Suppressor and Activator Functions Mediated by a Repeated Heptad Sequence in the Liver Fatty Acid-binding Protein Gene (Fabpl)

EFFECTS ON RENAL, SMALL INTESTINAL, AND COLONIC EPITHELIAL CELL GENE EXPRESSION IN TRANSGENIC MICE*

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A 35-nucleotide sequence in the liver fatty acid-binding protein gene (Fabpl) has been identified that interacts with nuclear proteins present in adult mouse liver, kidney, stomach, small intestine, and colon. The binding site consists of a direct heptad repeat (TTCTGNNNTT) separated by five nucleotides. Both heptads are required for formation of stable complexes with nuclear proteins in gel mobility shift assays. The in vivo functions mediated by the repeats were determined by comparing the expression of four Fabpl/human growth hormone fusion genes in multiple pedigrees of adult transgenic mice. The transgenes contained (i) nucleotides −596 to +21 of Fabpl linked to the human growth hormone reporter, (ii) 4 additional copies of the 35-base pair element placed at nucleotide −596 of Fabpl, (iii) 4 additional copies of the sequence placed just upstream of its endogenous site at nucleotide −132, and (iv) a sequence identical to (iii) but with all heptad repeats mutated within each of the 4 additional copies of the 35-base pair element. Transgene expression was defined by RNA blot hybridizations and by light and electron microscopic immunohistochemistry. The heptad repeat functions to suppress expression in tubular epithelial cells of the proximal nephron, in hepatocytes, in the mucus-producing pit cells of the gastric epithelium, and in absorptive enterocytes located in the proximal small intestine. There is a gradient of escape from enterocytic suppression as one moves from the proximal to distal small intestine. This escape progresses to involve successively less differentiated cells located closer and closer to the stem cell zone in crypts of Lieberkühn. The heptad repeat activates gene expression in the colonic epithelium so that all proliferating and nonproliferating cells in colonic crypts distributed from the cecum to the rectum support transgene expression. The heptad has no obvious sequence similarities to known transcription factor binding sites, suggesting that mediators of its in vivo activities are likely to be novel. One candidate factor is a 90-kDa protein identified in Southwestern blots. The 90-kDa protein also binds to an element in the matrix metalloproteinase-2 gene that functions as an enhancer in renal cells, shares sequence homology with the heptad, and generates similar-sized complexes in gel mobility shift assays as the Fabpl repeat. The heptad repeat represents a target for identifying transcription factors that regulate gene expression between gut and renal epithelia and that also regulate the differentiation program of the intestine's principal epithelial lineage as a function of its location along the duodenal-colonic axis. Finally, the Fabpl regulatory elements described in this report should be useful for delivering a variety of gene products throughout the colonic epithelium of transgenic mice.

Epithelial cells that line the intestine and the nephrons of the kidney share many common functions. The molecular mechanisms that regulate gene transcription within and between these epithelia are largely uncharacterized. The “liver” fatty acid-binding protein gene (Fabpl) provides a model for investigating these mechanisms.

In the adult mouse and rat, Fabpl is transcribed in hepatocytes and in polarized absorptive enterocytes, the principal epithelial cell lineage of the small intestine (1). Fabpl exhibits a cephalocaudal gradient of expression within the intestine; highest levels of its mRNA and protein products are encountered in differentiated enterocytes that overlie villi located in the middle third of the small intestine; levels diminish as one moves proximally or distally. The gene is silent in the gastric and distal colonic epithelium of both species and in all other epithelia not associated with the gastrointestinal tract.

The contribution of cis-acting suppressors to maintaining this pattern of expression was revealed from studies in transgenic mice (1). Seven fusion genes were produced by sequential deletions of the proximal 4000 nucleotides of rat Fabpl's 5'-nontranscribed domain and linkage of each truncated product to nucleotides +3 to +2150 of the human growth hormone (hGH) gene. The cellular and spatial patterns of expression of each fusion gene were defined in multiple pedigrees of adult transgenic mice. The expression pattern revealed is similar to that observed in vivo. These results suggest that the cis-acting suppressors that mediate this pattern are likely to be highly conserved.

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1 The abbreviations used are: Fabpl, liver fatty acid-binding protein gene; L-FABP, liver fatty acid-binding protein; GMSA, gel mobility shift assay; hGH, human growth hormone; MMP-2, matrix metalloproteinase-2 gene; DAB, 3,3'-diaminobenzidine; Fabpl, intestinal fatty acid-binding protein gene; BrdUrd, 5-bromo-2'-deoxyuridine; PBS, phosphate-buffered saline; oligos, oligodeoxynucleotides; bp, base pair(s).
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transgenic mice. Cis-acting suppressors of cecal and colonic transcription were identified between nucleotides −4000 and −1600. Suppressors of gastric expression are positioned outside of nucleotides −4000 to +21. Multiple suppressor elements, distributed between nucleotides −4000 and +21, help determine the intestinal epithelial cell lineage-specific patterns of Fabpl expression (1).

A suppressor of intestinal expression was also found. Although the endogenous gene remains silent in the mouse and rat kidney throughout adulthood, analyses of transgenic mice containing nucleotides −132 to +21 of Fabpl revealed that the steady state concentration of the hGH reporter mRNA was 10 times higher in kidney than in the four other tissues where it was expressed (1). Renal expression of hGH was confined to epithelial cells located in the proximal tubules of nephrons. The relative levels of hGH mRNA in the other tissues were small intestine > colon > stomach = liver. Addition of a 54-bp sequence spanning nucleotides −186 to −133 suppressed kidney hGH expression 40-fold relative to these four other tissues (1).

In the present report, we have determined that this 54-bp element contains a direct heptad repeat that binds nuclear proteins from kidney, small intestine, colon, and liver. The transcriptional regulatory activities of this repeat in kidney and gut epithelial cell lineages have been defined using transgenic mice. The results indicate that the heptad sequence binds novel transcription factors that modulate gene expression within and between different epithelia.

EXPERIMENTAL PROCEDURES

Preparation of Nuclear Extracts

Nuclear extracts were prepared from tissues harvested from 6- to 20-week-old male or female FVB/N mice. The entire extraction protocol was performed at 4 °C. The distal half of the small intestine was removed immediately after sacrifice, clamped at one end, infused with buffer A (10 mM HEPES, pH 7.6, 15 mM KCl, 2 mM MgCl2) supplemented with protease inhibitors (2 mM Pefabloc SC, 1 mM DTT) and desalted by passage through a Biospin 6GD column (Bio-Rad) equilibrated in buffer C. Ten-microliter aliquots of the column effluent were snap-frozen in liquid N2 and stored under liquid N2 until use. The protein content of each extract was determined using the Bio-Rad Protein Assay kit.

