Use of Transgenic Mice to Map *cis*-acting Elements in the Intestinal Fatty Acid Binding Protein Gene (*Fabpi*) That Control Its Cell Lineage-specific and Regional Patterns of Expression along the Duodenal–Colonic and Crypt–Villus Axes of the Gut Epithelium

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Abstract. The mouse intestinal epithelium is able to establish and maintain complex lineage-specific, spatial, and temporal patterns of gene expression despite its rapid and continuous renewal. A multipotent stem cell located near the base of each intestinal crypt gives rise to progeny which undergo amplification and allocation to either enterocytic, Paneth cell, goblet cell, or enteroendocrine cell lineages. Differentiation of these four lineages occurs during their geographically ordered migration along the crypt–villus axis. Gut stem cells appear to have a "positional address" which is manifested by differences in the differentiation programs of their lineal descendants along the duodenal–colonic (cephalocaudal) axis. We have used the intestinal fatty acid binding protein gene (*Fabpi*) as a model to identify *cis*-acting elements which regulate cell- and region-specific patterns of gene expression in the gut. Nucleotides −1178 to +28 of rat *Fabpi* direct a pattern of expression of a reporter (human growth hormone [hGH]) which mimics that of mouse *Fabpi* (*a*) steady-state levels of hGH mRNA are highest in the distal jejunum of adult transgenic mice and fall progressively toward both the duodenum and the mid-colon; and (*b*) hGH is confined to the enterocytic lineage and first appears as postmitotic, differentiating cells exit the crypt and migrate to the base of small intestinal villi or their colonic homologs, the surface epithelial cuffs. Nucleotides −103 to +28, which are highly conserved in rat, mouse and human *Fabpi*, are able to correctly initiate transgene expression in late fetal life, restrict hGH to the enterocytic lineage, and establish an appropriate cephalocaudal gradient of reporter expression. This cephalocaudal gradient is also influenced by *cis*-acting elements located between nucleotides −1178 and −278, and −277 and −185 that enhance and suppress (respectively) expression in the ileum and colon and by element(s) located upstream of nucleotide −277 that are needed to sustain high levels of hGH production after weaning. Nucleotides −277 to −185 contain part of a domain conserved between the three orthologous *Fabpi* genes (nucleotides −240 to −159), a 24-bp element (nucleotides −212 to −188) that binds nuclear factors present in colonic but not small intestinal epithelial cells, and a portion of a CCAAT/enhancer binding protein footprint (C/EBPα, nucleotides −188 to −167). Removal of nucleotides −277 to −185 (yielding I-FABP$^{-184\ to\ +28}$/hGH^{+3}) results in inappropriate expression of hGH in proliferating and nonproliferating epithelial cells located in the mid and upper portions of duodenal, jejunal, ileal, and colonic crypts without affecting the "shape" of the cephalocaudal gradient of transgene expression. Despite this precocious induction of hGH production, the hGH reporter remains restricted to the enterocytic lineage in both the small intestine and colon suggesting that these replicating crypt epithelial cells may already be allocated to this lineage at the point of induction of transgene expression. Alternatively, these replicating, hGH-positive cells may not be fully committed to the enterocytic lineage but nucleotides −184 to +28 contain *cis*-acting elements that bind factors which mediate lineage-specific expression. Together, these results indicate *Fabpi* will be a useful model system for describing the molecular mechanisms that maintain axial pattern formation in the perpetually renewed, lineal descendants of the multipotent crypt stem cell.

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THE adult mouse small intestine contains ~1.1 million flask-shaped crypts of Lieberkühn (Hagemann et al., 1970), each composed of an average of 530 cells (duodenum) to 360 cells (ileum) (Wright and Irwin, 1982). Cell division in the intestinal epithelium is confined to these crypts. Studies of normal, chimeric, and transgenic mice suggest that each crypt is monoclonal, containing a single active multipotent stem cell and its descendants (Cheng and LeBlond, 1974*e*; Ponder et al., 1985; Inoue et al., 1988; Winton et al., 1988, 1989; Potten and Loeffler, 1990; Winton and Ponder, 1990; Schmidt et al., 1985*a*, 1990; Cohn et al., 1991; Roth et al., 1991*b*). These descendants undergo amplification through several rounds of cell division (~ two thirds of the crypt's epithelial cell population pass through the cell cycle every 12 h) (Potten and Loeffler, 1987, 1990) and are allocated to four principal lineages. Differentiation occurs during a geographically ordered bipolar migration. Absorptive columnar enterocytes, mucus-producing goblet cells, and enteroendocrine cells differentiate as they migrate in vertical coherent bands from the crypt to the apex of a surrounding villus where they are exfoliated. Paneth cells differentiate during their descent to the base of the crypt (Cheng and LeBlond, 1974*a–d*; Potten and Allen, 1977; Partridge and Simpson, 1980; Schmidt et al., 1985*b*; Bjerknes and Cheng, 1981*a,b*). Proliferation, lineage allocation (commitment), differentiation/migration, and exfoliation are processes which occur perpetually and are completed rapidly in the small intestinal epithelium (e.g., it takes 2–3 d for an enterocyte to move from the upper crypt to the apical extrusion zone of a villus). A similar statement can be made about the colonic epithelium. A single active multipotent stem cell located at or near the base of each colonic crypt gives rise to each of the four epithelial cell lineages (Griffiths et al., 1988; Cohn et al., 1991; Roth et al., 1992*b*). The proliferative capacity of colonic crypts is comparable with that of small intestine crypts (Sunter et al., 1979). However, there are no villi in the colon. Cells migrate from colonic crypts to a hexagonal-shaped cuff of surface epithelial cells that surround the orifice of each crypt (gland) (Schmidt et al., 1985*b*).

Crypts form during the first two weeks of postnatal life. They arise from a polyclonal intervillus epithelium (i.e., one that has several active stem cells) through a complex process that involves: (*a*) progressive deepening (invagination); (*b*) a cell selection that results in "purification" of the nascent crypt from polyclonality to monoclonality; and (*c*) division (crypt fission) (Obuoforibo and Martin, 1977; Al-Nafussi and Wright, 1982; Cheng and Bjerknes, 1987; Schmidt et al., 1988). Studies of mouse aggregation chimeras indicate that each small intestinal villus in the adult mouse is supplied by several crypts, i.e., it is "polyclonal" (Schmidt et al., 1985*b*). The number of anatomically defined crypts that surround each villus varies as a function of location along the cephalocaudal (duodenal–ileal) axis: the crypt–villus ratio decreases from 14 in the duodenum to 6 in the ileum (Wright and Irwin, 1982). Moreover, a given crypt can supply cells to several adjacent villi. It is unclear whether one or more adjacent colonic crypts supply cells to a given surface epithelial cuff.

Since the intestinal epithelium undergoes perpetual and rapid renewal of its four terminally differentiated cell types along an anatomically well defined pathway (i.e., the crypt–villus or crypt–surface epithelial cuff axis), and since each crypt is composed of the progeny of a single active multipotent stem cell, the gut is an attractive system for: (*a*) inferring the biological properties of stem cells; (*b*) examining how stem cell hierarchies are established and maintained; (*c*) analyzing the relationships between passage through the cell cycle and lineage allocation (commitment); and (*d*) defining differentiation programs (Gordon et al., 1992). The mouse intestine is, in essence, a continuous developmental system. An intriguing additional feature is its capacity for morphoregulation (Edelman, 1992), i.e., to establish and maintain distinct regional differences in gene expression within specific cell lineages and in the relative proportions of the four lineages along the duodenal–colonic axis. Studies of jejunal, ileal, and colonic isografts, harvested just before conversion of the gut epithelium to a monolayer overlying nascent villi on embryonic days 15–16 and implanted into the subcutaneous tissues of young adult CBY/B6 nude mouse recipients, indicate that this regional differentiation of the gut epithelium can occur in the absence of luminal contents and suggest that a crypt stem cell has a "positional address" describing its location along the cephalocaudal axis (Rubin et al., 1991, 1992). Although axial pattern formation reflects position-dependent differences in the differentiation programs of crypt stem cell descendants, the molecular mechanisms that produce polarity in the gut epithelium remain unknown. The cellular basis is also unclear. For example, how much positional information is encoded in stem cells themselves? Do mesenchymal components, such as the pericryptal fibroblasts that proliferate and migrate along the crypt–villus axis, help specify the crypt stem cell's positional address and/or influence the differentiation programs of its progeny (Marsh and Trier, 1974*a,b*; Maskens et al., 1979; Haffen et al., 1983, 1987; Kedinger et al., 1986)?

Transcription of the mouse intestinal fatty acid binding protein gene (*Fabpi*)[1] located on chromosome 3 (Sweetser et al., 1987; Green et al., 1992) is confined to the gut epithelium: its mRNA and protein products first appear as differentiating enterocytes exit small intestinal and colonic crypts. Expression is sustained during subsequent migration to the apical extrusion zone of villi and to the surface epithelial cuff of colonic glands (Sweetser et al., 1988*a*). Distinct regional differences in the steady-state concentrations of these gene products also occur along the cephalocaudal axis: highest levels are present in the distal jejunum with progressive decreases occurring toward either the proximal duodenum or the mid-colon (Sweetser et al., 1988*a*). These cell-specific and region-specific patterns of *Fabpi* are evident at the time of its initial activation in late fetal life (E16—coinciding with the first appearance of the gut epithelial monolayer), and are maintained throughout adulthood (Sweetser et al., 1988*a*; Green et al., 1992). Analyses of intestinal isografts have shown that the differentiation-dependent induction of intestinal fatty acid binding protein (I-FABP) synthesis and the cephalocaudal variations in enterocytic I-FABP levels do not depend upon luminal signals (Rubin et al., 1991, 1992).

We have exploited these features of *Fabpi* to define *cis*-acting elements that regulate enterocyte-specific and region-specific patterns of gene expression in the mouse gut epithelium. Preliminary studies in transgenic mice indicated that

1. *Abbreviations used in this paper*: *Fabpi*, intestinal fatty acid binding protein gene; IGSS, immunogold/silver stained; hGH, human growth hormone.

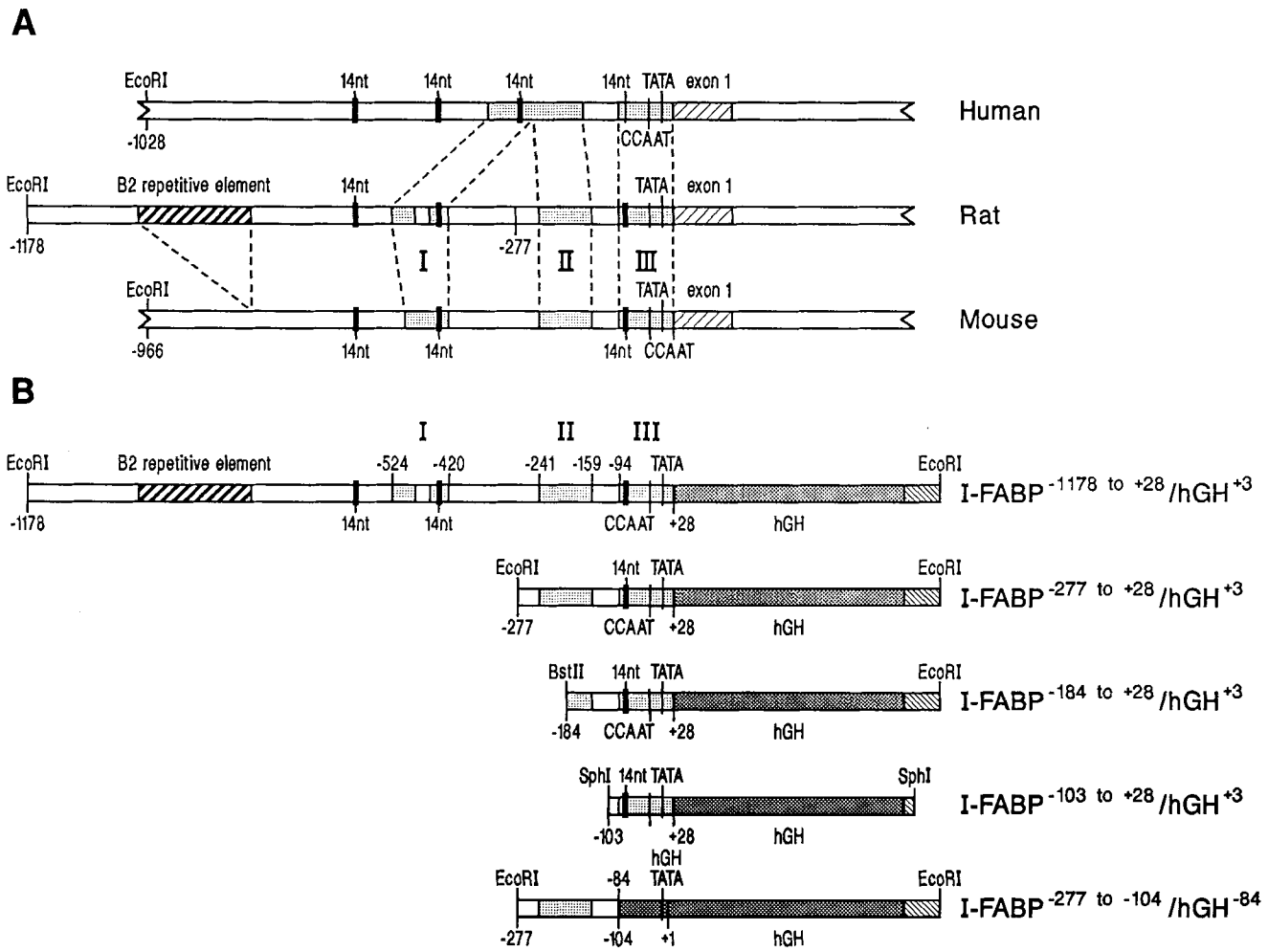


Figure 1. Summary of conserved elements present in the 5′ nontranscribed domains of human, rat, and mouse *Fabpi*. (*A*) Comparison of the orthologous *Fabpi* genes. Conserved domains I, II, and III are shown as stippled boxes and are described in Green et al., 1992. The positions of sequences homologous to a 14-bp element (consensus 5′-TGAACTTTGAACTT-3′) found in *Fabpi*, other members of its gene family which are expressed in the gut, and in several nonhomologous genes that are also transcribed in the intestine (Sweetser et al., 1987) are indicated by thick, black, vertical lines. The dark hatching shows a B2 repetitive element (Krayev et al., 1982) present in rat, but not human or mouse, *Fabpi*. (*B*) Summary of sequence elements present in I-FABP/hGH transgenes.

