-FABP gene are able to direct high levels of expression of its heterologous reporter (hGH). Expression was hepatocyte specific and formed intralobular gradients similar to those noted for the endogenous murine L-FABP gene. Perilobular hepatocytes generally contained higher

levels of L-FABP than hepatocytes near the central vein. One possible explanation for this difference is that perilobular hepatocytes located near the portal triads are exposed to higher concentrations of factors present in the nutrient-rich portal blood, which directly or indirectly regulate L-FABP gene expression. The similarity of the L-FABP- and hGH-staining patterns in these transgenic animals indicates that the sequences necessary for proper distribution of L-FABP within the hepatic parenchyma are contained within 600 bp, 5′ to the start of transcription.

Within the intestine, nucleotides −596 to +21 of the rat L-FABP gene function with remarkable efficiency to direct high levels of hGH expression. Like the endogenous murine L-FABP, hGH synthesis appears to be restricted to two of the four major classes of epithelial cells that arise from crypt progenitors—enterocytes and enteroendocrine cells. The function of L-FABP in enteroendocrine cells is unclear. Endocrine cells lining the intestinal epithelium secrete a wide variety of polypeptide hormones such as gastrin, gastric inhibitory polypeptide, cholecystokinin, and secretin in response to luminal contents (Holst 1986). L-FABP could deliver fatty acids or other hydrophobic ligands to intracellular compartments of these cells for biosynthetic purposes or mediate the effects of fatty acids on production of intestinal hormones (e.g., cholecystokinin; Holst 1986).

Comparison of the hGH distribution in the intestines of transgenic animals containing nucleotides −4000 to +21 and −596 to +21 of the rat L-FABP gene showed that elements located between nucleotides −597 and −4000 are responsible for suppression of L-FABP expression in cecum and colon. In addition, the function of the suppressor elements is independent of orientation. Because we do not know the precise location of these regulatory elements within the distal promoter region, we cannot conclude whether they function at various distances from the start site of transcription. Curiously, this same distal promoter region of the rat L-FABP gene did not significantly affect the gradient in hGH mRNA concentration observed along the horizontal axis of the *small* intestine. Ileal levels of hGH mRNA were substantially lower than proximal jejunal levels in transgenic mice containing either 596 or 4000 nucleotides from this portion of the rat L-FABP gene. Thus, only the proximal promoter sequences (nucleotides −596 to +21) are needed to obtain lower expression in the ileum. This suggests that the 5′ flanking element(s) responsible for the relatively lower levels of hGH (and L-FABP) mRNA observed in the distal small intestine may be distinct from the elements responsible for cecal and colonic suppression (nucleotides −4000 to −597). It is unclear to what extent, if any, luminal factors modulate or participate in this process.

We can make two additional conclusions from the data concerning L-FABP and hGH expression in the small intestine. First, based on our immunocytochemical studies, the gradient in both hGH and L-FABP reflects, at least in part, a net reduction of gene expression within enterocytes rather than a simple decrease in the number of cells that support synthesis of these mRNAs and their primary translation products. Second, the similar small intestinal gradients in hGH mRNA concentration observed among transgenics containing the long and short promoter constructs serve as a valuable internal control. Their similarity in the face of the dramatic differences in cecal, colonic, and renal expression suggest that the additional 3400 nucleotides present in the long promoter construct do not function as nonspecific suppressors of transcription, but rather as specific suppressors in certain cell types.

For example, our observations concerning kidney expression of hGH in animals containing long and short promoter L-FABP–hGH constructs suggest that nucleotides −597 to −4000 of rat L-FABP can express a suppressor function in renal proximal tubular epithelial cells. The ability of nucleotides −597 to +21 to direct anomalous expression of the reporter gene in these cells is intriguing in light of the morphologic and functional similarities between proximal renal tubular epithelial cells and intestinal enterocytes. Both are highly polarized absorptive cells with well-developed brush borders. They synthesize many of the same proteins including Na^+-coupled hexose, phosphate, and amino acid transport systems. Gradients in gene expression occur along the length of the nephron, just as they do along the horizontal axis of the intestine. Thus, regulation of certain genes that are expressed in both renal proximal tubular epithelial cells and enterocytes may entail the use of related *trans*-acting factors and similar DNA promoter sequences.