The integrity of nuclear proteins was verified using a gel mobility shift assay (GMSA) with a template consisting of an orphan steroid hormone receptor binding site. This previously characterized element (5'-TTCTTTGAGACTTTGAACTTCAACA-3') was derived from nucleotides −86 to −63 of the rat intestinal fatty acid-binding protein gene promoter (Fabpl) (2, 3) and formed complexes with nuclear extracts from all tissues.

Gel Mobility Shift Assays

Templates for GMSA were made by annealing two complementary single-stranded synthetic oligodeoxynucleotides (oligos). Oligos were end-labeled with 32P by using polynucleotide kinase (Boehringer Mannheim). Each gel shift reaction (total volume = 20 μl) contained 12.5 mM HEPES, pH 7.6, 40 mM KCl, 0.1 mM EDTA, 1 mM DTT, 7.5% glycerol (w/v), 32P-labeled probe (50 fmol), poly(d(CD)) (6 ng, Boehringer Mannheim), herring sperm DNA (6 ng, Sigma), and nuclear extract (50 μg–800 μg protein). Following a 15-min incubation at room temperature, the reaction was terminated by subjecting the mixture to polyacrylamide gel electrophoresis (5% of 29.1% acrylamide/N,N'-methylene-bisacrylamide in 45 mM Tris borate buffer, pH 8.0, 1 mM EDTA). All GMSA assays were performed at least twice and with at least two independent preparations of tissue nuclear protein extracts.

Southwestern Blots

Nuclear extracts (80 μg of protein/tissue) were fractionated by electrophoresis through 7% polyacrylamide gels containing 0.1% SDS according to the protocol described in Ausubel et al. (4) with the following exceptions: DTT was omitted from the sample loading buffer and the samples were not heated. Proteins were electrophoretically transferred to supported nitrocellulose membranes (Life Technologies, Inc.). Triplicate blots were preincubated for 30 min at 4 °C in 5% Blotto (25 mM HEPES, pH 7.6, 60 mM KCl, 1 mM DTT, 1.5 mM EDTA, 0.5 μg/ml sheared herring sperm DNA, and 5% nonfat dry milk (w/v)) followed by a 60-min incubation at the same temperature in 0.5% Blotto. Each blot was probed with one of three concatenated 32P-labeled double-stranded oligos: (i) 5'-ACAAACATTCTTGCTTGGCCATCTTGTATTTTATGC-3' (nucleotides −167 to −133 of rat Fabpl); (ii) 5'-ACAAACAGGGGTGCGCCAGGAGTATGTTATGC-3' (identical to (i) but with mutated bases indicated in lowercase); and (iii) 5'-ATGGTTGAGTTGTTGAGTTTGTACAGACACGCTAAACG-3' (nucleotides −1319 to −1285 of the rat matrix metalloproteinase 2 gene, Ref. 5). Concatenation was accomplished using the Rapid Ligation kit from Boehringer Mannheim and 40 pmol of the double-stranded oligo per 20-μl reaction. The specific activity of each double-stranded oligo was identical (6000 Ci/mmol). The entire reaction mixture was added to 5 ml of hybridization buffer (0.5% Blotto). Following an overnight incubation with the concatenated probe at 4 °C, blots were washed in 0.5% Blotto for 5 min and then in a solution containing 25 mM HEPES, pH 7.6, 60 mM KCl, 1 mM DTT, and 1 mM EDTA (3 washes of 5 min, each wash done at 4 °C). DNA-protein complexes were visualized using a storage PhosphorImaging system (Molecular Dynamics).

Generation of Transgenic Mice

Construction of Fabpl/hGH Fusion Genes—Three recombinant DNAs were produced. Fabpl5'−596/hGH was created as follows. A BamHI/EcoRI fragment spanning nucleotides −596 to +21 of Fabpl was ligated to RelaxFab (6) and ligated to BamHI/EcoRI-digested pBluescript II SK+ (Stratagene), yielding pTS9. Nucleotides +3 to +2150 of hGH were removed from pBluescript (1) with BamHI and ligated to BamHI-digested pTS9. pTS10 was identified by Southern analysis using the probe DNA with the 5'-end of the hGH gene joined to the 3'-end of the Fabpl sequence. Two pairs of complementary synthetic oligos were phosphorylated and annealed to one another.

5'-CGATTTAGAAAAACACTGCTGGCCATCTCAGTATTTTACGTGTA-3' − 3'ATTATCGTTTTAAGACGGAGCCAGGTTAACTGACTCGAAAATAGCAAT-GAACAAAACCTTGTGCATGCACATCTGATTTTATGACTGTA-3' − 3'AGTGGGTTTCTGACAAGTTCA-3'

Oligo 1

5'-ACAAACATTCTTGCTTGGCCATCTTGTATTTTATGC-3'

Oligo 2

Double-stranded oligo 1 was synthesized so that one end would be compatible for ligation to CslI-digested DNA and the other end would contain a 10-base overhang compatible for ligation with double-stranded oligo 2. Oligo 2 was synthesized so that one end would be compatible for ligation with oligo 1 and the other end compatible for ligation with EcoRI-digested DNA. These two double-stranded oligos each contain two copies of Fabpl nucleotides −172 to −133. Each copy, in turn, contains two copies of the heptad sequence underlined above. Both oligos were simultaneously ligated to ClaI/EcoRI-digested pTS10, producing pTS5.
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pt55 contains nucleotides −596 to +21 of Fabpl with four tandem repeats of nucleotides −172 to −133 added at Fabpl nucleotide −596 (Fabpl<sup>−596</sup>). Sequence analysis verified this arrangement. Fabpl<sup>−596</sup>−gHGH was liberated from pt55 by SaliI/XmnI digestion.

A similar strategy was used to create a second fusion gene, Fabpl<sup>−132</sup>−gHGH, which contains four copies of the same sequence as in Fabpl<sup>−596</sup>−gHGH (i.e. nucleotides −172 to −133) but inserted at Fabpl nucleotide −132.