nucleotides −1178 to +28 of rat *Fabpi* (Sweetser et al., 1987) are sufficient to produce a pattern of reporter expression in enterocytes distributed along the crypt-villus and duodenal-colonic axes which mimics that of the intact endogenous mouse and rat *Fabpi* genes (Sweetser et al., 1988*a*; Hauft et al., 1992). Removal of nucleotides −1178 to −278 restricted transgene expression to the proximal small intestine in the one pedigree that was available but did not affect expression along the crypt–villus axis, suggesting that distinct *cis*-acting regulatory elements may modulate gene expression along the two axes of the gut (Sweetser et al., 1988*a*). Recent comparison of the nucleotide sequences of mouse, rat and human *Fabpi* revealed three highly conserved domains in their 5′ nontranscribed regions as well as multiple copies of a 14-bp element. This element (consensus 5′-TGAACTTTGAACTT-3′) is a direct repeat of a 7-bp sequence and is present in comparable positions of the 5′ nontranscribed domains of the orthologous *Fabpi* genes (see Fig. 1 *A*). It is also represented in the 5′ nontranscribed domains of several other genes that are expressed in enterocytes including another member of the *Fabpi* gene family (*Crbp2* which encodes cellular retinol binding protein II) (Sweetser et al., 1987; Demmer et al., 1987). We have used this information and transgenic mice to conduct a more detailed functional analysis of the 5′ nontranscribed region of *Fabpi* to understand how its differentiation-dependent and region-specific patterns of expression are regulated in the enterocytic lineage.

Materials and Methods

Generation and Characterization of Transgenic Mice

Construction of I-FABP/hGH Fusion Genes. The I-FABP/human growth hormone (hGH) DNAs used to produce transgenic animals are illustrated in Fig. 1 *B*. I-FABP$^{-1178\ to\ +28}$/hGH^{+3}, representing nucleotides −1178 to +28 of rat *Fabpi* (Sweetser et al., 1987) linked to the human growth hormone gene beginning at its nucleotide +3 (Seeburg, 1982), is described in Sweetser et al. (1988*a*). I-FABP$^{-277\ to\ +28}$/hGH^{+3} was obtained by digesting pIFhGH1 (Sweetser et al., 1988*a*) with EcoRI yielding a 2.4-kb EcoRI fragment. I-FABP$^{-184\ to\ +28}$/hGH^{+3} and I-FABP$^{-103\ to\ +28}$/hGH^{+3} were retrieved from pIFhGH1 as 2.3-kb BstEII-EcoRI and 1.9-kb SphI fragments, respectively. I-FABP$^{-277\ to\ -104}$/hGH^{-84} was produced as follows. A SacI–PvuII restriction fragment containing nucleotides −277 to +28 of rat *Fabpi* was sub-

cloned into pUC13 DNA that had been digested with SacI and SmaI. The resulting recombinant plasmid DNA was digested with SphI and BamHI and a double-stranded oligonucleotide (5'-CAATGTAGCACAGAAACAGGTG-GGGTCAACAGTGGGAGAGAAGCCAGGGTATAAAAAGGGCCCAC AAGAGACCAGCTCAAG3'/5'GATCCTTGAGCTGGTCTCTTGTGGG CCCTTTTTATACCCTGGCCCCTTCTCTCCCACTGTTGACCCCACC TGTTTCTGTGCTACATTGCATG-3') representing nucleotides −84 to +3 of the hGH gene (Seeburg, 1982) was inserted in place of the SphI-BamHI fragment that contained nucleotides −103 to +28 of rat *Fabpi*. Finally, a 3.7-kb BamHI fragment from pIFhGH1 (i.e., a fragment which contains the hGH structure gene beginning at its nucleotide +3) was subcloned into the BamHI site of the recombinant plasmid. I-FABP$^{-277\text{ to }-104}$/hGH^{-84} was recovered as a 2.4-kB EcoRI fragment and used for pronuclear injections.

Production and Identification of Transgenic Mice. All restriction fragments used for pronuclear injections were purified by agarose gel electrophoresis followed by the GeneClean system (Biol01, Inc., La Jolla, CA). DNA fragments were injected into the pronuclei of oocytes obtained from mating C57BL/6J X C3H F1 mice. Oocytes were transferred on the day of injection to the infundibulum of the fallopian tubes of pseudopregnant Swiss Webster mice (Hogan et al., 1986). Live born mice were subsequently screened for the presence of the transgene by probing Southern blots of EcoRI digested tail DNA with a ^{32}P-labeled (Feinberg and Vogelstein, 1984) 2.2-kb BamHI-EcoRI fragment of pIFhGH1 containing the hGH gene beginning at its nucleotide +3. Founder mice were mated to C57BL/6J X C3H F1 animals to establish pedigrees that were obligate hemizygotes for the transgene. Lines were subsequently maintained by littermate breeding.

Calculation of Copy Number. To determine transgene copy number, genomic DNA was prepared from the liver or spleen of F_1 mice. 15 μg of DNA were digested with PvuII, an enzyme that cleaves the hGH gene at two sites yielding a 1-kb internal fragment from each copy of an I-FABP/hGH transgene. The products of EcoRI digestion were separated by agarose gel electrophoresis and transferred to nylon membranes (GeneScreen Plus, Dupont, New England Nuclear, Boston, MA). Southern blots were probed with the ^{32}P-labeled 2.2-kb BamHI-EcoRI fragment of pIFhGH1 described above, using hybridization and washing stringencies detailed in Sweetser et al. (1988*b*). The intensity of the hybridization signal generated by the 1-kb band was quantitated by scanning laser densitometry of the filter autoradiograph and compared to the intensities of signals produced by known amounts of cloned hGH DNA (added to 15 μg of liver or spleen DNA prepared from normal littermates and included in the same Southern blots as internal controls). Only signals in the linear range of film sensitivity were used to calculate transgene copy number.

Caging. Suckling, weaning, and adult mice were maintained in microisolator cages under a strictly controlled light cycle (lights on at 0600 and off at 1800 h). Weaning and weaned mice were given a standard autoclavable chow diet ad libitum. For pregnant mice, gestational age was calculated from the day a vaginal plug was first noted (designated day 1).

Preparation of Tissue RNA. Transgenic mice and their normal littermates were sacrificed by cervical dislocation and their tissues rapidly removed. 90 min before sacrifice, some animals received an intraperitoneal injection of 5-bromo-2'-deoxyuridine (BrdUrd; Sigma Chemical Co., St. Louis, MO) (dose = 30 mg/kg) to label cells in S-phase (Chwalinski et al., 1988) and 5-fluoro-2'-deoxyuridine (Sigma Chemical Co.) (dose = 1 mg/kg) to inhibit endogenous thymidylate synthetase activity (Brinkman and Dormier, 1972). The gastrointestinal tract was divided along its cephalocaudal axis as described by Sweetser et al., (1988*b*) yielding segments that are described in the text as stomach (ST), duodenum (DU), proximal and distal jejunum (PJ and DJ), ileum (IL), cecum (CE), proximal colon (PC) and distal colon (DC). A 0.2–0.5-cm section was obtained from the proximal, mid, and distal portions of each segment for subsequent immunocytochemical studies (see below). The rest of the tissue was quickly frozen in liquid nitrogen. Other tissues obtained at the time of sacrifice included brain, heart, lung, spleen, liver, kidney, pancreas, gonads, and skeletal muscle. Total cellular RNA was extracted from frozen, pulverized tissue using guanidinium isothiocyanate, phenol, and chloroform (Chomczynski and Sacchi, 1987). The integrity of RNA samples was assessed by formaldehyde agarose gel electrophoresis.

RNA Blot Hybridization Studies. Samples of total cellular RNA were fractionated by denaturing, formaldehyde-agarose gel electrophoresis and transferred to nylon membranes. RNA dot blots were used to quantitate steady-state levels of specific mRNAs as described in Sweetser et al. (1988*a*,*b*). Blots contained in vitro transcribed, purified mouse I-FABP mRNA and hGH mRNA standards (0.1–500 pg, Sweetser et al., 1988*a*,*b*; Green et al., 1992) and were probed with either a ^{32}P-labeled (Feinberg and Vogelstein, 1984) mouse I-FABP cDNA (Green et al., 1992) or a 150-bp BglII–PvuII fragment obtained from exon V of the hGH gene. Hybridization and washing conditions are detailed in our earlier publications (Sweetser et al., 1988*a*,*b*). The intensity of the hybridization signal produced by a given sample of tissue RNA was determined by counting the filters with a Betascope Model 603 scanner (Betagen, Waltham, MA) and compared with the signals obtained with the mRNA standards present in the same filter. This comparison allowed us to calculate the steady-state levels of specific mRNAs. Note that the distribution of hGH mRNA along the duodenal–colonic axis of the gut was compared and contrasted with the distribution of I-FABP mRNA by sequentially probing dot and/or Northern blots of RNAs prepared from a given mouse. Labeled DNA was removed after the first round of hybridization/washing by incubating the blot at 100°C in 1.5 mM NaCl/0.15 mM Na Citrate, pH 7.0/0.01% SDS. The stripped blot was then reprobed with the second ^{32}P-DNA. This direct comparison allowed us to conclude whether the distribution of reporter (hGH) mRNA was qualitatively and/or quantitatively similar to that of the *Fabpi* transcript and eliminated potential problems of interpretation due to animal-to-animal variation in (*a*) precise sites of division of the intestinal tract into duodenal, proximal and distal jejunal, ileal, cecal and colonic segments and (*b*) the relative steady-state levels of I-FABP mRNA in these segments.

Immunocytochemical Studies. Tissue samples were fixed in Bouin's solution (Sweetser et al., 1988*b*). Material obtained from the seven segments of intestine from a given animal were placed on a single slide together with samples of stomach, liver, pancreas, and kidney. All tissues were then embedded in paraffin and 5–10 μm sections were prepared. This allowed us to simultaneously survey cellular patterns of gene expression in several organs and to compare and contrast the steady state level of a specific protein as a function of cellular position along the two axes of the gut. The methods used for single- and double-label immunocytochemical studies are described in previous publications (Roth et al., 1990; Hermiston et al., 1992). Briefly, paraffin was removed from sections which were then overlaid with a primary antiserum (diluted as described below) and placed in a humidified chamber, maintained at 4°C, for 12–14 h. The primary antiserum was then removed and the sections washed several times in PBS. For single label studies, antigen-antibody complexes were detected with either fluorescent-labeled secondary antibodies (Jackson Immunoresearch Laboratories, West Grove, PA) or with gold-labeled secondary antibodies followed by silver enhancement (Amersham Corp., Arlington Heights, IL). For double and triple labeling, immunogold/silver stained (IGSS) sections were washed with water and PBS followed by addition of a second and/or third primary antiserum raised in a rabbit or a goat. Silver deposition during the IGSS procedure effectively abolishes the antigenicity of the first primary antiserum gold-labeled secondary antiserum complex, allowing detection of additional primary antisera with fluorescent-labeled secondary antibodies (Roth et al., 1990; Hermiston et al., 1992). The second and third primary antisera were detected with fluorescein-labeled donkey anti-goat and Texas red-labeled donkey anti-rabbit sera (Roth et al., 1990). The final dilutions of antisera used and their sources are as follows: goat anti-BrdUrd (1:2000) (Cohn and Lieberman, 1984); rabbit anti-rat I-FABP (Lowe et al., 1987); rabbit anti-hGH (1:2,000) (Dako Corp., Santa Barbara, CA), and goat anti-hGH (1:2,000) (McKeel and Askin, 1978). The immunostaining characteristics and specificities of these antisera have been described in our earlier publications (Sweetser et al., 1988*a*,*b*; Roth et al., 1990; Green et al., 1992).