Another important finding is that sequences present in the L-FABP gene that are sufficient to restrict expression of hGH along the horizontal axis of the intestine are not sufficient to suppress expression in the crypts of the small intestine. It is possible that different or additional *cis*- and/or *trans*-acting sequences participate in the establishment of the vertical and horizontal gradients in expression of hGH and, by implication, L-FABP. However, the mechanisms underlying the vertical gradients of L-FABP and hGH are unclear at present and may involve translational as well as transcriptional controls.

The mosaic patterns of expression observed for hGH but not L-FABP in heterozygotes derived from the various transgenic pedigrees were an unexpected result. Immunocytochemical studies indicated that the distribution of enterocytes having markedly altered levels of hGH expression was consistent with their derivation from single crypt progenitor cells. In the colon of animals containing the long promoter construct, the pattern suggested 'escape' from suppression—with small bands of markedly positive cells surrounded by broad areas of epithelial cells that did not express the transgene. In the small intestine, the opposite pattern was seen—with relatively infrequent islands of enterocytes with markedly reduced or absent hGH expression extending from the crypt up to the villus.

The mechanisms underlying this mosaicism remain ill defined at present. It is unlikely that it is due to random X chromosomal inactivation, as this pattern

occurs in the intestines of both male and female animals derived from several transgenic pedigrees. We cannot so readily dismiss somatic mutation as an underlying cause. Rearrangements or loss of transgene sequences may occur at a higher frequency than mutations in the endogenous gene. Moreover, although we could not detect mosaicism in the livers of transgenic mice, a rare, single hepatocyte that has undergone mutation to a nonexpressing phenotype may be more difficult to detect than in the intestine, where the event will be amplified because of the clonal structure of the crypts (Griffiths et al. 1988).

Alternatively, the absence of mosaic L-FABP expression in these mice may indicate that additional sequences are missing from the transgenes that are involved in regulation of endogenous L-FABP expression. Subtle differences may exist among crypt progenitors in these heterozygous transgenic mice that can be operationally defined in their daughter cells using these transgenes. For example, differences in the types and/or levels of expression of positive (or negative) *trans*-acting factors in these crypt cell lineages, combined with the absence of certain *cis*-acting regulatory sequences, may account for the observed local variation in transgene expression.

The generality of these proposed mechanisms underlying horizontal (and vertical) gradient formation in the intestine needs to be established by using other model transgenes. Although further studies of structure/activity relationships in the rat L-FABP gene remain to be performed, it is clear that domains contained within 4 kb of its start site of transcription can be used to promote efficient expression of gene products in liver, as well as specific regions of the gastrointestinal tract.

Experimental procedures

Construction of recombinant DNAs containing rat L-FABP and hGH gene sequences

An *Eco*RI–*Pvu*II fragment encompassing nucleotides −596 to +21 of the rat L-FABP gene promoter was inserted into *Eco*RI–*Sma*I-digested M13mp19 (Fig. 1). A 2.15-kb *Bam*HI fragment of MThGH 111 (Palmiter et al. 1983), which contained the entire structural gene for hGH (beginning at nucleotide +3), was isolated and subcloned into the *Bam*HI site of the mp19 L-FABP clone. The L-FABP–hGH fusion gene was excised as a 2.7-kb *Eco*RI fragment and introduced into pBR325. The resulting clone was termed pLFhGH2.