5′-AAATTAGAACAAACCTCGCCATGCATTTATCCTGTAAG-3′; TTATACCTTGTTGAGACCGCAGGTTAGCATAACAAAAATAGCAAACATCCCTGCATTTATCCTGTAAG-3′; TGGTTGGAGACCGCAGGTTAGCATAACAAAAATAGCAAACATCCCTGCATTTATCCTGTAAG-3′

**Oligo 3**

5′-ACAAACACTCTCCTGCATTTATCCTGTAAG-3′

**Oligo 4**

Double-stranded oligo 3 was synthesized so that one end would be compatible for ligation to BstBI-digested DNA and the other end would contain a 10-base overhang compatible for ligation to double-stranded oligo 4. Double-stranded oligo 4 was synthesized so that one end would be compatible for ligation to oligo 3 and the other end to Bst BI-digested DNA. Oligos 3 and 4 and BstBI-digested pt510 were incubated with T4 DNA ligase, yielding pt54 (Fabpl<sup>−132</sup>−gHGH). Sequence analysis verified the correct positioning of the tandem repeats. Fabpl<sup>−132</sup>−gHGH was then liberated from pt54 by vector sequences by EcoRI/XmnI digestion.

The same strategy used to create Fabpl<sup>−596</sup>−gHGH was exploited to generate the third fusion gene, Fabpl<sup>−132</sup>−gHGH mutated at −132−gHGH, which contains nucleotides −596 to +21 of Fabpl with four tandem repeats added at Fabpl nucleotide −132. These repeats were mutated copies of nucleotides −172 to −133, where each purine in each heptad repeat was replaced with a pyrimidine from the opposite base pair and vice versa.

5′-AGAACAAACTTCTGCCTTGCCCATTCTGATTTTGATT-3′; TCTTGTTTGAAGACGGAACGGGTAGAAGCTAAA-5′

**Oligo 5**

5′-ACAAACACTCTCCTGCATTTATCCTGTAAG-3′

**Oligo 6**

The resulting plasmid containing Fabpl<sup>−132</sup>−gHGH was designated pt54 and its insert excised with EcoRI/XmnI.

DNA fragments containing the three fusion genes were each purified from their vector sequences by agarose gel electrophoresis, extracted from the gel using the Qiaex system (Qiagen), and then used for nucleofusion injection into fertilized FVB/N oocytes (7). Injected oocytes were transferred to pseudopregnant Swiss Webster mice using standard nuclear injection into fertilized FVB/N oocytes (7). Injected oocytes were maintained by crosses to non-transgenic FVB/N littermates. Serum gHGH levels were measured by radioimmunoassay (Nichols Diagnostic).

Pedigrees from transgenic founder animals were established and maintained by crosses to non-transgenic FVB/N littermates. Serum gHGH levels were measured by radioimmunoassay (Nichols Diagnostic). Pedigrees with high serum levels of gHGH (>1 μg/ml) had to be maintained by ovarian transplantation (9). All mice were housed in microisolators on a 12-h light/dark cycle and given an irradiated diet (Pico rodent chow 20, PMI Feeds) ad libitum. Mice were determined to be specific pathogen-free based on the results of screening sentinel animals for hepatitis, minute, lymphochromatiosis, ectromelia, polyoma, Sendai, pneumonia, and mouse adenosviruses, enteric pathogens, and parasites.

**Analyses of Transgene Expression**

Transgenic mice and their normal littermates were sacrificed between postnatal days 35 (P35) and 42. Some animals received an intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdUrd, 120 mg/kg body wt) and 5-fluoro-2′-deoxyuridine (12 mg/kg) 90 min prior to their death.

Immediately after sacrifice, several tissue samples were recovered. The small intestine was divided into quadrants. A 2-cm segment was taken from the center of each quadrant and rapidly frozen in liquid nitrogen for subsequent RNA isolation. The colon was divided in half and a 2-cm segment was taken from the centers of the proximal and distal halves, also for isolation of RNA. The stomach was divided in half along its greater and lesser curvatures. One-half was used for RNA extraction. A wedge of liver and one-half of one kidney were also used to prepare RNA. The remainder of these tissues, plus portions of heart, lung, spleen, pancreas, skin, white adipose tissue, skeletal muscle, and brain were fixed in Bouin’s solution at room temperature for 8–18 h, rinsed in ethanol, and embedded in paraffin for immunohistochemical analysis.

**Quantitative RNA Hybridization Studies**—Total cellular RNA was isolated from frozen pulverized tissues using RNeasy columns (Qiagen). RNA samples were fractionated by denaturing formaldehyde agarose gel electrophoresis and transferred to nylon-1 membranes by capillary blotting (Life Technologies, Inc.). The membranes were probed with gHGH<sup>−172</sup>−gHGH labeled with <sup>32</sup>P using random primers (1). Each blot contained reference RNA samples containing known amounts of gHGH mRNA. The intensity of the hybridization signal from mouse tissue RNA sample was quantitated using a storage PhosphorImaging system. Blots were stripped and reprobed with a <sup>32</sup>P-labeled human glyceraldehyde-6-phosphate dehydrogenase cDNA (American Tissue Culture Collection). gHGH mRNA concentrations in tissue RNAs were calculated by comparing the sample signal to the signals from the RNA standards after normalization to glyceraldehyde-6-phosphate dehydrogenase mRNA levels.

**Single and Multi-label Immunohistochemistry**—Five-micron thick sections prepared from paraffin-embedded samples of stomach, small intestine, cecum, colon, liver, kidney, pancreas, spleen, white adipose tissue, skeletal muscle, brain, heart, and lung were incubated overnight at 4 °C with sheep anti-gHGH (Cortex Biochem; diluted 1:10,000) and rabbit anti-BrdUrd (1:1000) in blocking buffer (1% bovine serum albumin, 0.02% non-fat dry milk, 0.3% Triton X-100 in PBS). Bound antibodies were detected by using biotinylated donkey anti-sheep immunoglobulins (1:1000) and anti-biotin peroxidase conjugate (Vector Laboratories) for 30 min. Metal-enhanced 3′,3′-diaminobenzidine (DAB, Pierce Chemical) was used as the peroxidase substrate (incubation = 5 s at room temperature).

Sections were counterstained with hematoxylin and eosin.