Measurement of hGH Levels in Serum by Radioimmunoassay. Serum was collected from transgenic mice at various ages. Serum from each animal was assayed in duplicate for hGH using a sensitive radioimmunoassay (Nichols Institute, San Juan Capistrano, CA) whose limits of detection is 0.5 ng/ml of hGH. It does not recognize mouse growth hormone as assessed by surveys of sera obtained from nontransgenic littermates (data not shown).

Preparation of Nuclear Extracts from Intestinal Epithelial Cells

A modification of the procedure of Gorski et al. (1986) was used to isolate nuclear proteins from small intestinal and colonic epithelial cell populations. The intestine was removed en bloc (i.e., from the gastroduodenal junction to the rectum) from 2–3-mo-old mice and then divided at the ileal/cecal border. A 22-gauge, blunt-end needle, attached to a 30-ml syringe, was inserted into the duodenum and the lumen of the small intestine perfused with an ice cold solution of TBS buffer (10 mM Tris, pH 7.6, 150 mM NaCl). The small bowel was then placed in ice cold homogenization buffer (10 mM Hepes, pH 7.6, 25 mM KCl, 1 mM EDTA, 1 mM DTT, 2 M sucrose, 2 mM benzamidine, 0.2 mM phenylmethylsulfonylfluoride, 0.5 mM spermidine, 0.15 mM spermine, 10% glycerol [vol/vol]). The mucosa was

extruded by scraping the serosal side of the proximal two thirds of the small intestine with the blunt end of a spatula. The remaining fibromuscular tissue was discarded. After removal of luminal contents, the colon was minced in ice cold homogenization buffer rather than scraped. The scraped mucosa from each small intestine or the minced colonic tissue was brought to a total volume of 8 ml with homogenization buffer (maintained at 4°C). Typically, material obtained from the small intestine or colon of three animals was pooled and adjusted to a final volume of 28 ml. *N*-acetyl-L-cysteine (Sigma Chemical Co.) and Triton X-100 were then added to final concentrations of 50 mM and 0.5%, respectively. The mixture was homogenized with a Tissumizer (Tekmar Co., Cincinnati, OH) set at maximum speed (3–5 30-sec bursts were applied with the tube containing the homogenate placed on ice). Aliquots of the resulting 28-ml suspension were examined using phase-contrast microscopy to assure that the majority of cells had been disrupted. The 28-ml suspension was subsequently layered over 10 ml of homogenization buffer and nuclei pelleted by centrifugation at 80,000 g_{ave} for 50 min at 4°C. The resulting pellet was resuspended in 2 ml of ice cold nuclear extraction buffer (10 mM Hepes, pH 7.6, 0.1 mM EDTA, 3 mM $MgCl_2$, 100 mM KCl, 2 mM benzamidine, 0.2 mM phenylmethylsulfonylfluoride, 1 mM DTT, 10% glycerol) using a loose fitting pestle and Dounce homogenizer. NaOH was then added (final concentration = 10 mM) to an aliquot of nuclei, and the absorbance of the resulting lysate determined at 260 nm. This information was used to adjust the volume of the remaining nuclear suspension to 10–20 A_{260}/ml using ice cold nuclear extraction buffer. One-tenth volume of 4 M $(NH_4)_2SO_4$, pH 8.0, was slowly added to the nuclear suspension and the mixture gently rocked for 30 min at 4°C. Chromatin was pelleted from the lysate by centrifugation at 100,000 g_{ave} for 60 min at 4°C. The supernatant fraction was collected and 0.34 g of $(NH_4)_2SO_4$ added per milliliter. After an overnight incubation on ice, precipitated material was recovered by centrifugation at 100,000 g_{ave} for 25 min. The pellet was resuspended in an ice cold buffer composed of 25 mM Hepes, pH 7.6, 40 mM KCl, 0.1 mM EDTA, 1 mM DTT, 2 mM benzamidine, 0.2 mM phenylmethylsulfonylfluoride, and 10% glycerol and dialyzed against the same buffer at 4°C (three changes of 100 vol of dialysis buffer, 2–3 h per exchange). The concentration of protein in the nuclear extract was determined using the method of Bradford (1976). Aliquots were then frozen in liquid nitrogen. Nuclear extracts were also prepared from liver, kidney, and spleen using a protocol identical to that described above for colon.

Mobility Shift Studies

DNA binding assays were performed in 25-μl reactions. The mixture contained 50 fmoles of template DNA labeled at one of its 5′ ends with [^{32}P] using T4 polynucleotide kinase (New England Biolabs, Beverly, MA), 0.5–1.0 μg of poly dI-dC (Pharmacia Fine Chemicals, Piscataway, NJ), varying amounts of one of the double-stranded oligodeoxynucleotide competitors described in the legend to Fig. 3, 25 mM Tris, pH 8.0, 50 mM KCl, 0.1 mM EDTA, and 5 mM 2-mercaptoethanol. The solution was incubated at room temperature for 30 min and the products analyzed by electrophoresis through 5 or 8% polyacrylamide gels (running buffer = 25 mM Tris, 190 mM glycine, 1 mM EDTA, final pH = 8.5). After electrophoresis, gels were dried and subjected to autoradiography at −80°C using Kodak XAR-5 film (Eastman Kodak Co., Rochester, NY) and an intensifying screen.

DNAse I Footprint Analysis Using Recombinant C/EBPα and Fabpi

E. coli-derived C/EBPα was a kind gift of Steve McKnight (Carnegie Institution of Washington, Baltimore, MD) and was prepared as described in Landschulz et al. (1988). The template DNA used for these studies was prepared by digesting pIFhGH1 with EcoRI. The resulting 2.4 kb EcoRI fragment was purified by agarose gel electrophoresis and labeled with [^{32}P] using T4 polynucleotide kinase. Digestion with BamHI yielded a 312-bp EcoRI–BamHI fragment containing nucleotides −277 to +28 of rat *Fabpi*. This DNA was purified by agarose gel electrophoresis. Alternatively, pIFhGH1 was digested with BamHI, the 3.7-kb fragment labeled with [^{32}P] at its 5′ ends, and then incubated with EcoRI yielding a 312-bp EcoRI–BamHI fragment that was labeled at nucleotide +28 of *Fabpi* rather than at nucleotide −277. ~3,000 cpm of either one of these [^{32}P]DNAs was added to a reaction mixture (45 μl) containing C/EBPα (final concentration = 30 ng/μl), 1 μg unlabeled poly dI-dC, 50 mM KCl, 25 mM Tris-HCl, pH 7.9, 6 mM $MgCl_2$, 0.5 mM EDTA, and 10% glycerol. After a 10-min incubation on ice and a 2-min incubation at 23°C, 5 μl of DNAase I (prepared in 25 mM $CaCl_2$) was introduced into the mixture at varying final concentrations which were determined empirically as a function of both the DNAse I activity and the specific activity of the labeled DNA fragment. After a 1-min incubation at 23°C, DNAse I digestion was terminated by adding 110 μl stop buffer (1% SDS, 200 mM NaCl, 20 mM EDTA, and 200 μg/ml proteinase K), placing the mixture at 55°C for 30 min, and then extracting it with phenol and chloroform. The labeled DNA products were recovered by ethanol precipitation, dissolved in loading dye (0.1% bromophenol blue, 0.1% xylene cyanol, 80% formamide, 50 mM Tris-borate, pH 8.3, and 1 mM EDTA), and fractionated by electrophoresis through a 7% polyacrylamide gel containing 8 M urea.

Results

Comparative Sequence Analyses of Orthologous Fabpi Genes and DNA Binding Assays Using Nuclear Extracts Prepared from Small Intestinal and Colonic Epithelial Cells Provide a Design Rationale for Conducting Functional Mapping Studies

As noted in the introduction, earlier studies in transgenic mice indicated that nucleotides −1178 to +28 of rat *Fabpi* contain sufficient information to direct cellular and regional patterns of expression of at least two reporters, hGH (Sweetser et al., 1988*a*) and SV-40 T antigen (Hauft et al.,

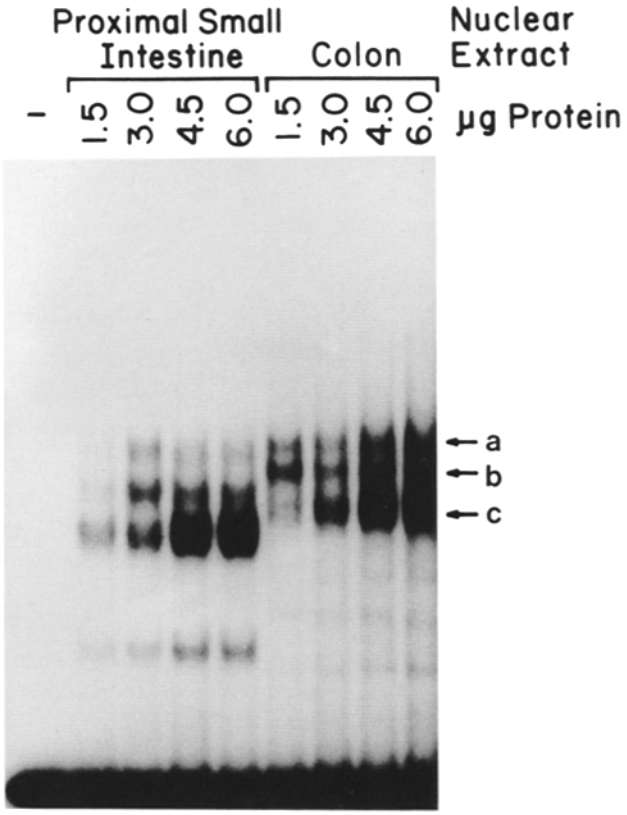
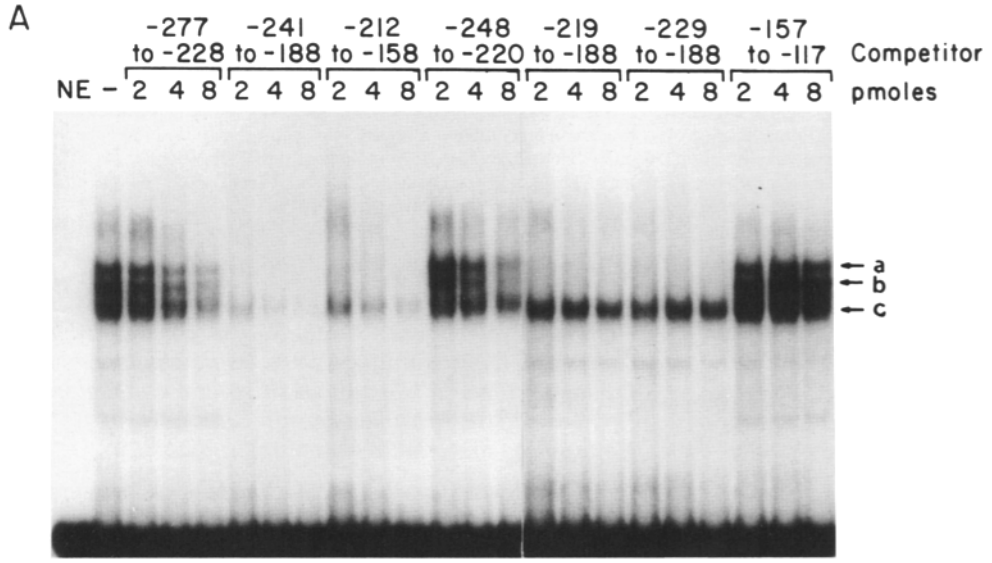


Figure 2. Gel retardation studies using nuclear extracts prepared from the mouse proximal small intestinal epithelium or colon and nucleotides −241 to −188 of rat *Fabpi*. Nuclear extracts were prepared from duodenal and jejunal mucosal scrapings and from the colon as described in Materials and Methods. A ^{32}P-labeled double-stranded oligonucleotide template spanning nucleotides −241 to −188 of rat *Fabpi* was incubated with the indicated amount of protein from each of these nuclear extracts and the products analyzed by electrophoresis through nondenaturing 8% polyacrylamide gels. An autoradiograph of the gel is shown. Note that extracts from these different regions of the cephalocaudal axis of the gut produce different patterns of retarded bands.