To construct the long promoter L-FABP–hGH fusion gene, a 3.4-kb *Eco*RI genomic fragment containing nucleotides −4000 to −597 of the rat L-FABP gene (Sweetser et al. 1986) was first blunt-ended using the Klenow fragment of DNA polymerase I and dNTPs. *Hin*dIII linkers were attached, and the resulting 3.4-kb fragment subsequently subcloned into the *Hin*dIII site of pUC13. This strategy preserved the original *Eco*RI sites in the rat L-FABP DNA sequence. An empirically determined amount of ethidium bromide was then included in an *Eco*RI restriction digest to linearize the plasmid randomly at one of the three *Eco*RI sites. The linearized plasmid was then purified by agarose gel electrophoresis and ligated to the 2.7-kb *Eco*RI fragment of pLFhGH2. The correct 5′–3′ orientation and site of integration was selected from the six possible permutations using analytical *Hin*dIII and *Bam*HI digests. This construct was called pLFhGH4. Another clone containing the upstream 3.4-kb fragment in the reverse orientation was also isolated and called pLFhGH5.

Generation and characterization of transgenic mice

Pronuclear injections A 2.7-kb *Eco*RI fragment from pLFhGH2 DNA and a 6-kb *Hin*dIII fragment from pLFhGH4 and pLFhGH5 DNAs were purified by preparative electrophoresis through agarose gels followed either by electrophoresis onto and elution from DEAE–cellulose paper (Schleicher and Schuell) or extraction using the Geneclean DNA purification system (BIO 101, La Jolla, California).

Procedures for isolation of zygotes from C57BL/6J females mated to males, microinjection of 2000–4000 copies of recombinant DNAs into their male pronuclei, and subsequent transfer of the morulae to pseudopregnant C57BL/6J × SJL/J F_1 hybrid females were similar to those described previously (Wagner et al. 1981).

Determination of transgene copy number Southern blots of restriction-endonuclease-digested DNAs were probed with a ^{32}P-labeled 2.7-kb *Eco*RI fragment from pLFhGH2. Hybridization and washing conditions were identical to those described in Kozak and Birkenmeier (1983). The number of copies of the transgene in mice from each of these pedigrees was calculated using laser densitometric measurements of bands present in autoradiographs of Southern blots. These blots also contained known amounts of digested recombinant plasmids.

Radioimmunoassays of serum hGH levels Serum was obtained from mice by retroorbital phlebotomy at the time of their sacrifice. hGH was measured by a double-antibody radioimmunoassay. Highly purified hGH for iodination and standards was kindly provided by the National Hormone and Pituitary Program (Baltimore, Maryland). A monospecific, polyclonal guinea pig anti-hGH serum (Daughaday et al. 1987) was used. hGH was undetectable (<0.5 ng/ml) in the serum of normal siblings of the transgenic mice (see Table 1), indicating that the antibody employed did not cross-react with mGH. (The level of mGH in the serum of normal mice after weaning is ~10 ng/ml; Sinha et al. 1972.) Triplicate determinations were performed for each serum sample assayed for hGH.

Analysis of the tissue distribution of L-FABP and hGH mRNAs

Isolation of total cellular RNA Transgenic mice and their normal littermates were sacrificed by cervical dislocation. Brain, heart, lung, skeletal muscle, spleen, kidney, pancreas, testis, liver, plus eight regions of the gastrointestinal tract were then dissected rapidly. Regional dissection of the intestinal tract was accomplished as follows. The duodenum was considered to be a 4-cm segment of small intestine extending from the pylorus. The remainder of the small intestine was divided into three equal-length segments. These segments were operationally defined as the proximal jejunum, distal jejunum, and ileum. The cecum was separated from the colon which, in turn, was divided into two equal-length portions. Tissues were frozen in liquid nitrogen, and RNA was extracted using the guanidine isothiocyanate procedure of Chirgwin et al. (1979). The integrity of RNA samples was established by denaturing methylmercury gel electrophoresis (Bailey and Davidson 1976). Prior to freezing, a number of tissue sections were excised and fixed for histological studies. These included a portion of liver, the

gastroduodenal junction, as well as two 5-mm-long segments one-quarter the distance from the proximal and distal ends of each of the jejunal and ileal sections. Similar length biopsies were taken from the middle of the duodenal, cecal, proximal, and distal colonic segments.