Triple label fluorescent visualization of liver fatty acid-binding protein (FABP), gHGH, and BrdUrd in the intestinal epithelium was accomplished using the following multistep protocol: (i) bovine pancreas α-chymotrypsin (1 mg/ml in 0.1% CaCl<sub>2</sub>) for 15 min at 37 °C (antigen unmasking step); (ii) rabbit anti-FABP sera (1:1000). Ref. 6) overnight at 4 °C; (iii) normal donkey serum (1:50, Jackson ImmunoResearch) for 1 h at room temperature; (iv) Cy3-conjugated goat anti-rabbit immunoglobulin (1:500, Jackson ImmunoResearch) for 1 h at room temperature; (v) sheep anti-gHGH (1:1000) overnight at 4 °C; (vi) Cy3-donkey anti-sheep Ig (1:500, Jackson ImmunoResearch) for 1 h at room temperature; (vii) normal goat serum (1:50, Jackson ImmunoResearch) for 1 h at room temperature; (viii) goat anti-BrdUrd (10); 1:1000, biotinylated using a kit from Boehringer Mannheim) overnight at 4 °C;
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Fabpl Contains a Direct Heptad Repeat That Binds Proteins Present in Kidney Nuclear Extracts—Gel mobility shift assays (GMSA) were performed to identify transcription factor binding sites that mediate suppression of Fabpl in renal proximal tubular epithelial cells. Nuclear extracts, prepared from adult FVB/N mouse kidneys, formed a prominent complex with a double-stranded oligo spanning nucleotides 187 to 123 of rat Fabpl (Fig. 1A plus Fig. 1B, lane 5). A 64-fold molar excess of unlabeled double-stranded template markedly reduced the amount of labeled complex formed (Fig. 1B, lanes 2–5).

A series of shorter double-stranded oligos, derived from nucleotides 187 to 123, were used as templates and competitors in subsequent gel mobility shift assays. The results, summarized in Fig. 1, A–C, revealed that nucleotides 167 to 133 encompassed the nuclear factor binding site(s). This 35-base sequence contains a direct repeat, 5′-TTCTGNNNTT-3′, separated by a 5-nucleotide “spacer”:

\[-161 \quad 139\]

\[\text{TTCGCTTCGCCCATTCCGATT}\]

Sequence 1

A sequence centered on the direct repeat with six extra bases at both ends (nucleotides 167 to 133) was as effective a competitor as the unlabeled 177 to 133 template itself (Fig. 1C, lanes 3 and 8).

Three prominent complexes formed when gel mobility shift assays were performed with kidney nuclear extracts and nucleotides 167 to 133 (designated A, B, and C in lane 2 of Fig. 2A). Complex formation was abolished by a 128-fold molar excess of the unlabeled double-stranded template (Fig. 2A, lane 3).

To test whether the heptad repeats contained within nucleotides 161 and 139 were required for binding kidney nuclear proteins, competition assays were performed with mutated oligos. The mutations involved replacement of purines in each heptad with a pyrimidine from the opposite base pair and vice versa. When all purines in both repeats were mutated (oligo 13 in Fig. 2B) the ability to effectively compete with the wild type template was lost (Fig. 2A, lane 5). In addition, the “completely” mutated oligo 13 was unable to function as a template for binding, i.e. neither complex A, B, or C was observed when radiolabeled double-stranded oligo 13 was incubated with kidney nuclear proteins (data not shown). An oligo with the TTCTG component of

![Fig. 1. Nucleotides 167 to 133 of Fabpl contain elements that bind kidney nuclear proteins.](http://www.jbc.org/)

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component of the heptads is important for assembly of the B and C complexes.

Oligos with either the upstream or the downstream heptad repeat mutated were not effective competitors for complex formation with the native –167 to –133 template nor were they able, when radiolabeled, to form detectable complexes with kidney nuclear proteins (data not shown). To further test the hypothesis that both heptads are necessary for complex formation under the conditions of the GMSA, we attempted to block formation of these complexes with a 128-fold molar excess of an oligo spanning the upstream heptad with five extra bases at both ends (sequence 16 in Fig. 2B) and an oligo encompassing the downstream heptad with five extra bases at both ends (sequence 17). Neither sequence alone nor both oligos together were effective competitors (lanes 8–10 in Fig. 2A).

Kidney nuclear proteins were fractionated by SDS-polyacrylamide gel electrophoresis. Nitrocellulose blots of the gels were probed with double-stranded oligos containing both heptads (nucleotides –167 to –133; sequence 11) or with both heptads mutated (sequence 13). A prominent ~90-kDa reactive protein was seen when blots were probed with the wild type sequence (Fig. 3A). Binding to this protein was markedly reduced with the mutant oligo (Fig. 3B). This Southwestern blotting result provides further evidence that kidney nuclear protein(s) specifically bind to the heptad repeats.

Kidney nuclear extracts were also subjected to Superdex 200 gel filtration chromatography using nondenaturing conditions. Column fractions were analyzed by GMSA employing radiolabeled double-stranded oligo 11 as the template. Complex forming activity was restricted to fractions in two size classes. Nuclear proteins that eluted with a estimated size of 70,000 formed single complexes with mobilities equivalent to that of “C.” Proteins that eluted in the size range of 200,000–240,000 formed complexes B and C (data not shown).

An Enhancer Element in the Matrix Metalloproteinase-2 Gene with Homology to the Fabpl Heptad Repeat Forms Similar Complexes with Kidney Nuclear Proteins—The TRANSFAC database (12) was used to search nucleotides –167 to –133 of Fabpl for known transcription factor binding sites. No significant similarities were detected. However, significant sequence homology was noted between the heptad repeat and an enhancer element in the rat matrix metalloproteinase-2 gene (MMP-2). MMP-2 is normally expressed at high levels in the developing mouse kidney and lung (13). In adult animals, expression is markedly repressed in these tissues. Using cultured glomerular mesangial and epithelial cells, Harendza et al. (5) identified an enhancer spanning nucleotides –1322 to –1282 of MMP-2. Fig. 4A shows the homology between this enhancer and Fabpl’s heptad repeats.

An oligo representing nucleotides –1319 to –1285 of rat MMP-2 competes for complex formation with nucleotides –167 to –133 of rat Fabpl as effectively as the Fabpl sequence itself (compare lanes 3 and 4 in Fig. 4B). In addition, the MMP-2 sequence forms complexes with nuclear proteins prepared from adult FVB/N kidney (lane 7). These complexes have mobilities indistinguishable from those formed with nucleotides –167 to –133 of Fabpl (compare lanes 2 and 7 in Fig. 4B). Both the MMP-2 sequence and the Fabpl sequence compete for complex formation with the radiolabeled MMP-2 template (lanes 8 and 9). Finally, the oligo with the mutated heptad repeats (sequence 13 in Fig. 2B) is not as effective a competitor for complex formation between kidney nuclear proteins and the MMP-2 template as the oligo with the authentic Fabpl heptad repeats (compare lanes 9 and 10 in Fig. 4B).