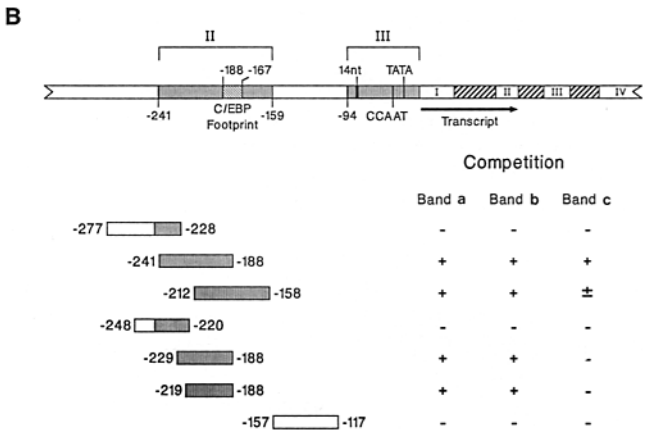


Figure 3. Localization of the binding site for colonic nuclear factor(s) to nucleotides −212 to −188 of rat *Fabpi*. (*A*) Nuclear extracts from colon (3 μg total protein) were incubated with 0.05 pmole of a ^{32}P-labeled 54-bp fragment spanning nucleotides −241 to −188 plus the indicated amount of unlabeled oligonucleotide competitors. The reaction products were fractionated by nondenaturing PAGE. Note that all competitors which contain the nucleotide sequence 5′-TGCTGTAGTCGGAGACAGAGTAGGT-3′ (nucleotides −212 to −188) compete for binding of colonic nuclear factors to bands *a* and *b*. Competition for binding to band *c* was only observed with the same oligonucleotide that was used as the labeled template (−241 to −188, data not shown) and, to a lesser extent, by the oligonucleotide which spans nucleotides −212 to −158. *NE*, no extract added; "−" no oligonucleotide competitor added. (*B*) Summary of the results presented in *A*, demonstrating the relationships of the various oligonucleotide competitors to the conserved sequence elements present in *Fabpi* and to a C/EBPα binding site identified by DNAse I footprint analysis (see Fig. 4). The sequences of the unlabeled double-stranded oligonucleotides used as competitors are as follows: −277 to −228; 5′-AATTCGAGCTCGGATTAAAGGTGGAAGCCATCACACTTGACCCTAATTCTTGGA-3′; −248 to −220; 5′-CACTTGACCCTAATTCTTGGAATAAAAAT-3′; −241 to −188; 5′-CCCTAATTCTTGGAATAAAAATGCCTACATGCTGTAGTCGGAGACAGAGTAGGT-3′; −229 to −188, 5′-GAATAAAAATGCCTACATGCTGTAGTCGGAGACAGAGTAGGT-3′; −219 to −188, 5′-GCCTACATGCTGTAGTCGGAGACAGAGTAGGT-3′; −212 to −158; 5′-TGCTGTAGTCGGAGACAGAGTAGGTATGGTTACCAAATTTGAATGCAGTTGAATC-3′; −157 to −117, 5′-TCAGCAATAGATTCAAAGAAAGCACTAGAAGAGAAACTAAA-3′.

1992), that mimic that of the intact endogenous *Fabpi* gene. The cellular and regional patterns of *Fabpi* expression are quite similar in humans, rats, and mice (Shields et al., 1986; Sweetser et al., 1988*a*; Green et al., 1992). Therefore, we reasoned that the three conserved sequence elements located in the 5′ nontranscribed domains of the orthologous human, rat, and mouse *Fabpi* genes (labeled I–III in Fig. 1 *A*) and sites in their 5′ nontranscribed domains that specifically bind intestinal nuclear proteins are likely to contain *cis*-acting regulatory sequences important for establishing and maintaining cell-specific and/or region-specific patterns of expression.

Nuclear proteins were prepared from the duodenal/jejunal epithelium and from the colon of adult mice (see Materials and Methods). Gel mobility shift studies were then performed using these extracts, or control extracts from liver, kidney, and spleen, and DNA fragments derived from nucleotides −277 to +28 of rat *Fabpi* (i.e., those nucleotides sufficient to support reporter expression in villus-associated enterocytes located in the proximal half of the small intestine of transgenic mice). Nuclear extracts from the proximal small intestine and the colon produced distinct patterns of shifted bands when a ^{32}P-labeled DNA fragment spanning nucleotides −241 to −188 was used as a template (Fig. 2). Markedly different patterns were noted with liver, kidney, and spleen extracts (data not shown). Moreover, incubation of proximal small intestinal or colonic nuclear extracts with other, double-stranded oligodeoxynucleotide templates derived from nucleotides −277 to +28 yielded a set of shifted bands that included subsets which migrated in a similar fashion to those shown in Fig. 2 and subsets that were distinct (data not shown). Analysis of these results revealed that two prominent shifted bands were only present when colonic extracts were incubated with nucleotides −241 to −188 (la-

beled *a* and *b* in Figs. 2 and 3 *A*). Addition of proteinase K to the reaction mixture containing this template DNA and colonic nuclear extracts resulted in a single band that had the same mobility as the free ^{32}P-labeled 54-bp oligodeoxynucleotide, indicating that the shifted bands were likely due to the binding of proteins present in the colonic nuclear extract (data not shown).

The region(s) in the −241 to −188 fragment that reacted with colonic nuclear proteins to produce bands *a* and *b* was further defined in a series of competitive binding studies using unlabeled double stranded oligodeoxynucleotides (Fig. 3, *A* and *B*). All oligonucleotides that contained a 24-base sequence representing nucleotides −212 to −188 of rat *Fabpi* (5′-TGCTGTAGTCGGAGACAGAGTAGGT-3′) were efficient competitors as judged by their ability to reduce the amount of labeled bands *a* and *b* but not band *c* (Fig. 3 *A*). Other oligonucleotides that did not contain this 24-bp element (e.g., −248 to −220 or −157 to −117) were ineffective competitors even when present in >100-fold molar excess relative to the −241 to −188 template (Fig. 3, *A* and *B*). Finally, each of the oligonucleotides used in the competition experiments was labeled with ^{32}P, incubated with colonic nuclear extracts, and the reaction products subjected to native gel electrophoresis. Their patterns of reactivity with colonic nuclear proteins (as judged by gel shifts) mirrored their ability to reduce the amount of labeled bands *a* and *b* (data not shown). Together, these data suggest that nucleotides −212 to −188 contain a binding site(s) for one or more proteins represented in colonic, but not proximal small intestinal, nuclear extracts.

Members of the CCAAT/enhancer binding protein (C/EBP) family of transcription factors (Cao et al., 1991; Williams et al., 1991; Ron and Habner, 1992) have been shown to affect the transcriptional activities of several genes during cellular differentiation (Umek et al., 1991), including a member of the *Fabpi* gene family known as *aP2* (Hunt et al., 1986) which is induced during adipocyte differentiation (Herrera et al., 1989; Christy et al., 1989; 1991; Cao et al., 1991). These observations, together with recent studies which indicate that C/EBPα and related leucine zipper proteins are produced in the mouse intestine (Birkenmeier et al., 1989; Cao et al., 1991), caused us to examine whether nucleotides −277 to +28 contained any binding sites that reacted with *E. coli*–derived C/EBPα (Landschulz et al., 1988). DNAse I footprinting revealed a single protected site that spanned nucleotides −188 to −167 of rat *Fabpi* (Fig. 4). This footprint lies within one of the three conserved *Fabpi* domains shown in Fig. 1 *A* (domain II) and is immediately adjacent to the colonic nuclear factor binding site. No other nuclear proteins were present in the recombinant C/EBPα preparation used for these studies thereby precluding the formation of heterodimers with other C/EBP isoforms (Williams et al., 1991) or with other *trans*-acting factors that might, in turn, alter its binding site specificity (Lamb and McKnight, 1991). Unfortunately, we have been unable to obtain unambiguous footprints in this region of *Fabpi* using either colonic or small intestinal nuclear extracts. This failure may be due to a number of causes including, for example, (*a*) partial degradation of C/EBPα and other transcription factors by intracellular and/or luminal (pancreatic) proteases during preparation of our nuclear extracts and (*b*) C/EBPα's extraordinarily low concentration in the gut epithelium.

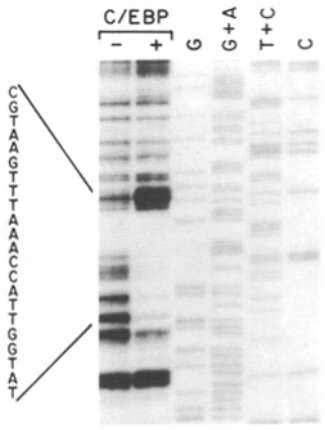


Figure 4. DNAse I footprint analysis reveals a binding site for recombinant C/EBPα located between nucleotides −188 and −167 of rat *Fabpi*. *E. coli*-derived rat C/EBPα was incubated with a ^{32}P-labeled, 312-bp fragment of rat *Fabpi* spanning nucleotides −277 to +28. DNAse I was subsequently added to the reaction mixture and the digestion products were analyzed by electrophoresis through a 7% polyacrylamide gel containing 8 M urea. Parallel lanes contained the products of sequencing reactions (Maxam and Gilbert, 1977) performed on the same labeled DNA fragment. The sequence of *Fabpi* that was protected from DNAse I digestion by C/EBPα is shown.

Only the most sensitive immunocytochemical techniques such as the immunogold/silver intensification staining method combined with reflected light polarization microscopy are able to detect C/EBPα in jejunal villus-associated enterocytes. Moreover, enterocytic levels decrease markedly in the distal small intestine and the protein is undetectable in the colonic epithelium (C. Chandrasekaran, K. A. Roth, and J. I. Gordon, unpublished observations).

The results presented above provided us with a rationale for constructing several recombinant DNAs composed of elements from the 5′ nontranscribed domain of rat *Fabpi* linked to the human growth hormone (hGH) gene beginning at its nucleotide +3 (hGH was selected because it had been used as a reporter in the earlier functional studies of *Fabpi*). I-FABP$^{-277\ to\ +28}$/hGH^{+3} contains conserved domains II and III, a single copy of the 14-bp repeat (see Fig. 1 *B*), the binding sites for the protein factor(s) present in colonic nuclear extracts, and C/EBPα. I-FABP$^{-184\ to\ +28}$/hGH^{+3} contains the proximal half of conserved domain II and all of conserved domain III but lacks the colonic nuclear factor binding site and the 5′ four bases of the C/EBPα footprint. I-FABP$^{-103\ to\ +28}$/hGH^{+3} contains conserved domain III with its associated 14-bp element, TATA and CCAAT boxes. A final construct, I-FABP$^{-277\ to\ -104}$/hGH^{-84}, contains conserved domain II plus the colonic factor and C/EBPα binding sites linked to hGH beginning at its nucleotide −84. (Other investigators have linked putative tissue specific enhancer elements to hGH, beginning at its nucleotide −84, to test their functional properties; e.g. Hammer et al., 1987; Ornitz et al., 1987.) A number of transgenic pedigrees were established containing each of these fusion genes (Table I).

Nucleotides −1178 to −278 Modulate Expression of Fabpi in the Distal Half of the Small Intestine and in the Colon

The highest steady-state level of I-FABP mRNA in 20-wk-old nontransgenic mice is encountered in the distal jejunum and ileum (60–70 pg/μg total cellular RNA). Its concentration in duodenum and colon ranges from 10–15% of that achieved in the distal jejunum (Fig. 5 *A*). Transgenic mice containing 200–600 copies of I-FABP$^{-1178\ to\ +28}$/hGH^{+3} ex-

Table I. Summary of Transgenic Pedigrees

Transgene	Number of liveborn mice screened	Number of founders identified	Designation of pedigrees that expressed transgene	Transgene copy number/ haploid genome
I-FABP$^{-1178\text{ to }+28}$/hGH^{+3}	40	2	1*‡	600
			4*‡	200
I-FABP$^{-277\text{ to }+28}$/hGH^{+3}	200	11	54‡	60
			8*	2-4
			18	8
I-FABP$^{-184\text{ to }+28}$/hGH^{+3}	117	20	2§	4
			12	1-2
			36*	1
			39	1-2
			50	20
			55	2
			89	1
I-FABP$^{-277\text{ to }-104}$/hGH^{-84}	111	10	none	ND
I-FABP$^{-103\text{ to }+28}$/hGH^{+3}	190	8	35§	8
			40	2
			46§	4

* Sterile founder.
‡ See Sweetser et al. (1988*a*).
§ Poor fertility in these transgenic animals limited the number of mice that were available for detailed analysis.

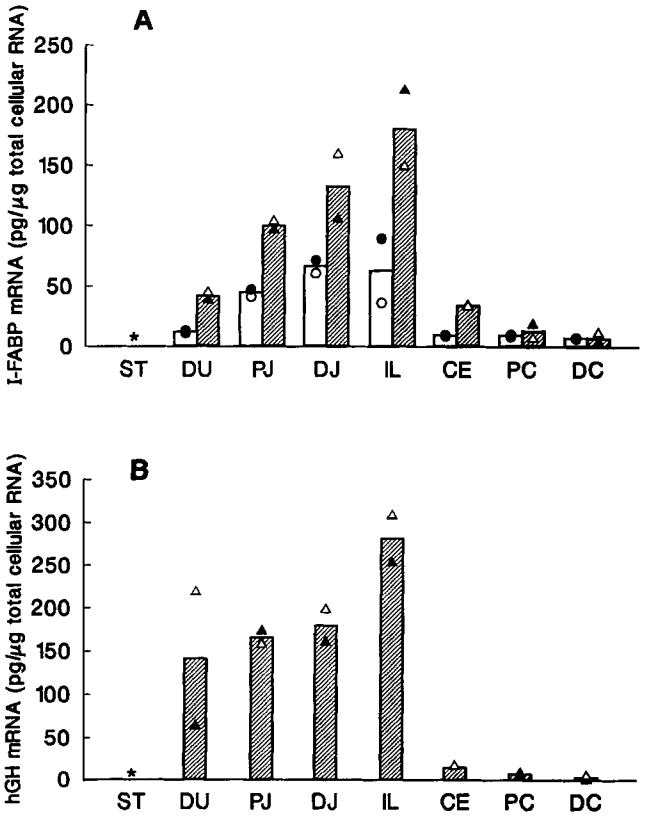


Figure 5. Comparison of the distribution of I-FABP and hGH mRNAs along the gastric-colonic axis of 21-wk-old I-FABP$^{-1178\text{ to }+28}$/hGH^{+3} mice (▨) and their normal littermates (□) representing two different pedigrees. Total cellular RNA was prepared from stomach (*ST*), duodenum (*DU*), proximal jejunum (*PJ*), distal jejunum (*DJ*), ileum (*IL*), cecum (*CE*), proximal colon (*PC*), and distal colon (*DC*). Steady-state levels of I-FABP and hGH mRNA were determined by quantitative RNA dot blot hybridizations. Two transgenic mice from I-FABP$^{-1178\text{ to }+28}$/hGH^{+3} lines 1 (△) and 4 (▲) (Sweetser et al., 1988*a*) were analyzed together with normal littermates (○,●). The bars represent the average of the data obtained from individual animals. The asterisks indicate that no hGH mRNA was detected (limits of sensitivity of the RNA blot hybridization assay = 0.2 pg hGH mRNA/μg total cellular RNA).

hibit a pattern of hGH mRNA distribution along the duodenal-colonic axis that is similar to that of I-FABP mRNA although their levels of I-FABP mRNA are up to three times higher than in normal littermates (Fig. 5, *A* and *B*). The increased steady-state concentration of I-FABP mRNA could reflect the effect of transgene copy number: the 200–600 copies of I-FABP$^{-1178\text{ to }+28}$/hGH^{+3}/haploid genome may bind nuclear transcription factors and thereby affect their ability to interact with the intact endogenous *Fabpi* gene.