RNA blot hybridizations Dot blots containing four concentrations (0.5, 1, 2, and 3 μg) of each sample of tissue RNA were made using nitrocellulose filters and a template manifold (Bethesda Research Labs). Yeast tRNA was added to each sample prior to denaturation so that the total concentration of RNA (yeast tRNA plus mouse tissue RNA) was identical for all dots in a blot (3 μg). Dot blots were probed with either a 150-bp *Bgl*II–*Pvu*II fragment from exon V of the hGH gene or a 335-bp *Pvu*II–*Eco*RI fragment recovered from pJG418, which contains a full-length rat L-FABP cDNA (Gordon et al. 1983). Each of these two probes was labeled with [α^{32}P]dATP to a sp. act. of ~1000 cpm/pg using hexanucleotide primers (Feinberg and Vogelstein 1983).

Stringencies selected for hybridization and washing of these dot blots have been described (Demmer et al. 1986). Northern blots indicated that they result in the reaction of the L-FABP cDNA probe with a unique 750 nucleotide-long mRNA species in samples of normal C57BL/6J × LT/Sv mouse liver and intestinal RNA (data not shown). These conditions do not produce any detectable cross-reaction between L-FABP cDNA and mRNAs encoding other family members [e.g., I-FABP (Alpers et al. 1984) and CRBPII (Li et al. 1986)]. When Northern blots of liver and intestinal RNA from transgenic mice from the various pedigrees were analyzed using these same stringencies and the hGH exon V probe, a unique 950-bp mRNA was visualized, in agreement with the size of hGH mRNA reported previously (Palmiter et al. 1983).

RNA primer extension analysis Primer extension studies were performed to verify that initiation of transcription of the various L-FABP–hGH transgenes had occurred at the proper sites in intestine, colon, liver, and kidney. A 19-base-long oligodeoxynucleotide was synthesized for these studies: (5′-GCCATTGCCGCTAGTGAGC-3′) complementary to nucleotides +70 to +88 of exon I of the hGH gene (Seeburg 1982). The protocol used for primer extension has been detailed in a previous publication (Sweetser et al. 1986).

RNA solution hybridization analysis A 245-bp *Rsa*I–*Sma*I fragment from exon V of the hGH gene was subcloned into pGEM2. A 335-bp *Pvu*II–*Eco*RI fragment from the cloned rat L-FABP double-stranded cDNA, described above, was also subcloned into this vector. A labeled 172-nucleotide antisense hGH cRNA probe was generated from the pGEM–hGH subclone by first linearizing it with *Bgl*I and then adding it to a reaction mixture containing SP6 RNA polymerase and [α^{32}P]UTP (Melton et al. 1984). A ^{32}P-labeled, 337-nucleotide-long antisense L-FABP cRNA was similarly produced by in vitro runoff transcription of pGEM2–L-FABP DNA after linearizing it with *Hin*dIII. Only heterozygous F_1 and F_2 transgenics were used for these analyses. Total cellular RNA samples (1.0 and 2.0 μg for measurement of L-FABP mRNA and 25 ng–2.0 μg for hGH mRNA analysis) were hybridized and processed as described previously (Melton et al. 1984). Standard curves were constructed by incubating each of the ^{32}P-labeled cRNAs with varying amounts (0.1–100 pg) of ^{3}H-labeled L-FABP and hGH mRNA transcribed in vitro from the pGEM recombinant DNAs with T7 DNA polymerase.