The MMP-2 element reacts with two prominent proteins in Southwestern blots of kidney nuclear proteins: one has the
Fig. 3. Southwestern blots of tissue nuclear extracts reveal that the heptad repeats mediate binding to a 90-kDa protein. Nuclear extracts from the indicated tissues (80 µg of protein/lane) were fractionated by electrophoresis through 7% polyacrylamide gels containing 0.1% SDS. Nitrocellulose blots of triplicate gels were probed with 32P-labeled double-stranded oligo 11 (Fabpl nucleotides 167 to 133 containing both heptad repeats; A), with oligo 13 (all bases in each heptad repeat mutated; B), or with an oligo encompassing nucleotides 1319 to 1285 of the rat matrix metalloproteinase-2 gene (C).

Fig. 4. An enhancer element in the rat matrix metalloproteinase 2 gene has sequence similarities to the Fabpl heptad repeats and forms similar size complexes with mouse kidney nuclear proteins. A, alignment of the two sequences. B, GMSA using radiolabeled double-stranded oligos representing the sequences shown in A as templates. FVB/N kidney nuclear extracts, and 128-fold molar excesses of various unlabeled double-stranded oligos as competitors.

same mobility as the 90-kDa protein that binds nucleotides 167 to 133 of Fabpl and the other is larger (140 kDa; Fig. 3C). This result provides further evidence that the heptad repeats in Fabpl and the homologous MMP-2 enhancer sequence may interact with similar proteins.

Nuclear Extracts from Several Tissues Contain Proteins That Recognize the Heptad Repeat—Nuclear extracts were prepared from a variety of adult FVB/N mouse tissues and assayed for their ability to form complexes with nucleotides 167 to 133 of Fabpl. The specificity of complex formation was defined based on the ability of unlabeled template to compete for complex formation with tissue nuclear proteins and by the inability of oligo 13 (both heptads mutated) to function as a competitor. Complexes with similar mobilities to those obtained with kidney nuclear proteins (lanes 2–4 of Fig. 5) were generated with extracts prepared from liver (lanes 5–7) and the distal half of the small intestine (lanes 11–13). Specific complexes also formed with colonic and stomach nuclear proteins (lanes 8–10 and 14–16, respectively). The major colonic complex co-migrated with the major complex formed with kidney nuclear extracts (complex C). However, none of the complexes formed with stomach extracts co-migrated with those formed with kidney extracts. Thus, all the tissues that support Fabpl/hGH expression in adult FVB/N transgenic mice contain nuclear proteins that recognize the direct heptad repeats.

Southwestern blots of nuclear extracts prepared from these tissues indicated that the heptad repeats mediate binding to a protein in liver which has the same size as the reactive protein in kidney. No bands were detectable in small intestinal, colonic, or stomach extracts (Fig. 3, A and B). GMSAs performed using varying amounts of nuclear extracts disclosed that these other tissues had <10% of the complex forming activity/µg of protein as kidney extracts (data not shown).

None of the seven Fabpl/hGH transgenes that had been analyzed previously are expressed in heart or spleen nor are the endogenous rat and mouse genes transcribed in these tissues (1). Nonetheless, cardiac nuclear proteins form specific complexes with nucleotides 167 to 133 (lanes 17–19 in Fig. 5). The major complex generated in the GMSA had a mobility similar to the major complex (C) formed with kidney extracts (compare lanes 2 and 17). Specific complexes also formed with splenic nuclear extracts (lanes 20–22). The more slowly migrating splenic complexes had mobilities similar to those generated from kidney, liver, and distal small intestinal (ileo) nuclear proteins (complexes A and B). These findings indicate that nuclear factor(s) that bind to the heptad repeat are present in tissues that do not support expression of Fabpl or Fabpl/hGH transgenes.

In Vivo Analyses

The in vivo function of the protein binding site(s) encompassed by Fabpl nucleotides 167 to 133 were initially tested using two different fusion genes. Four tandem copies of this sequence plus a 5' spacer of five nucleotides (i.e. nucleotides 172 to 133) were added to the 5' end of Fabpl–596 to +21/hGH, creating Fabpl-4x at +596/hGH. In the other construct, four tandem copies were placed adjacent to nucleotide –132 of Fabpl in Fabpl–596 to +21/hGH (i.e. next to the "endogenous" 35-bp sequence spanning nucleotides 167 to 133). This construct was designated Fabpl-4x at –132/hGH. We reasoned that any differences in hGH expression between comparably aged FVB/N mice with Fabpl–596 to +21/hGH, Fabpl-4x at +596/hGH, and Fabpl-4x at –132/hGH transgenes should reveal the function(s) of the 35-bp element and the positional dependence of the function(s).
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The 35-Base Pair Element Can Function as a Renal Suppressor—Seven transgenic mice with Fabpl<sup>4x at −596</sup>/hGH were identified from 63 live born animals. Six of these seven founder animals expressed the transgene as determined by the presence of hGH in the serum and hGH mRNA in various tissues. All of the expressing founder animals were sterile due to high serum hGH levels (6.0–66.3 μg/ml). Lines were established by ovarian transplantation from the two female founders (G<sub>26</sub> and G<sub>27</sub>.

Blot hybridization analyses were performed using RNAs prepared from kidney, stomach, each quarter of the small intestine, the proximal and distal halves of the colon, and liver. Only two differences were noted between 6-week-old FVB/N Fabpl<sup>4x at −596</sup>/hGH mice and comparably aged Fabpl<sup>596 to +21</sup>/hGH animals. Kidney expression was suppressed 17-fold in mice containing 4 extra copies of the 35-bp element (reference standard = jejunal hGH mRNA levels). Colonic expression was slightly augmented (range = 117–314% of jejunal hGH mRNA levels in Fabpl<sup>596 to −314</sup>/hGH animals versus 64–81% in Fabpl<sup>596 to +21</sup>/hGH mice).

Multilabel immunohistochemical studies of 6-week-old mice from each pedigree revealed that the cellular patterns of Fabpl<sup>4x at −596</sup>/hGH expression in stomach, small intestine, colon, and liver were indistinguishable from the extensively characterized patterns of Fabpl<sup>596 to +21</sup>/hGH expression in similarly aged animals (see Refs. 1, 6, 14–17). Immunoreactive hGH was undetectable in any cell type in Fabpl<sup>4x at −596</sup>/hGH kidney using sensitive immunofluorescence detection methods (data not shown). In addition, Fabpl<sup>4x at −596</sup>/hGH was not expressed at detectable levels in heart, spleen, or any of the other tissues surveyed (brain, lung, pancreas, skeletal muscle, white adipose, and skin).