Removal of nucleotides −1178 to −278 (containing conserved domain I and its associated 14-bp element plus a 14-bp element 5′ to domain I) (Fig. 1 *A*), produces a marked reduction in reporter expression in the distal small intestine and no detectable hGH mRNA in the colon. This was observed in multiple members of two pedigrees of mice that were analyzed at several stages of postnatal development (2, 3, 12, and 18–21 weeks of age, Fig. 6) and in a sterile 32-wk-old founder mouse (G_o8, data not shown). The presence of 2–60 copies of the I-FABP$^{-277\text{ to }+28}$/hGH^{+3} transgene had no qualitative or quantitative effect on the regional patterns of expression of *Fabpi* along the cephalocaudal axis of the gut as judged by RNA blot hybridization studies of these animals and their normal littermates (data not shown). Surveys of stomach, brain, lung, liver, pancreas, kidney, spleen, muscle, and gonadal RNAs prepared from mice in three I-FABP$^{-277\text{ to }+28}$/hGH^{+3} pedigrees failed to detect any hGH mRNA (limits of detection = 0.2 pg hGH mRNA/μg total cellular RNA). These data confirm that *cis*-acting elements

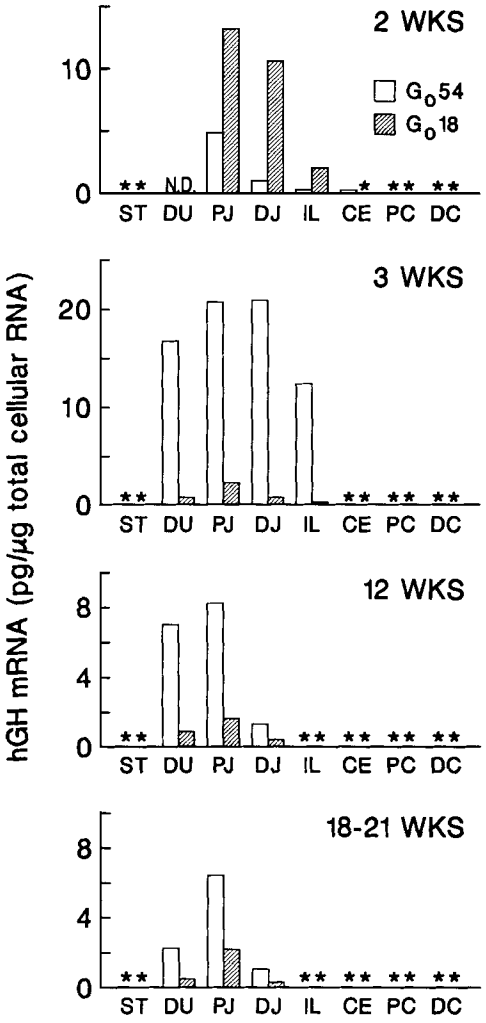


Figure 6. Distribution of hGH mRNA along the gastric–colonic axis of 2–21-wk-old I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenic mice from two different pedigrees. Total cellular RNA was prepared from intestinal segments harvested from members of I-FABP$^{-277\ to\ +28}$/hGH^{+3} lines 18 and 54 (see Table I). The concentration of hGH mRNA in these preparations was determined as described in Materials and Methods. *N.D.*, not determined because the yield of RNA from the duodenum of these 2-wk-old transgenic mice was not sufficient to allow accurate quantitation of hGH mRNA levels. (*) Not detectable.

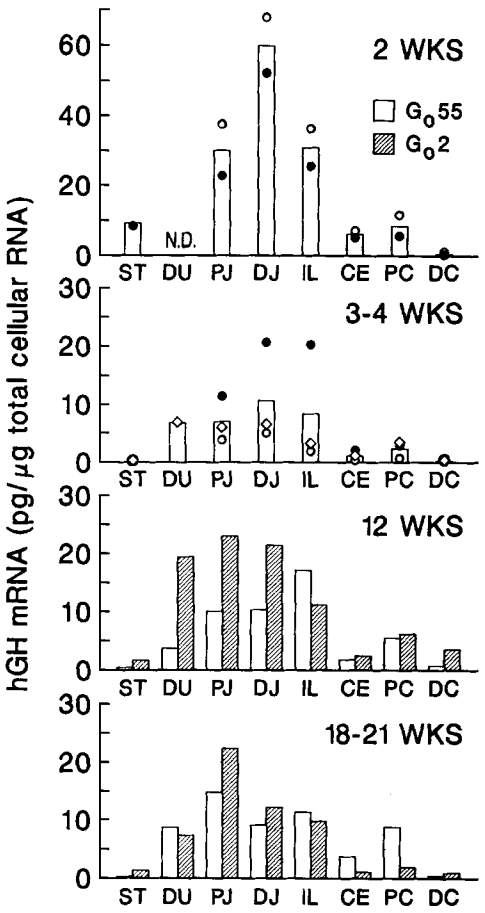


Figure 7. Distribution of hGH mRNA along the gastric–colonic axis of 2–21-wk-old I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice. RNA was prepared from members of I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice belonging to lines 2 and 55 (see Table I). Measurements of hGH mRNA levels during postnatal weeks 2–4 were only made on mice derived from $G_0$55 (n = 2–3 littermates for each of these time points, each animal is represented by either a closed circle, an open circle circle or an open triangle). *N.D.*, not determined (see legend to Fig. 6).

located between nucleotides −1178 to −278 support transgene expression in the distal intestine and colon and contribute to establishment of a proper cephalocaudal distribution of reporter mRNA.

Nucleotides −277 to −184 Contain cis-acting Elements That Suppress Transgene Expression in Distal Small Intestinal and Colonic Enterocytes and in Their Crypt Epithelial Cell Populations

Removal of nucleotides −277 to −185 (containing a portion of conserved domain II, the colonic nuclear factor binding site, and four nucleotides of the C/EBPα footprint) "restored" reporter expression in the distal small intestine and colon. Of the 20 founder mice containing I-FABP$^{-184\ to\ +28}$/hGH^{+3} that were identified, obligate hemizygous mice from six established lines that contained hGH-positive sera were characterized. Fig. 7 shows that the concentration of hGH mRNA in ileal and colonic segments harvested from 2–21-wk-old members of two representative I-FABP$^{-184\ to\ +28}$/hGH^{+3} pedigrees ranged from 20–80% of jejunal levels. Inappropriate expression of the transgene occurred in the stomach but in none of the other 8 tissues examined. (Immunocytochemical surveys of portions of the zymogenic, mucoparietal and pure mucous zones of the stomach (defined according to Lee et al., 1982 and Roth et al., 1992*a*), failed to reveal hGH-positive cells. Our inability to find reporter-positive cells despite the presence of hGH mRNA in stomach RNA could either be due to a sampling error or the fact that the steady-state levels of hGH in gastric epithelial cell lineages (Karam and Leblond, 1992) were below the limits of detection by the IGSS technique.) The relative and absolute concentrations of I-FABP mRNA along the cephalocaudal axis were similar in I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenics and in their comparably aged normal littermates (data not shown).

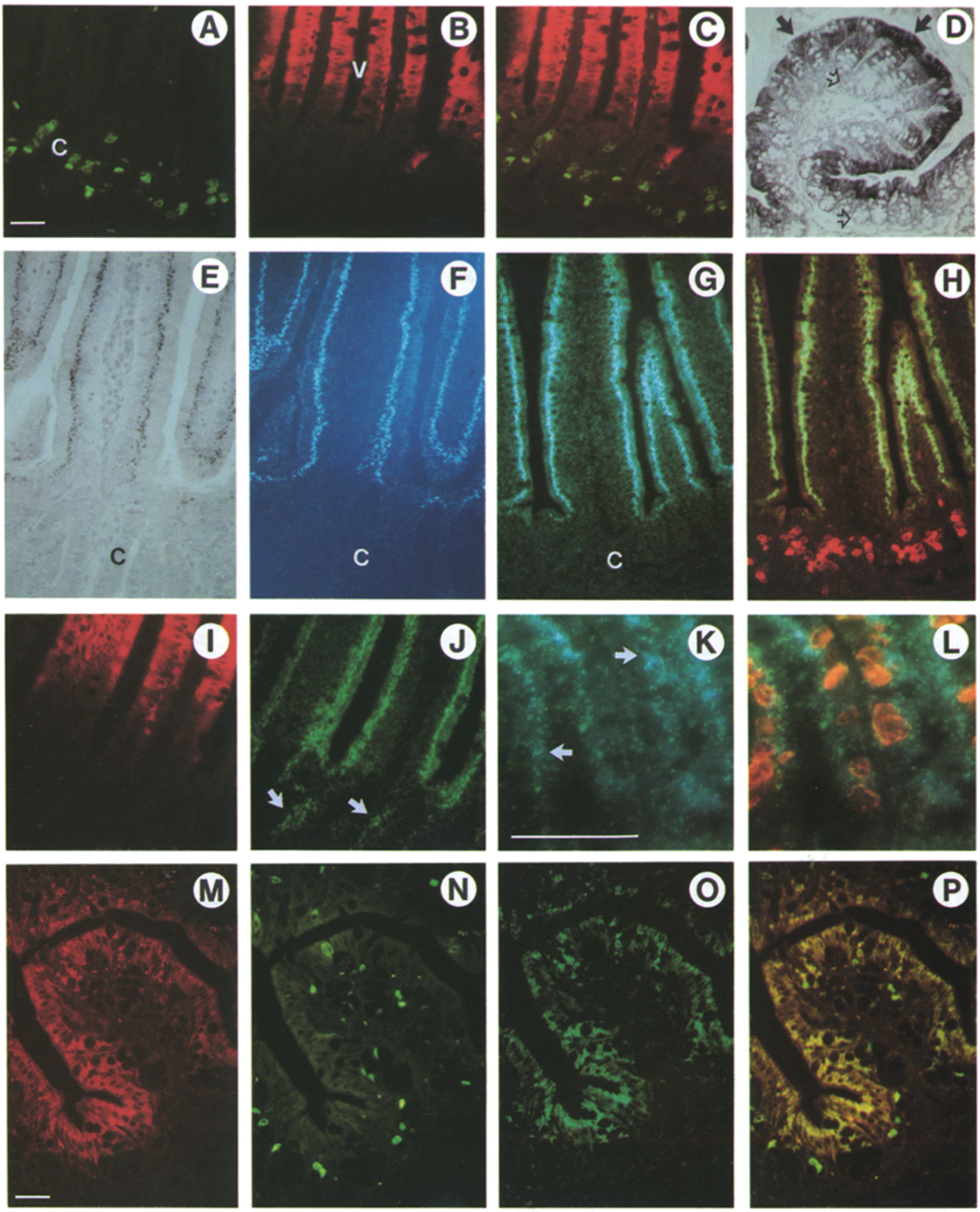


Figure 8. Multilabel immunocytochemical studies of the distribution of I-FABP and hGH along the crypt–villus and crypt–surface epithelial cell cuff axes of adult I-FABP$^{-1178\ to\ +28}$/hGH^{+3}, I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice and their normal littermates. (*A–D*) *Fabpi* is activated in enterocytes as they exit crypts and migrate to the base of small intestinal villi or their colonic homologues, the surface epithelial cuff. Sections from the small intestine (*A–C*) and proximal colon (*D*) from a nontransgenic mouse illustrate the normal cellular distribution of I-FABP. In *A–C* the section was incubated with goat anti-BrdUrd and rabbit anti-I-FABP sera. Antigen–antibody

Removal of nucleotides −277 to −185 also affects cellular patterns of transgene expression along the crypt–villus and crypt–surface epithelial cuff axis of the gut. Double-label immunocytochemical studies of normal and transgenic mice, treated with 5-bromo-2′-deoxyuridine (BrdUrd) 90 min before sacrifice to label cells in S phase, reveal that I-FABP is first detectable in nonproliferating enterocytes as they exit small intestinal and colonic crypts and migrate onto the base of villi or the surface epithelial cuffs (Green et al., 1992 and Fig. 8, *A–D*). (Comparative immunocytochemical and RNA blot hybridization studies of transgenic mice and their normal littermates treated with BrdUrd or vehicle alone indicated the nucleoside analog had no qualitative or quantitative effect on the cellular and regional patterns of *Fabpi* and I-FABP/hGH expression.) None of the proliferating, BrdUrd-positive cells located in the lower and middle thirds of these crypts contain I-FABP. Adult I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenics only express hGH in nonproliferating, villus-associated enterocytes located in the proximal two thirds of the small intestine (Fig. 8, *G* and *H*). In contrast to I-FABP$^{-1178\ to\ +28}$/hGH^{+3} (Fig. 8, *E* and *F*) and I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenic animals, adult I-FABP$^{-184\ to\ +28}$/hGH^{+3} mice produce hGH in villus enterocytes and in both proliferating and nonproliferating epithelial cells located in the mid and upper thirds of the crypt (Fig. 8, *I–L*). The hGH reporter is also present in enterocytes located in the upper portion of colonic crypts and in the surface epithelial cuff (Fig. 8, *M–P*). Despite its inappropriate production in proliferating crypt epithelial cells, hGH was not detectable in differentiating or differentiated members of the goblet, Paneth, or enteroendocrine cell lineages.