Immunocytochemical studies Sections 5 μM thick were prepared from tissue samples that had been fixed in Bouin's solution (0.9% picric acid, 9% formaldehyde, 5% acetic acid) and embedded in paraffin. The unlabeled antibody peroxidase–antiperoxidase (PAP) method (Sternberger 1979) was used to examine the cellular distribution of L-FABP and hGH. Endogenous tissue peroxidases were neutralized by preincubating the sections in 0.3% H_2O_2 for 30 min. A 1 : 500 dilution of the L-FABP antibody or a 1 : 2000 dilution of hGH antibody was applied to the tissue sections and allowed to incubate at room temperature for 2 hr (hGH) or at 4°C overnight (L-FABP) prior to addition of the secondary linker antisera and the PAP conjugate (Sternberger–Meyer Immunocytochemicals). After treatment with diaminobenzidine (Sigma), nuclei were counterstained with hematoxylin. The polyclonal, monospecific goat anti-hGH sera has been described (McKeel and Askin 1978). Polyclonal L-FABP antisera was raised in rabbits using rat L-FABP that had been purified form *Escherichia coli* containing a suitably constructed prokaryotic expression vector (Lowe et al. 1984). The purity of the antigen was established by denaturing SDS-polyacrylamide gel electrophoresis and by automated sequential Edman degradation (Lowe et al. 1984). The specificity of the antisera produced was established using several criteria. (1) The antisera immunoprecipitated a single 15-kD radiolabeled protein from wheat germ and reticulocyte lysate cell-free translation systems that had been programmed with total rat intestinal RNA. The [^{35}S]methionine and [^{3}H]lysine-labeled primary translation product that comigrated with authentic L-FABP was shown to be L-FABP after automated sequential Edman degradation (Lowe et al. 1984). (2) The antibody reacted with a single 15-kD protein when used to probe Western blots of rat or mouse liver or intestinal cytosolic extracts (Burnette 1981). Finally (3), the antibody did not react with the purified homologous *E. coli*-derived rat intestinal FABP (Lowe et al. 1987), as judged by Ouchterolony double immunodiffusion analysis (Kabat and Meyer 1961).

Several control experiments were performed to confirm that staining in tissues arose from specific interactions with hGH or L-FABP: (1) 5-μm sections of intestine and liver obtained from age- and gender-matched normal littermates did not produce staining using the hGH antisera; (2) L-FABP staining observed in these tissues was blocked by adsorption of the antisera with rat L-FABP but not with rat I-FABP or CRBP II (homologous proteins also found in the intestine); and (3) no staining was observed when preimmune sera was substituted for either primary antibody.

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References

Alpers, D.H., A.W. Strauss, R.K. Ockner, N.M. Bass, and J.I. Gordon. 1984. Cloning of a cDNA encoding rat intestinal fatty acid binding protein. *Proc. Natl. Acad. Sci.* **81:** 313–317.

Apfelbaum, T.F., N.O. Davidson, and R.M. Glickman. 1987. Apolipoprotein A-IV synthesis in rat intestine: Regulation by dietary triglyceride. *Am. J. Physiol.* **252**: G662–G666.

Asp, N.-G., E. Gudmand-Höyer, B. Andersen, N.-O. Berg, and A. Dahlqvist. 1975. Distribution of disaccharidases, alkaline phosphatase, and some intracellular enzymes along the human small intestine. *Scand. J. Gastroenterol* **10**: 647–651.

Bailey, J.M. and N. Davidson. 1976. Methyl mercury as a reversible denaturing agent for agarose gel electrophoresis. *Anal. Biochem.* **70**: 75–85.

Bass, N.M. 1985. Function and regulation of hepatic and intestinal fatty acid binding protein. *Chem. Phys. Lipids.* **38**: 95–114.

Bassuk, J.A., P.N. Tsichlis, and S. Soroff. 1987. Liver fatty acid binding protein is the mitosis-associated polypeptide target of a carcinogen in rat hepatocytes. *Proc. Natl. Acad. Sci.* **84**: 7547–7551.

Burnette, W.N. 1981. 'Western blotting': Electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radio iodinated protein A. *Anal. Biochem.* **112**: 195–203.