The Renal Suppressor Activity of the 35-Base Pair Element Is Position-dependent—We generated five FVB/N Fabpl<sup>4x at −132</sup>/hGH founders that expressed the transgene. Members of the three pedigrees established by ovarian transplantation exhibited identical patterns of transgene expression. Renal suppressor activity is retained when four extra copies of the 35-bp element are placed adjacent to the endogenous copy at −132. However, the suppression is less than when the copies are placed at −596: 3-fold versus >17-fold. Immunohistochemical studies indicated that renal suppression in Fabpl<sup>4x at −132</sup>/hGH mice is not associated with a change in cell type specificity; hGH is confined to proximal tubular epithelial cells (Fig. 6A), just as it is in Fabpl<sup>596 to +21</sup>/hGH animals.

The 35-Base Pair Element Can Function as a Suppressor of Gene Expression in Liver and Stomach—Moving the four tandem repeats of the 35-bp element from nucleotide −596 to −132 results in the acquisition of two “new” suppressor activities. Reporter expression is abolished in hepatocytes as assayed by measurements of hGH mRNA in liver RNA (Fig. 7) or by immunohistochemistry (data not shown). Expression is also silenced in the stomach: mucus-producing pit cells support expression of a variety of Fabpl<sup>596 to +21</sup>/reporter transgenes (17, 18), but immunoreactive hGH is not detectable in the pit cell or any other gastric epithelial lineage of Fabpl<sup>4x at −132</sup>/hGH animals (data not shown; cf. Fig. 7).

The 35-Base Pair Element Can Function as a Suppressor of Gene Expression in Proliferating and Differentiating Enteroctyes Depending upon Their Location Along the Duodenal-Ileal Axis of the Small Intestine—The adult mouse small intestinal epithelium undergoes continuous renewal (reviewed in Ref. 19). Cellular proliferation is confined to ~1 million flask-shaped mucosal invaginations known as crypts of Lieberkühn, several of which surround the base of each villus. Each crypt contains multipotent stem cells, located near its base. This stem cell gives rise to the four principal epithelial cell lineages of the small intestine. Enteroctyes (comprising >80% of the epithelial cells), goblet cells, and enteroendocrine cells differentiate as they undergo an orderly upward migration from a crypt to the tip of an adjacent villus. Cells are removed at the villus tip by apoptosis and/or exfoliation (20). The cycle is completed every 2–5 days (21–25). In contrast, Paneth cells, which elaborate anti-microbial peptides and growth factors, differentiate as they migrate to the base of the crypt where they reside for ~20 days (26, 27).

Throughout the length of the small intestine, Fabpl<sup>596 to +21</sup>/hGH and Fabpl<sup>4x at −596</sup>/hGH are active in proliferating and non-proliferating crypt epithelial cells (Fig. 6, B and C). Moving the 4 tandem copies of the 35-bp sequence to nucleotide −132 of Fabpl results in suppression of hGH expression in members of the enterocytic lineage. Light and electron microscopic immunohis-
tochemical studies of 6-week-old Fabpl<sup>4x</sup> at <sup>-132</sup>hGH mice indicated that in the very proximal portion of the small intestine (i.e. within 3 cm of the gastroduodenal junction), hGH is not detectable in proliferating and nonproliferating cells located in the upper half of the crypt or in most differentiated enterocytes, save a small subpopulation of scattered cells in the upper three-quarters of the villus (Figs. 6, D and E, and 8B). In contrast, hGH is expressed in each of the three other epithelial lineages: Paneth cells (Fig. 8G), rare enteroendocrine cells (Fig. 8C), and members of the goblet cell lineage. The latter include oligomucus (Fig. 8D), granule goblet, and common goblet cells (Fig. 8F) as well as “intermediate cells” (Fig. 8E) that have morphologic features demonstrating expression of hGH (green) throughout the crypt in both proliferating (blue) and nonproliferating epithelial cells. The open arrows point to the crypt-villus junction. D-I, the 35-bp element functions as a suppressor of gene expression in proliferating and differentiating enterocytes depending upon their location along the duodenal-ileal axis of a P42 Fabpl<sup>4x</sup> at -132 hGH mouse. D, crypt-villus units from the proximal duodenum (first 3 cm of the small intestine) stained with sheep anti-hGH (detected with peroxidase-conjugated donkey anti-sheep Ig and DAB). The section was counterstained with hematoxylin and eosin. Placement of four copies of the 35-bp element at nucleotide -132 silences hGH expression in all but a few scattered villus epithelial cells located in the upper two-thirds of these duodenal vili (e.g. open arrow). The closed arrowheads point to the crypt-villus junction. The closed arrow in the upper right corner of the panel points to a cluster of hGH-positive cells in the upper third of a villus sectioned perpendicular to its crypt-villus axis. The hGH-positive cells were identified as enteroocytes and goblet cells by EM immunohistochemistry (see Fig. 8, B and F). Although not detectable with light microscopic methods, EM immunohistochemistry established that hGH is also present in Paneth cells located at the base of all proximal duodenal crypts (see Fig. 8G). E, high power view of two proximal duodenal crypts stained with sheep anti-hGH, Cy3-donkey anti-sheep Ig, biotinylated goat anti-BrdUrd, and Cy5-conjugated donkey antihGH mouse. The section was incubated with rabbit anti-liver fatty acid binding protein (L-FABP; visualized with Cy5-conjugated donkey anti-rabbit Ig) and biotinylated goat anti-BrdUrd (visualized with Cy2-streptavidin). Rapidly dividing crypt cells are marked in S-phase by incorporation of BrdUrd and appear blue. The crypt-villus junction is indicated by open arrows. L-FABP (red-orange) is confined to post-mitotic villus-associated enterocytes and is not expressed in dividing or non-dividing crypt epithelial cells. hGH is detected in the supranuclear Golgi apparatus of villus enterocytes (yellow, e.g. closed arrows) and in BrdUrd-positive and -negative crypt cells (where hGH is seen as green staining material). C, high power confocal view of one of the crypts shown in B