Nucleotides −103 to +28 of Fabpi Are Sufficient to Establish Enterocyte-specific and Region-appropriate Patterns of Transgene Expression along the Duodenal–Colonic Axis

Nucleotides −277 to −104 of *Fabpi* were linked to a neutral promoter/reporter (the hGH gene beginning at its nucleotide −84 [see above]) to test the possibility that this 174-bp fragment which contains conserved domain II, plus the colonic factor and C/EBPα binding sites but no copies of the 14-bp element (Fig. 1 *B*), can modulate expression along the crypt–villus and/or duodenal–colonic axes. Ten I-FABP$^{-277\ to\ -104}$/hGH^{-84} pedigrees were established (Table I). No hGH expression was detected in intestinal or extraintestinal tissues harvested from one-wk-old and/or adult animals that were members of each of these lines. Thus, it appears likely that sequences contained within nucleotides −277 to −104 of *Fabpi* are not sufficient to direct intestinal expression of *Fabpi*. However, we could not exclude the possibility that these 174 bp could function as a tissue-specific element if it were linked to a different heterologous promoter/reporter.

In contrast, *cis*-acting elements contained within 103 bp of the start site of transcription of *Fabpi* appear to be sufficient to establish an appropriate cephalocaudal gradient of hGH expression and to confine this reporter to the enterocytic lineage. This conclusion is based on the following observations. We identified seven founder transgenic mice containing nucleotides −103 to +28 linked to nucleotide +3 of hGH. This sequence contains conserved domain III, an associated 14-bp element and the TATA and CCAAT boxes of *Fabpi* (Fig. 1 *B*). Three of these founders expressed hGH and

complexes were then detected with fluorescein-labeled donkey anti-goat and Texas red-labeled donkey anti-rabbit sera, respectively. S phase cells labeled with BrdUrd are confined to the lower and middle thirds of crypts, labeled *c* in *A*. Diffuse enterocytic staining for I-FABP is seen in the villus epithelium, labeled *v* in *B*. Double exposure of BrdUrd and I-FABP immunostaining in *C* clearly illustrates the distinct geographic distributions of S phase cells and I-FABP immunoreactive enterocytes. In the proximal colon (*D*), I-FABP immunoreactivity can be seen as a dark black precipitate using the IGSS detection method and is largely confined to the surface epithelial cuff. Immunoreactive cells in the surface epithelial cuff are indicated by a closed arrow while I-FABP-negative cells deep in the crypt are indicated by open arrows. (*E* and *F*) I-FABP$^{-1178\ to\ +28}$/hGH^{+3} directs a pattern of hGH accumulation in differentiating members of the enterocytic lineage which mimics the pattern of accumulation of I-FABP. A section from the proximal small intestine of a 20-wk-old transgenic mouse stained for hGH by the IGSS method shows intense Golgi-associated staining of villus enterocytes (*E*). The crypt cell zone (labeled *c*) appears negative for hGH in this section which was lightly counterstained with hematoxylin. Visualization of this section with the sensitive, reflected light polarization microscopy technique (*F*) fails to reveal any traces of hGH immunoreactivity in the crypt cell zone (*c*) while villus-associated enterocytes exhibit intense Golgi labeling. (*G* and *H*) hGH is restricted to villus-associated enterocytes in the proximal small intestine of I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenic mice. hGH immunoreactivity in *G*, detected by the IGSS method and visualized with reflected light polarization microscopy, is found in villus-associated enterocytes while cells in the crypt (*c*) are unlabeled. The crypt cell zone is clearly identified in *H* by the presence of BrdUrd-labeled cells (visualized with Texas red–labeled secondary antibodies). (*I–L*) I-FABP$^{-184\ to\ +28}$/hGH^{+3} is inappropriately expressed in small intestinal crypts. A section of proximal small intestine from a transgenic mouse was incubated with rabbit anti-I-FABP and goat anti-hGH sera. Antigen–antibody complexes were detected with Texas red–labeled-, and fluorescein-labeled donkey anti-rabbit and anti-goat secondary antibodies, respectively. (*L*) I-FABP immunoreactivity is restricted to villus-associated enterocytes. The hGH reporter seen in *J* extends into the crypt cell zone (*arrows*). This crypt cell expression can be further defined in animals treated with BrdUrd 90 min before sacrifice. A high power view shows immunoreactive hGH in the Golgi apparatus of crypt epithelial cells (*K*), some of which are in S phase as indicated by their BrdUrd-labeled nuclei (*L*; double exposure of hGH-labeled cells [IGSS, reflected light polarization microscopy] and BrdUrd-labeled cells [Texas red]). (*M–P*) I-FABP$^{-184\ to\ +28}$/hGH^{+3} is expressed in both crypt and surface epithelial cuff cell populations in the colon. (*M*) I-FABP immunoreactivity is present in the uppermost region of colonic crypts and in surface epithelial cuff cells. (*N*) scattered BrdUrd-labeled cells are seen in the lower segments of the crypt. (*O*) hGH immunoreactivity is seen in both the surface epithelial cuff and in the mid-crypt region. The triple exposure in *P* shows extension of hGH immunoreactivity down to BrdUrd-labeled cells. (Note: the turnover rate of epithelial cells in the colon is longer than that in the small intestine, resulting in fewer BrdUrd-labeled cells/crypt after a 90-min labeling period than observed in the small intestine.) Bar, 25 μm.

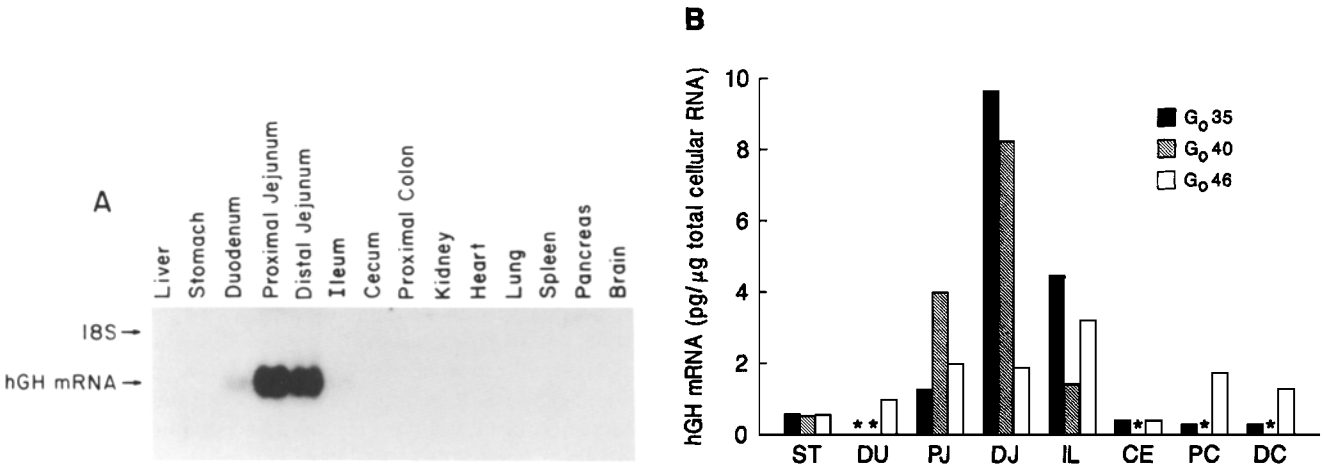


Figure 9. Distribution of hGH mRNA along the gastric–colonic axis of 2-wk-old I-FABP$^{-103\ to\ +28}$/hGH^{+3} transgenic mice from three different pedigrees. (*A*) A blot of total cellular RNA, prepared from portions of the cephalocaudal axis of the gut and 7 extraintestinal tissues of a G_o35-derived mouse, was probed with a ^{32}P-labeled, 150-bp fragment recovered from exon V of hGH. Each lane contains 15 μg of total cellular RNA. An autoradiograph of the RNA blot is shown. (*B*) Quantitation of hGH mRNA levels along the cephalocaudal axis of the intestine of representative members of pedigrees 35, 40, and 46 (see Table I). (*) Not detectable.

were used to establish pedigrees. F1 and/or F2 mice from two of these lines exhibited gastrointestinal-specific expression of the reporter (e.g., see Fig. 9 *A*) while another (derived from G_o46) demonstrated expression in stomach, small and large intestine, kidney, lung, brain, spleen and pancreas (data not shown). However, all three pedigrees demonstrated a duodenal–colonic distribution of hGH mRNA (Fig. 9 *B*) which mimicked that of I-FABP mRNA in the same preparation of total cellular RNA (data not shown). Peak levels of reporter mRNA were encountered in the distal jejunum of 2-wk-old suckling mice and ranged from 2–10 pg/μg total cellular RNA. Immunocytochemical studies demonstrated that removal of nucleotides −184 to −104 had no effect on the restriction of hGH expression to the enterocytic lineage (see Fig. 11 *K–N*). Moreover, the presence of 2–8 copies of the I-FABP$^{-103\ to\ +28}$/hGH^{+3} transgene had no qualitative or quantitative effect on the regional or cellular patterns of mouse *Fabpi* expression (see Fig. 11, *L* and *N*).

Developmental Patterns of Fabpi/hGH Expression

As noted in the introduction, *Fabpi* not only represents a sensitive marker of enterocytic differentiation along the crypt–villus and crypt–surface epithelial cuff axis, it is also a molecular marker of the process of cytodifferentiation that the gut undergoes during late fetal life. This process is initiated in the proximal small intestine during E15–E16 and proceeds in a proximal–distal "wave" that travels along the cephalocaudal axis during late gestation transforming the stratified intestinal endoderm to a monolayer of epithelial cells that overlie nascent villi. Transcriptional activation of *Fabpi* occurs in villus-associated enterocytes located in the proximal small intestine at the same time that morphologic transformation of its endoderm is initiated (i.e., E15–16) (Green et al., 1992). Initiation of *Fabpi* expression follows the wave of cytodifferentiation that proceeds rapidly along the duodenal–colonic axis (Rubin et al., 1989, 1992; Green et al., 1992).

We measured serum hGH levels and performed multilabel immunocytochemical surveys of late gestation and suckling I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH^{+3}, and I-FABP$^{-103\ to\ +28}$/hGH^{+3} transgenic mice to determine whether their transgenes exhibited appropriate developmental stage-specific patterns of activation and whether the cellular patterns of reporter accumulation along the evolving duodenal–colonic and crypt–villus axis mimicked those of *Fabpi*. hGH first appears in the serum of I-FABP$^{-277\ to\ +28}$/hGH^{+3} and I-FABP$^{-184\ to\ +28}$/hGH^{+3} mice at the time that *Fabpi* is activated in the proximal small intestine (Fig. 10, *A* and *B*). hGH is also present in the serum of I-FABP$^{-103\ to\ +28}$/hGH^{+3} mice on the day of birth, which was the earliest time point we surveyed (Fig. 10 *C*). Serum hGH concentration peaks during the suckling period (postnatal days 1–14) and then declines during weaning (postnatal days 15–28) in mice containing each of these three I-FABP/hGH transgenes (Fig. 10 *A–C*). The postnatal decreases in serum hGH levels documented during the suckling/weaning period are accompanied by decreases in the steady-state levels of hGH mRNA along the cephalocaudal axis of the gut (compare the upper two panels in Figs. 6 and 7 with Fig. 10, *A* and *B*). I-FABP mRNA concentrations do not fall during the suckling/weaning period (e.g., see Green et al., 1992). However, a number of gene products show marked changes in their levels at the suckling/weaning transition in both the rat and mouse. Many of these alterations have been ascribed to increases in glucocorticoid levels which occur at this stage of development (reviewed in Henning et al., 1987). Therefore, we wondered whether the glucocorticoid responsive element present in intron I of hGH (Moore et al., 1985) mediated the observed declines in I-FABP/hGH expression. However, administration of hydrocortisone acetate (25 μg/g body weight per day) to G_o18-derived I-FABP$^{-277\ to\ +28}$/hGH^{+3} mice, from postnatal days 7–10, produced only minimal (<50%) reductions in serum hGH levels compared with transgenic littermates that had been treated with vehicle alone.