Capron, F., B. Coltoff-Schiller, A.B. Johnson, G.M. Fleischner, and S. Goldfischer. 1979. Immunocytochemical localization of hepatic ligandin and Z protein utilizing frozen sections for light and electron microscopy. *J. Histochem. Cytochem.* **27**: 961–966.

Chirgwin, J.M., A.E. Przybyla, R.J. MacDonald, and W.J. Rutter. 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **24**: 5294–5299.

Custer, R.P. and S. Soroff. 1984. Target polypeptide of a carcinogen is associated with normal mitosis and carcinogen-induced hyperplasias in adult hepatocytes. *Proc. Natl. Acad. Sci.* **81**: 6738–6742.

Daughaday, W.H., R.H. Starkey, S. Saltman, J.R. Gavin, B. Mills-Dunlap, and E. Heath-Monnig. 1987. Characterization of serum growth hormone (GH) and insulin-like growth factor I in active acromegaly with minimal elevation of serum GH. *J. Clin. Endocrinol. Metab.* **65**: 617–623.

Davies, P.H. and M. Messer. 1984. Intestinal cathepsin B and D activities of suckling rats. *Biol. Neonat.* **45.**: 197–202.

Demmer, L.A., M.S. Levin, J. Elovson, M.A. Reuben, A.J. Lusis, and J.I. Gordon. 1986. Tissue-specific expression and developmental regulation of the rat apolipoprotein B gene. *Proc. Natl. Acad. Sci.* **83**: 8102–8106.

Feinberg, A.P. and B. Vogelstein. 1983. High specific activity labeling of DNA restriction endonuclease fragments. *Anal. Biochem.* **132**: 6–13.

Ferguson, A., V.P. Gerskowitch, and R.I. Russell. 1973. Pre- and postweaning disaccharidase patterns in isografts of fetal mouse intestine. *Gastroenterology* **64**: 292–297.

Glatz, J.F.C. and J.H. Veerkamp. 1986. Intracellular fatty acid binding proteins. *Int. J. Biochem.* **17**: 13–22.

Gordon, J.I., D.H. Alpers, R.K. Ockner, and A.W. Strauss. 1983. The nucleotide sequence of rat liver fatty acid binding protein mRNA. *J. Biol. Chem.* **258**: 3356–3363.

Gordon, J.I., D.P. Smith, D.H. Alpers, and A.W. Strauss. 1982. Cloning of a complementary deoxyribonucleic acid encoding a portion of rat intestinal preapolipoprotein AIV messenger ribonucleic acid. *Biochemistry* **21**: 5424–5431.

Griffiths, D.R.F., S.J. Davies, D. Williams, G.T. Williams, and E.D. Williams. 1988. Demonstration of somatic mutation and crypt clonality by x-linked enzyme histochemistry. *Nature* **333**: 461–463.

Holst, J.J. 1986. Hormonal regulation of digestive secretions. In *Molecular and cellular basis of digestion* (ed. P. Desnuelle, H. Sjöström, and O. Norén), pp. 79–112. Elsevier Science Publishers, Amsterdam.

Jolma, V.M., K. Kendall, O. Koldovsky. 1980. Differences in the development of jejunum and ileum as observed in fetal-rat intestinal isografts—possible implications related to the villus size gradient. *Amer. J. Anat.* **158**: 211–215.

Kabat, E.A. and M.M. Mayer, 1961. *Experimental immunochemistry*, pp. 85–90. Charles C. Thomas, Springfield, Illinois.

Kendall, K., J. Jumawan, and O. Koldovsky. 1979. Development of jejunoileal differences of activity of lactase, sucrase and acid β-galactosidase in isografts in fetal rat intestine. *Biol. Neonat.* **36**: 206–214.

Koldovsky, O. 1979. Developement of sucrase activity: Effect of maternal hormonal status and fetal programming of jejunoileal differences. *Ciba Found. Symp* **70**: 147–168.