**Fig. 6. Light microscopic immunohistochemical studies of the effect of the heptad repeat on cellular patterns of transgene expression.** A. section of kidney from a P42 Fabpl<sup>4x</sup> at -132 hGH mouse was incubated with sheep anti-hGH sera. Antigen-antibody complexes were visualized with peroxidase-conjugated donkey anti-sheep immunoglobulins and metal-enhanced DAB. The section was counterstained with hematoxylin and eosin. hGH present in epithelial cells of the proximal nephron appears red-brown. The arrows point to a glomerulus composed of hGH-negative cells. B. confocal micrograph of a section of crypt-villus units from the proximal jejunum of a P42 Fabpl<sup>4x</sup> at -132 hGH mouse. The section was incubated with rabbit anti-liver fatty acid binding protein (L-FABP; visualized with Cy3-conjugated donkey anti-rabbit Ig), sheep anti-hGH (detected with Cy3-conjugated anti-sheep Ig), and biotinylated goat anti-BrdUrd (visualized with Cy2-streptavidin). Rapidly dividing crypt cells are marked in S-phase by incorporation of BrdUrd and appear blue. The crypt-villus junction is indicated by open arrows. L-FABP (red-orange) is confined to post-mitotic villus-associated enterocytes and is not expressed in dividing or non-dividing crypt epithelial cells. hGH is detected in the supranuclear Golgi apparatus of villus enterocytes (yellow, e.g. closed arrows) and in BrdUrd-positive and -negative crypt cells (where hGH is seen as green staining material). C, high power confocal view of one of the crypts shown in B...
FIG. 7. The 35-bp element can also function as a transcriptional activator throughout the colon and a transcriptional suppressor in liver and stomach. Total cellular RNA was prepared from F35-P42 Fabpl<sup>-596</sup> to -21<sup>hGH</sup> and Fabpl<sup>+</sup> at -132<sup>hGH</sup> mice. The small intestine was divided into quarters (designated duodenum (DU), proximal jejunum (PJ), distal jejunum (DJ), and ileum (IL)). The cecum (CE) was separated from the rest of the colon which, in turn, was divided into proximal and distal halves (abbreviated PC and DC, respectively). Other abbreviations include KID (kidney), LIV (liver), and ST (stomach). Steady state levels of hGH mRNA were determined as described under "Experimental Procedures." Values from individual animals belonging to each pedigree are plotted. Each animal in each pedigree is assigned a symbol. The average value in each tissue for all mice in a given pedigree is indicated by the height of the bar. hGH mRNA levels in the various tissue RNAs prepared from each animal are expressed as a percentage of the steady state concentration in that animal’s proximal jejunal RNA.

 intermediate between those of granule goblet and Paneth cells. There is gradient of escape from this enterocyte-specific suppression along the duodenal-ileal axis. Proceeding distally through the next 25% of the small intestine of Fabpl<sup>1</sup> at -132<sup>hGH</sup> mice, the percentage of villus enterocytes that are hGH-positive increases. In addition, their distribution expands to include the lower quarter of the villus (Fig. 6F). By the midpoint of the small intestine, hGH production has generalized to hypothesized (28) to represent Paneth cells undergoing transformation to goblet cells, goblet cells undergoing transformation to Paneth cells, or a bi-potential precursor of both lineages.

<sup>2</sup> Approximately 10% of goblet cells in the normal adult mouse intestine contain very small electron dense cores within their mucin granules. As these “granule goblet cells” move up villi and differentiate, they secrete their dense core mucin granules which are then replaced by "common" mucin granules that lack electron-dense cores (21). "Intermediate" cells are rare (28). Their granules contain electron-dense cores intermediate in size between those in granule goblet cells and those in the apical granules of young Paneth cells. Intermediate cell granules contain mucins as well as several gene products produced in mature Paneth cells (E. M. Garabedian, L. J. J. Roberts, and J. I. Gordon, manuscript submitted for publication). Intermediate cells have been hypothesized (28) to represent Paneth cells undergoing transformation to goblet cells, goblet cells undergoing transformation to Paneth cells, or a bi-potential precursor of both lineages.
Regulating Gene Expression in Kidney and Gut Epithelia

| transgene          | kidney (tubular epithelium) | liver (hepatocytes) | stomach (pit cell lineage) | small intestine | cecum | colon |
|--------------------|-----------------------------|---------------------|-----------------------------|-----------------|------|------|
| Fabpl596 to 92 hGH | +                           | +                   | +                           | proximal 0%     | 25% | 50% |
| Fabpl4x at 596 hGH | -                           | +                   | +                           | 75%             |     |     |
| Fabpl4x at 102 hGH | -                           | -                   | -                           | distal 100%     |     |     |
| Fabpl4x mutated at 132 hGH | + | + | + | proximal 0% | 25% | 50% |

Fig. 9. Summary of the position-dependent transcriptional suppressor and activator activities mediated by the heptad repeat in Fabpl. Note that the proximal to distal variations in Fabpl4x at 132/hGH expression shown within the small intestinal epithelium refer to the enterocytic lineage. The transgene is expressed in goblet, enteroendocrine, and Paneth cells throughout the duodenal-ileal axis. The other three transgenes are expressed in all four small intestinal epithelial cell lineages positioned in the proximal, middle, and distal small intestine.

all villus enterocytes as well as nonproliferating cells in the uppermost portion of the crypt (Fig. 6, G and H). EM immunohistochemical studies disclosed that the transgene remains active in the goblet and Paneth cell lineages, just as it is in the proximal small intestine (data not shown).

In the distal half of the small intestine, there is a progressive increase in hGH expression in proliferating and nonproliferating cells located in the crypt. The more distal the location of a crypt, the deeper the cellular location of hGH expression within that crypt. In the terminal 10% of the small intestine, hGH is expressed in the putative stem cell zone positioned five cell layers above the crypt base (Fig. 6H) and in differentiating and differentiated members of all four lineages distributed along the length of the crypt-villus axis (Fig. 6I).

The 35-Base Pair Element Can Function as an Activator of Gene Expression throughout the Colonic Epithelium—The colon lacks villi and Paneth cells. Multipotent stem cells, thought to be located at the crypt base (29), give rise to three epithelial cell types, enterocytes (colonocytes), goblet cells, and enteroendocrine cells. Differentiation occurs as cells migrate up each colonic crypt and onto a hexagonal-shaped surface epithelial cuff surrounding the crypt orifice.

Moving the 4 tandem repeats of the 35-bp sequence to −132 does not change the pattern of hGH expression in the cecum or proximal half of the colon. The reporter is present in all crypt epithelial cells, as judged by light or EM immunohistochemistry (e.g. Fig. 6J). However, moving the elements to −132 produces a marked activation of transgene expression from the mid-crypt to the rectum. Greater than 99% of crypts in the mid- and distal thirds of the colon are hGH-positive (Fig. 6K). These hGH-positive crypts extend to the rectum (Fig. 6L). All proliferating and nonproliferating cells in distal colonic crypts appear to support transgene expression, whether judged by light or EM immunohistochemistry. This includes enterocytes (colonocytes) and goblet cells, as well as cells located at the presumptive stem cell zone at the crypt base. Expression is sustained as cells migrate upward to the surface epithelial cuff surrounding the crypt orifice.