Surveys of 1–14-d-old I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH, and I-FABP$^{-103\ to\ +28}$/hGH^{+3} mice indicated that the presence of the transgene had no effect on the cellular patterns of I-FABP accumulation along the na-

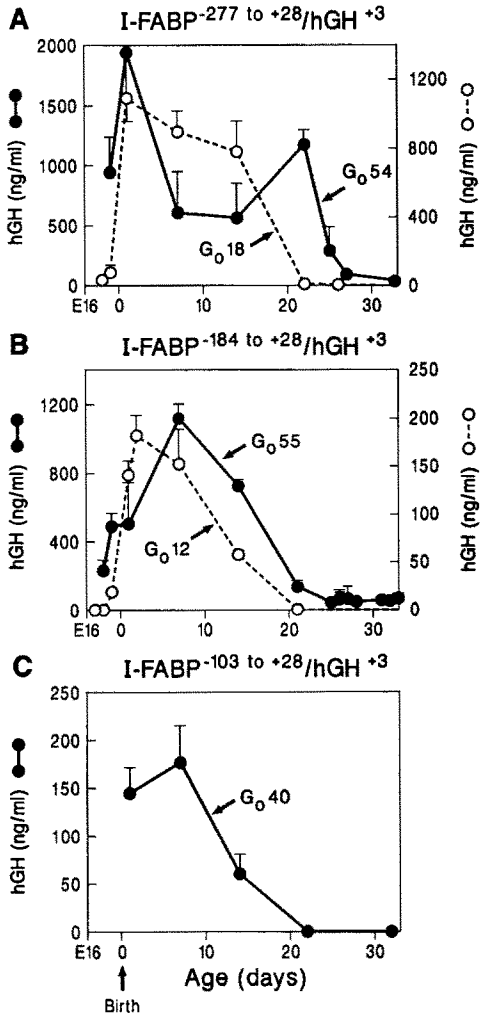


Figure 10. Serum hGH levels in E16-E20, suckling, and weaning I-FABP/hGH transgenic mice. 1–15 mice from the indicated pedigrees were examined at each of the ages shown. hGH concentrations were determined in each serum sample in duplicate and the results were averaged. Data are expressed as the average serum hGH concentration among comparably aged mice from a given line ± SEM. The limits of detection of the assay are 0.5 ng/ml serum. (*A*) Data derived from members of I-FABP$^{-277\ to\ +28}$/hGH^{+3} lines 18 and 54; (*B*) values documented in members of I-FABP$^{-184\ to\ +28}$/hGH^{+3} pedigrees 12 and 55; (*C*) serum hGH levels in members of I-FABP$^{-103\ to\ +28}$/hGH^{+3} line 40. Further analysis of I-FABP$^{-277\ to\ +28}$/hGH^{+3} and I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice from the same pedigrees indicated that hGH levels remained constant from the 35–250 days of life (data not shown).

scent crypt–villus and duodenal–colonic axes (Fig. 11 and data not shown). Like I-FABP, hGH was confined to enterocytes overlying nascent villi in I-FABP$^{-277\ to\ +28}$/hGH^{+3} mice (Fig. 11, *A–G*). Removal of nucleotides −277 to −185 produced inappropriate expression of hGH in the intervillus epithelium but still restricted this reporter to the enterocytic lineage in small intestinal villi (Fig. 11, *H* and *I*). Finally, distinct differences in the steady-state levels of hGH were evident in enterocytes distributed along the duodenal–colonic axis. These differences were noted at the time of initial activation of each of the transgenes (e.g., Fig. 11, *H* and *J*). They were subsequently maintained during the suckling period and parallel cephalocaudal differences in the steady-state concentration of hGH mRNA (Figs. 6, 7, and 9).

Discussion

Fig. 12 describes the functional elements that have been identified in the 5′ nontranscribed domain of *Fabpi* from the studies described above. Analysis of I-FABP/hGH transgenic mice have provided insights about: (*a*) the mechanisms which permit cephalocaudal differences in gene expression to be sustained in a continuously renewing gut epithelial cell lineage; (*b*) how gene transcription is regulated in enterocytes as they differentiate during their migration along the crypt–villus axis; (*c*) the relationship between proliferation and lineage allocation; and (*d*) the "temporal" gradient of gene expression that affects the gut epithelium.

Our results suggest that the spatial patterns of *Fabpi* expression observed along the duodenal–colonic and crypt–villus (or crypt–surface epithelial cuff) axes reflect the contributions of positive and negative *cis*-acting transcriptional regulatory elements which lie within 1 kb of its start site of transcription. Remarkably, nucleotides −103 to +28 of rat *Fabpi* are sufficient to direct lineage-appropriate expression and to establish a duodenal–colonic distribution of reporter mRNA accumulation in enterocytes which mimics that of mouse I-FABP mRNA. These 132 bp include (*a*) a sequence which extends from nucleotide −94 to −1 that is conserved between three orthologous *Fabpi* genes and (*b*) a 14-bp element which is represented in at least one other family member (*Crbp2*) whose enterocyte-specific and geographic patterns of expression resemble those of *Fabpi*. However, the functional significance of these elements remains to be defined. A detailed search of a relational database of known transcriptional regulatory elements (Ghosh, 1990) failed to reveal any members, other than TATA and CCAAT box elements, represented within nucleotides −103 to +28. The gradient of I-FABP mRNA levels observed along the cephalocaudal axis appears to be modulated by two additional *cis*-acting spatial regulatory elements: a domain or domains located between nucleotides −1178 and −277 which promotes expression in the ileum and colon and a domain or domains located between nucleotides −277 and −184, which operates to suppress ileal and colonic expression (Fig. 12). This latter portion of the 5′ nontranscribed domain of *Fabpi* includes a 24-bp element, spanning nucleotides −212 to −188, that binds nuclear factors present in colonic but not proximal small intestinal epithelial cells. The suppressor and colonic factor binding domains have no obvious sites recognized by known transcription factors and are contained within a portion of *Fabpi* (nucleotides −277 to −104) that is not sufficient to support expression of the hGH reporter in any lineage in any portion of the gut epithelium. (The use of various combinations of a limited number of positive and negative elements could permit diverse regional patterns of expression of genes along the cephalocaudal axis of the gut. For example, it will be interesting to determine which of these elements in *Fabpi*, if any, are represented in a homologous gene encoding ileal lipid binding protein [ILBP]. *Ilbp* expression is limited to ileal, villus-associated enterocytes in weaning and adult mice [Sacchettini et al., 1990; M. Crossman, S. Hauft, K. Roth, and J. Gordon, unpublished observations].)

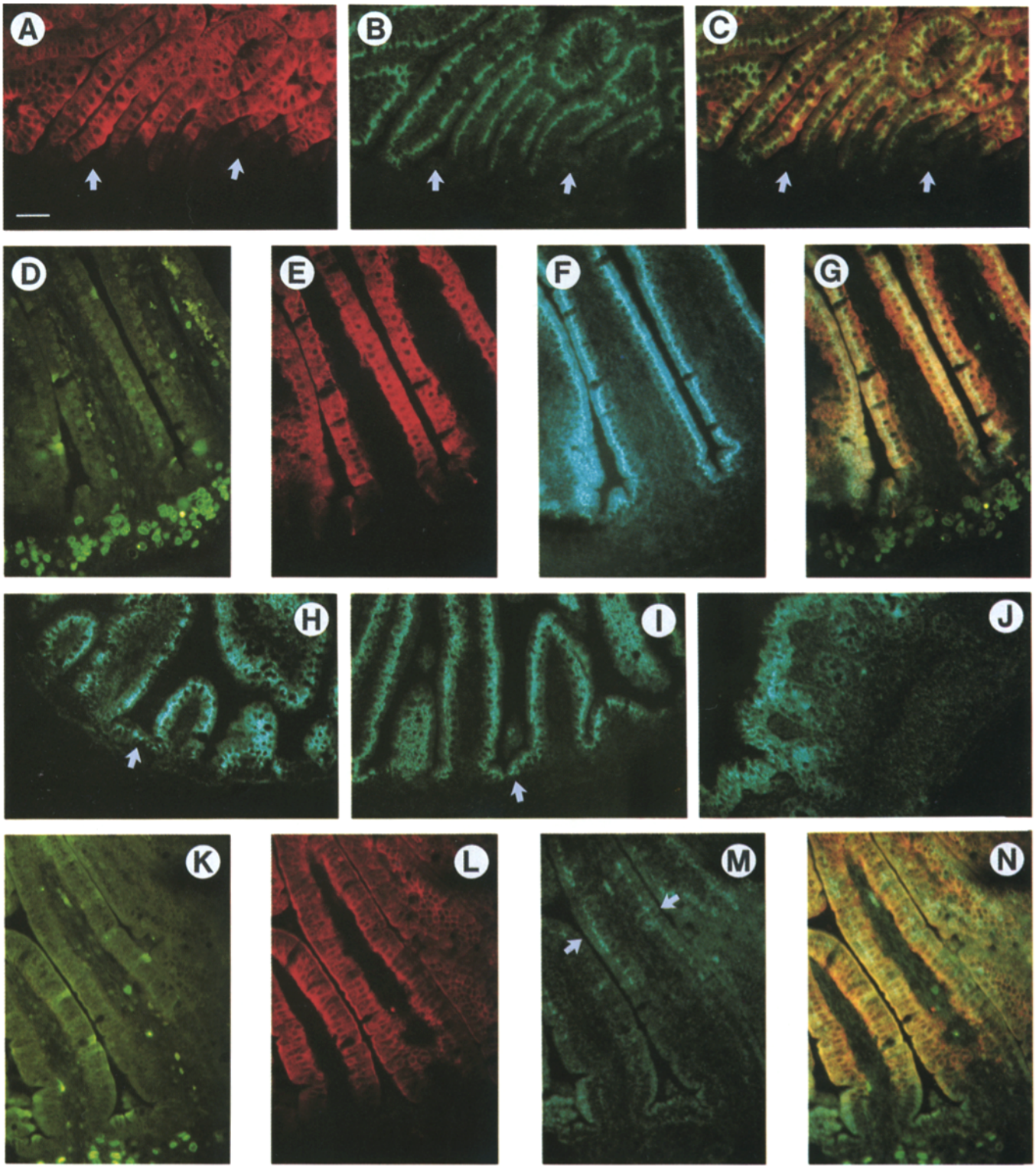


Figure 11. Comparison of cellular distribution of I-FABP and hGH along the nascent crypt–villus axis of proximal small intestinal segments prepared from suckling I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH^{+3}, I-FABP$^{-103\ to\ +28}$/hGH^{+3} transgenic mice and their normal littermates. (*A–G*) transgene expression in developing I-FABP$^{-277\ to\ +28}$/hGH^{+3} mice is limited to villus-associated enterocytes. A section of small intestine from a 1-d-old I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenic mouse was incubated with rabbit anti-I-FABP and goat anti-hGH sera and visualized with Texas red and fluorescein-conjugated secondary antibodies, respectively. Diffuse cytoplasmic I-FABP immunoreactivity (*A*) and intense Golgi staining for hGH (*B*) are seen in villus-associated enterocytes. The colocalization of I-FABP and hGH immunoreactivity and the lack of intervillus expression of either protein is seen in the double exposed photomicrograph in *C* (the intervillus region is indicated by *arrows*). In a 14-d-old I-FABP$^{-277\ to\ +28}$/hGH^{+3} transgenic mouse, the crypts are well formed and purification from polyclonality to monoclonality has been completed. The hGH reporter remains restricted to villus-associated enterocytes (*D–G*). BrdUrd-labeled cells, detected with fluoresceinated secondary antibodies, clearly define the proliferative zone in the crypt (*D*). I-FABP immunoreactivity, detected with Texas red–labeled secondary antibodies in *E*, is restricted to villus-associated enterocytes. hGH immunoreactivity, detected

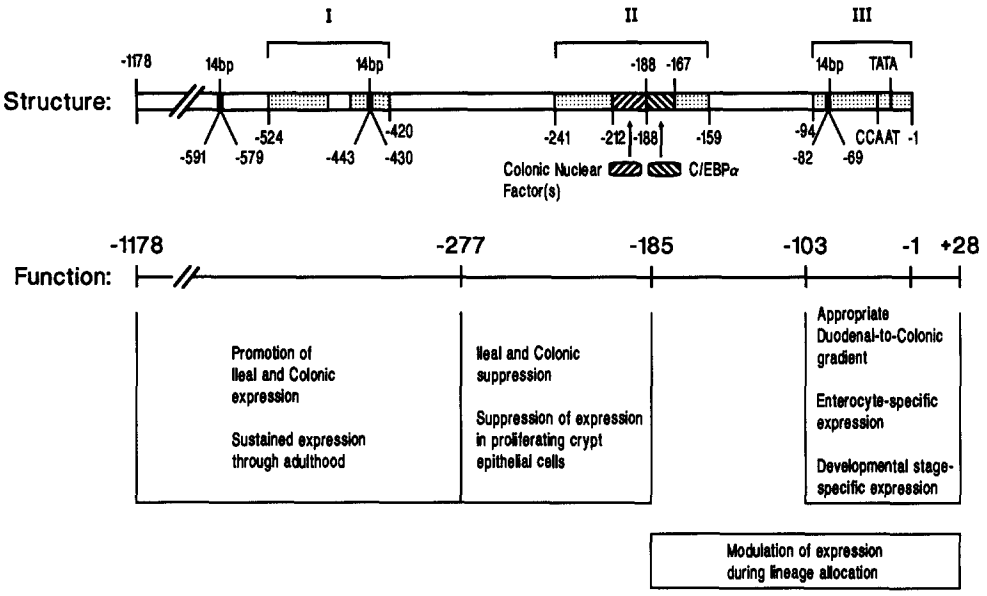


Figure 12. Summary of elements in the 5′ nontranscribed domain of rat *Fabpi* that regulate its patterns of expression along the duodenal–colonic and crypt–villus (or crypt–surface epithelial cuff) axes. See text for discussion.