Kozak, L.P. and E.H. Birkenmeier. 1983. Mouse sn-glycerol-3-phosphate dehydrogenase: Molecular cloning and genetic mapping of a cDNA sequence. *Proc. Natl. Acad. Sci.* **80**: 3020–3024.

Lebenthal, E. and P.C. Lee. 1983. Review article. Interactions of determinants in the ontogeny of the gastrointestinal tract: A unified concept. *Pediatr. Res.* **17**: 19–24.

Li, E., L.A. Demmer, D.A. Sweetser, D.E. Ong, and J.I. Gordon. 1986. Rat cellular retinal binding protein II: Use of a cloned cDNA to define its primary structure, tissue specific expression, and developmental regulation. *Proc. Natl. Acad. Sci.* **83**: 5779–5783.

Lowe, J.B., A.W. Strauss, and J.I. Gordon. 1984. Expression of a mammalian fatty acid-binding protein in *Escherichia coli*. *J. Biol. Chem.* **259**: 12696–12704.

Lowe, J.B., J.C. Sacchettini, M. Laposata, J.J. McQuillan, and J.I. Gordon. 1987. Expression of rat intestinal fatty acid-binding protein in *Escherichia coli*. Purification and comparison of ligand binding characteristics with that of *Escherichia coli*-derived rat liver fatty acid-binding protein. *J. Biol. Chem.* **262**: 5931–5937.

Madera, J.L. and J.S. Trier. 1987. Functional morphology of the mucosa of the small intestine. In *Physiology of the gastrointestinal tract* (ed. L.R. Johnson) pp. 1209–1249. Raven Press, New York.

McKeel, D.W., Jr. and F.B. Askin. 1978. Ectopic hypophyseal hormonal cells in benign cystic teratoma of the ovary. *Arch. Pathol. Lab. Med.* **102**: 122–128.

Melton, D.A., P.A. Krieg, M.R. Reboglieti, T. Maniatis, K. Zinn, and M.R. Green. 1984. Efficient in vitro synthesis of biologically active RNA and RNA hybridization probes from plasmids containing a bacteriophage SP6 promoter. *Nucleic Acids Res.* **12**: 7035–7056.

Morello, D., G. Moore, A.M. Salmon, M. Yaniv, and C. Babinet. 1986. Studies on the expression of an H-2K/human growth hormone fusion gene in giant transgenic mice. *EMBO J.* **5**:1877–1883.

Nordström, C., A. Dahlqvist, and L. Josefsson. 1968. Quantitative determination of enzymes in different parts of the villi and crypts of rat small intestine. Comparisons of alkaline phosphatase, disaccharidases and dipeptidases. *J. Histochem. Cytochem.* **15**: 713–721.

Norén, O., H. Sjöström, E.M. Danielsen, G.M. Cowell, and H.

Skovbjerg. 1986. The enzymes of the enterocyte plasma membrane. In *Molecular and cellular basis of digestion* (ed. P. Desnuelle, H. Sjöström, and O. Norén) pp. 335–366. Elsevier Sciences Publishers, Amsterdam.

Ockner, R.K. and J.A. Manning. 1974. Fatty acid-binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *J. Clin. Invest.* **54**: 326–338.

Ornitz, D.M., R.D. Palmiter, R.E. Hammer, R.L. Brinster, G.H. Swift, and R.J. MacDonald. 1985. Specific expression of an elastase-human growth hormone fusion gene in the pancreatic acinar cells of transgenic mice. *Nature* **313**: 600–602.

Palmiter, R.D., G. Norstedt, R.E. Gelinas, R.E. Hammer, and R.L. Brinster. 1983. Metallothionein-human GH fusion genes stimulate growth of mice. *Science* **222**: 809–814.

Ponder, B.A.J., G.H. Schmidt, M.M. Wilkinson, M.J. Wood, M. Monk, and A. Reid. 1985. Mouse intestinal crypts are each derived from a single progenitor cell. *Nature* **313**: 689–691.

Potten, C.S., R. Schofield, and L.G. Lajtha. 1979. A comparison of cell replacement in bone marrow, testis and three regions of surface epithelium. *Biochim. Biophys. Acta* **560**: 281–299.

Schmidt. G.H., M.M. Wilkinson, and B.A.J. Ponder. 1985. Cell migration pathway in the intestinal epithelium: An in situ marker system using mouse aggregation chimeras. *Cell* **40**: 425–429.

Seeburg, P.H. 1982. The human growth hormone gene family: Nucleotide sequences show recent divergence and predict a new polypeptide hormone. *DNA* **1**: 239–249.

Shields, H.M., F.A. Bair, M.L. Bates, S.T. Yedlin, and D.H. Alpers. 1982. Localization of immunoreactive alkaline phosphatase in the rat small intestine at the light microscopic level by immunocytochemistry. *Gastroenterology* **82**: 39–45.

Shields, H.M., M.L. Bates, N.M. Bass, C.J. Best, D.H. Alpers, and R.K. Ockner. 1986. Light microscopic immunocytochemical localization of hepatic and intestinal types of fatty acid-binding proteins in rat small intestine. *J. Lipid Res.* **27**: 549–557.

Simon, P.M., M. Kedinger, F. Raul, J.F. Grenier, and K. Haffen. 1979. Developmental pattern of rat intestinal brush-border enzymic proteins along the villus-crypt axis. *Bichem. J.* **178**: 407–413.

Sinha, Y.N., F.W. Selby, U.J. Lewis, and W.P. Vanderlaan. 1972. Studies of GH secretion in mice by a homologous radioimmunoassay for mouse GH. *Endocrinology* **91**: 784–792.

Skovbjerg, H. 1981. Immunoelectrophoretic studies on human small intestinal brush border proteins—The longtitudinal distribution of peptidases and disaccharidases. *Clin. Chim. Acta.* **112**: 205–212.

Smith, M.W. 1985. Expression of digestive and absorptive function in differentiating enterocytes. *Annu. Rev. Physiol.* **47**: 247–260.

Sternberger, L.A. 1979. *Immunocytochemistry*, pp. 122–127. John Wiley, New York.

Sweetser, D.A., R. Heuckeroth, and J.I. Gordon. 1987a. The metabolic significance of mammalian fatty acid binding proteins: Abundant proteins in search of a function. *Annu. Rev. Nutr.* **7**: 337–359.

Sweetser, D.A., J.B. Lowe, and J.I. Gordon. 1986. The nucleotide sequence of the rat liver fatty acid binding protein gene. Evidence that exon 1 encodes an oligopeptide domain shared by a family of proteins which bind hydrophobic ligands. *J. Biol. Chem.* **261**: 5553–5561.

Sweetser, D.A., E.H. Birkenmeier, I.J. Klisak, S. Zollman, R.S. Sparkes, T. Mohandas, A.J. Lusis, and J.I. Gordon. 1987b. The human and rodent intestinal fatty acid binding protein genes. A comparative analysis of their structure, expression and linkage relationships. *J. Biol. Chem.* **262**: 16060–16071.

Wagner, T.E., P.C. Hoppe, J.D. Jollick, D.R. Scholl, R.L. Hodinka, and J.B. Gault. 1981. Microinjection of a rabbit β-globin gene into zygotes and its subsequent expression in the adult mice and their offspring. *Proc. Natl. Acad. Sci.* **78**: 6376–6380.

Young, G.P., S.T. Yedlin, and D.H. Alpers. 1981. Distribution of soluble and membranous forms of alkaline phosphatase in the small intestine of the rat. *Biochim. Biophys. Acta* **676**: 257–265.

Mechanisms underlying generation of gradients in gene expression within the intestine: an analysis using transgenic mice containing fatty acid binding protein-human growth hormone fusion genes.

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