As noted in the introduction, the differentiation programs of the four principal gut epithelial cell lineages are expressed as these descendants of a single, active multipotent crypt stem cell undergo a spatially constrained amplification and bipolar migration along the crypt–villus axis. Nucleotides −277 to +28 of rat *Fabpi* are sufficient to recapitulate the normal pattern of activation of mouse *Fabpi* in differentiating enterocytes as they exit the crypt and begin their upward journey in vertical coherent bands to the apical extrusion zone of villi. Removal of nucleotides −277 to −185 results in inappropriate expression of hGH in proliferating epithelial cells located in the mid portion of intestinal crypts. Despite this precocious activation, accumulation of the reporter is subsequently restricted to differentiated enterocytes; hGH is not detectable in the differentiated products of the Paneth, goblet, or enteroendocrine cell lineages. This finding suggests several conclusions. First, many of the regulatory factors necessary for transcription of *Fabpi* have apparently already been synthesized and are functional within the replicating descendants of the multipotent crypt stem cell. Second, the inappropriate crypt expression of I-FABP$^{-184\text{ to }+28}$/hGH^{+3} is not the result of a general de-repression of transgene expression since enterocyte-specific and region-appropriate patterns of reporter production are observed. Rather, expression of *Fabpi* appears to be specifically suppressed in crypt epithelial cells through the action of *cis*-acting regulatory elements located between nucleotides −277 and −184. This finding provides the first clues about the mechanisms that normally modulate gene expression during translocation/differentiation of enterocytes along the crypt–villus axis. The CCAAT-enhancer binding proteins have been implicated in (*a*) activation of genes that confer a specialized phenotype to a differentiated cell although they do not appear to be able to independently specify a terminal differentiation program, and (*b*) in the production of a nonproliferating state (reviewed in Umek et al., 1991). Although C/EBPα footprints to nucleotides −188 to −167 of *Fabpi* in vitro, its role in regulating differentiation-dependent activation of *Fabpi* in nonproliferating members of the enterocytic lineage remains to be established. For example, the cellular patterns of C/EBPα expression have yet to be fully defined in the gut epithelium, nor have those of C/EBPβ and C/EBPδ which are also expressed in the intestine and form mixed dimers with one another and C/EBPα (Cao et al., 1991).

The third conclusion which can be made from these results deals with the point at which allocation to a particular gut cell lineage occurs during the processes of amplification and migration. The fact that I-FABP$^{-184\text{ to }+28}$/hGH^{+3} expression occurs in replicating crypt epithelial cells yet is ultimately restricted to the enterocytic lineage in both small intestine and colon suggests that the replicating cells are already committed to the enterocytic lineage or that the proliferating cells are not fully committed and nucleotides −184 to +28 contain *cis*-acting elements which bind lineage specific sup-

with IGSS, is also confined to villus enterocytes (*F*). Triple exposure of BrdUrd, I-FABP, and hGH immunoreactivity in *G* confirms that I-FABP and hGH are not detectable in crypt epithelial cells. (*H–J*) I-FABP$^{-184\text{ to }+28}$/hGH^{+3} directs transgene expression to nascent intestinal crypts in neonatal mice. hGH immunostaining of the small intestine from both 1-d-old (*H*) and 7-d-old (*I*) transgenic animals shows labeling of cells in the intervillus epithelium (indicated by *arrows*). (*J*) Immunostaining of the proximal colon from a 7-d-old I-FABP$^{-184\text{ to }+28}$/hGH^{+3} mouse reveals the reporter in the upper segments of the developing crypt–surface epithelial cuff axis. (*K–N*) expression of I-FABP$^{-103\text{ to }+28}$/hGH^{+3} in a 2-wk-old mouse. A section of small intestine was incubated with antibodies against BrdUrd, I-FABP, and hGH. The fluorescein-labeled BrdUrd-immunoreactive cells in *K* define the crypt cell zone, while Texas red–labeled I-FABP immunoreactive cells in *L* define the villus epithelium. Relatively weak, focal hGH immunoreactivity, visualized by the IGSS method, is apparent in *M* (indicated by *arrows*). Multiexposure of the section in *N*, indicates that hGH is limited to villus-associated enterocytes. The staining was generally too weak to make conclusions about whether the reporter is also present in proliferating and/or nonproliferating crypt epithelial cells. Bar, 25 μm.

pressor factors produced by cells after they are subsequently allocated to enteroendocrine, goblet and Paneth cell lineages. Alternatively, suppression of hGH synthesis after allocation to these lineages could reflect loss of a positive factor, produced in uncommitted cells, that binds to elements located between nucleotides −184 to +28 (this loss would presumably occur during differentiation of enteroendocrine, goblet and Paneth cells but not enterocytes). The apparent restriction of I-FABP$^{-184\ to\ +28}$/hGH expression to the enterocytic lineage is interesting in light of our previous studies of transgenic mice that contain nucleotides −4000 to +21 or −596 to +21 of the homologous liver fatty acid binding protein gene (*Fabpl*) linked to a variety of reporters including hGH (Sweetser et al., 1988*b*; Hansbrough et al., 1991). The intact, endogenous mouse *Fabpl* gene is normally restricted to the enterocytic lineage, although a very small subpopulation of enteroendocrine cells located in proximal small intestinal villi contains detectable levels of this cytoplasmic long chain fatty acid binding protein (Sweetser et al., 1988*b*; Roth et al., 1990). Like *Fabpi*, *Fabpl* is activated as enterocytes exit small intestinal crypts. L-FABP$^{-4000\ to\ +21}$ and L-FABP$^{-596\ to\ +21}$ direct inappropriate expression of these reporters in proliferating and nonproliferating small intestinal and colonic crypt epithelial cells (Sweetser et al., 1988*b*; Trahair et al., 1990; Roth and Gordon, 1990; Cohn et al., 1991; Hansbrough et al., 1991; Rubin et al., 1991, 1992; Roth et al., 1991*b*). The cellular distribution of hGH in the crypts of L-FABP$^{-4000\ to\ +28}$/hGH^{+3} and L-FABP$^{-596\ to\ +21}$/hGH^{+3} animals is somewhat different than the cellular distribution observed in comparably aged I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice: the L-FABP/hGH transgenes are first activated in cells that are closer to the base of crypts where the multipotent stem cell is presumed to reside (Potten and Loeffler, 1990) and the steady-state level of their hGH product is maintained at higher levels in both proliferating and nonproliferating cells during their translocation through the crypts (Sweetser et al., 1988*b* and data not shown). Precocious expression of hGH in the crypts of multiple pedigrees of L-FABP$^{-4000\ to\ +28}$/hGH^{+3} and L-FABP$^{-596\ to\ +28}$/hGH^{+3} mice is associated with "appropriate" production of the reporter in enterocytes and inappropriate production in fully differentiated members of the enteroendocrine, the goblet cell, and the Paneth cell lineages (Roth et al., 1990; 1991*a*,*b*; 1992*b*; Roth and Gordon, 1990; Trahair et al., 1990; Cohn et al., 1991). One possible interpretation of these results is that nucleotides −4000 to +28 do not contain lineage-specific suppressor elements comparable with those postulated to be located between nucleotides −184 to +28 of *Fabpi*. Alternatively, these two L-FABP/hGH transgenes may be activated in crypt epithelial cells that are uncommitted, or less committed, than those cells which initially support I-FABP$^{-184\ to\ +28}$/hGH^{+3} activation (i.e., L-FABP/hGH may be initially expressed in the multipotent stem cell or one of its immediate descendants). Whatever the mechanisms, transgenes appear to be potentially useful probes for operationally defining where and how lineage allocation occurs in the crypt. For example, crossing I-FABP$^{-184\ to\ +28}$/hGH^{+3} transgenic mice with mice that contain transgenes composed of L-FABP$^{-596\ to\ +21}$ linked to a different reporter would allow assessment of which crypt epithelial cell populations support expression of one or both transgenes and their relative locations along the basal-apical axis. Moreover, the recent development of methods for preparing primary cultures of suckling crypt and villus epithelial cell populations (Evans et al., 1992), may allow mice containing both I-FABP$^{-184\ to\ +28}$/reporter and L-FABP$^{-596\ to\ +21}$/reporter transgenes to be used for isolation of gut stem cells, their early descendants, and/or members of specific lineages if the reporters encode gene products that allow for positive and negative selection ex vivo.

A fourth conclusion that can be made from the experiments described here is that nucleotides −103 to +28 of rat *Fabpi* contain *cis*-acting elements which are sufficient to produce appropriate developmental stage-specific activation of hGH expression along the cephalocaudal axis of the gut but lack elements necessary to sustain similar levels of expression beyond the suckling/weaning period. Addition of nucleotides −184 to −104 or −277 to −104 did not modify the proper temporal pattern of activation of reporter production during E16-20 nor did they "prevent" a several fold reduction in steady state hGH mRNA levels between postnatal weeks 2 and 21. Analyses of I-FABP$^{-1178\ to\ +28}$/hGH^{+3} or I-FABP$^{-1178\ to\ +28}$/SV-40 T antigen transgenic mice suggest that nucleotides −1178 to +28 of rat *Fabpi* contain *cis*-acting elements that are sufficient to sustain reporter mRNA levels through at least the first nine months of life (Sweetser et al., 1988*a*; Hauft et al., 1992 and data not shown). It is tempting to speculate that the conserved domain I, which spans nucleotides −594 to −420, and/or its associated 14-bp repeat (Fig. 12) may function as a positive transcriptional element which is involved in sustaining *Fabpi* expression during postnatal life. It appears unlikely that the postnatal fall in hGH mRNA and protein levels (the latter evident by immunocytochemical surveys of sections of intestine and by measurement of serum hGH concentrations) is mediated by glucocorticoids, thyroxine, or by luminal contents. Although marked increases in glucocorticoids and thyroxine levels occur at the suckling/weaning transition and mediate a number of changes in gene expression within the enterocytic lineage (Malo and Menard, 1983; Henning, 1987), analysis of normal mice and rats or mice with congenital hypothyroidism, produced by a recessive mutation known as *hyt*, indicates that neither precocious administration of glucocorticoids, adrenalectomy, nor thyroid hormone deficiency affects *Fabpi* expression (Green et al., 1989; N. Nanthakumar and S. J. Henning, personal communication). Moreover, studies of intestinal isografts harvested from E16 mice and implanted into the subcutaneous tissues of adult CBY/B6 recipients reveal that *Fabpi* expression is sustained for up to 2 mos in villus-associated enterocytes distributed along the duodenal-colonic axis (Rubin et al., 1992). One obvious question is what role the reporter plays in the postnatal decreases in I-FABP$^{-277\ to\ +28}$/hGH^{+3}, I-FABP$^{-184\ to\ +28}$/hGH^{+3} and I-FABP$^{-103\ to\ +28}$/hGH^{+3} expression. hGH production is extinguished in aging transgenic mice containing L-FABP/hGH fusion genes (Cohn et al., 1991). However, the temporal and spatial pattern of silencing of L-FABP$^{-4000\ to\ +21}$/hGH^{+3} or L-FABP$^{-596\ to\ +21}$/hGH^{+3} differs from that of I-FABP/hGH: it is restricted to the colon and occurs in a "wave" that progresses from the distal to proximal portions of the large bowel over a 6–12-mo period. The decision to extinguish L-FABP/hGH expression appears to be made at the level of the crypt stem cell or one of its immediate descendants, and is coordinated between adjacent colonic

crypts, producing patches of wholly reporter negative colonic glands (Cohn et al., 1991). In contrast, the reduction in I-FABP/hGH expression is more rapid, affects the entire cephalocaudal axis, and produces a diminution in the steady state levels of hGH in all villus-associated enterocytes. It does not involve the progressive appearance of coherent, wholly positive bands of hGH-negative enterocytes extending from a crypt to the apex of a villus, as would be expected if the decision to suppress transgene expression were made at the level of a single crypt stem cell that supplies a polyclonal villus (see introduction, and Roth et al., 1991*b*).[2]

Although the mechanism of postnatal suppression of I-FABP/hGH expression is currently unknown, these observations emphasize that mice containing *Fabpi*/reporter transgenes represent powerful model systems for further defining how gene expression is regulated in specific gut epithelial cell lineages as a function of their "positional address" along the duodenal–colonic and crypt–villus axes and as a function of their "temporal address" in this rapidly yet continuously renewing system.

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