RNA hybridization studies established that steady state levels of hGH mRNA were 2–3-fold higher in the distal compared with proximal colon of Fabpl4x at 132/hGH mice (Fig. 7).

The Suppressor and Activator Activities of the 35-Base Pair Element, Revealed by Placement at Nucleotide −132, Are Dependent upon the Heptad Repeat—Mice with an additional transgene (Fabpl4x mutated at −132/hGH) were generated to determine whether the position-dependent suppressor and activator activities of the 35-bp element were mediated by its heptad repeats. Fabpl4x mutated at −132/hGH is analogous to Fabpl4x at −133/hGH, except each of its 4 copies of the 35-bp element contains the seven nucleotide substitutions per heptad that abolished complex formation in the gel mobility shift assay (see sequence 13 in Fig. 2B). Seven founder Fabpl4x mutated at −132/hGH transgenic mice that contained ≥50 ng of hGH/ml serum were identified among the 96 live born animals screened. All of these founders plus offspring derived from two of them were analyzed. Immunohistochemical studies revealed that all animals had similar phenotypes.

Mutation of the heptad repeat abolished the effects of placing 4 copies of the 35-bp element at −132. Suppression in hepatocytes and in the pit cell lineage of the stomach was lost; P35-P42 mice with Fabpl4x mutated at −132/hGH had detectable levels of immunoreactive hGH in these cell types (data not shown). Suppression of transgene expression was also lost in crypts located in the proximal half of the small intestine; proliferating and nonproliferating epithelial cells distributed throughout the crypt were hGH-positive (Fig. 6M). Finally, transgene expression was no longer detectable in crypts located in the distal half of the colon (Fig. 6N).

As with Fabpl4x at −132/hGH, Fabpl4x mutated at −132/hGH is not expressed in heart, spleen, brain, lung, pancreas, skeletal muscle, white adipose, or skin.

DISCUSSION

Fig. 9 summarizes the effects of manipulating the position and sequence of the heptad repeat on transgene expression. The pleiotropic activities mediated by the heptad repeat were determined by comparing the pattern of expression of three Fabpl/hGH transgenes containing (i) nucleotides −596 to +21 of Fabpl; (ii) nucleotides −596 to +21 but with four additional copies of its nucleotides −172 to −133 (containing the direct heptad repeats) placed at −132; and (iii) a sequence identical to (ii) but with all heptad repeats mutated within each of the four additional copies of nucleotides −172 to −133 (the “endogenous” copy was not mutated). The suppressor and activator activities were revealed when four additional copies of nucleo-
tides –172 to –133 were added at nucleotide –132 of Fabp1.596 to +21. When the heptad repeats in these four additional copies were mutated, the pattern of expression was indistinguishable from that of the “parental” Fabp1.596 to +21 sequence without any inserts. These results allowed us to conclude that the suppressor and activator activities were dependent upon the interactions of trans-acting factors with the heptad repeat, rather than to a change in the relative spacing of cis-acting elements within –596 to +21 due to insertion of an additional 160 bp of DNA.

Comparison of the pattern of expression of another transgene containing four additional copies of nucleotides –172 to –133 just 5’ to nucleotides –596 to +21 (Fabp1<sup>4+</sup> at –596) with the pattern of expression produced by Fabp1<sup>4-</sup> at –132 allowed us to conclude that renal suppressor and colonic activation activities can be still be expressed even when these elements are moved >400 bp upstream.

The function of the heptad repeat illustrates a central theme of gene regulation within the rapidly self-renewing small intestinal epithelium: controlling expression in a given epithelial lineage as a function of its state of differentiation and location along the duodenal-ileal axis. The endogenous Fabp1 gene is turned on just as members of the enterocytic lineage exit the crypt. Expression is sustained as cells complete their migration up the villus to the apical extrusion zone. The heptad repeat in Fabp1 acts as an enterocyte lineage-specific suppressor. Remarkably, this suppressor activity is expressed at different points in the lineage’s differentiation program depending upon the location of enterocytes along the duodenal-ileal axis. In the proximal small intestine, the heptad suppresses expression in proliferating undifferentiated, differentiating, and fully differentiated enterocytes. As one travels distally toward the ileum, there is a smooth gradient of escape from this suppression that progresses from mature enterocytes in the upper villus to involve successively less differentiated cells located closer and closer to the stem cell zone in the crypt.

The function of the heptad repeat is distinctive among previously characterized cis-acting elements that regulate gene expression in the small intestinal epithelium. For example, functional mapping studies of cis-acting sequences in other members of the Fabp1 gene family conducted in transgenic mice have disclosed domains that regulate cell lineage-specific, differentiation-dependent, and cephalocaudal patterns of transcription (11, 32, 33). Like the heptad repeat, a 20-bp element in the homologous intestinal fatty acid-binding protein gene (Fabpi) functions as a differentiation-dependent suppressor of gene expression in the enterocytic lineage. However, the interaction of the 20-bp element with transcription factors causes uniform suppression of expression throughout a crypt, regardless of whether the crypt is located in the proximal, mid, or distal portions of the small intestine. This 20-bp Fabpi element has no obvious sequence similarities to the Fabp1 heptad repeat (or to other known transcription factor binding sites). Thus, the heptad repeat represents a novel target for identifying transcription factors that affect the interrelationship between differentiation and axial position in the intestinal epithelium. Although a number of transcription factors have been identified in the intestine (reviewed in Ref. 34), none have been assigned an in vivo role in regulating this interrelationship.

The ability of the heptad repeat to mediate suppression in the stomach, liver, and kidney, and activation in the colon, suggests that it also represents a target for identifying transcription factors that regulate gene expression between different epithelia. One candidate factor is a 90-kDa protein identified in Southwestern blotting experiments. Evidence supporting its candidacy includes the following: (i) an oligo containing the heptad repeats binds to a 90-kDa protein, whereas binding is markedly reduced when the heptad sequences are mutated; (ii) the 90-kDa protein also binds to an element in the matrix metalloproteinase-2 gene that functions as an enhancer in renal cells, shares sequence homology with the heptad, and generates similar-sized complexes in gel mobility shift assays as the Fabp1 heptads; and (iii) among the nuclear extracts prepared from tissues where the heptad repeat affects gene expression, the 90-kDa protein is most abundant in kidney nuclear extracts which also have the highest specific activity for complex formation in the GMSA. The heptad repeat bears no obvious sequence similarities to known transcription factor binding sites. Thus, the mediators of its in vivo activities are likely to be novel.

Finally, the various combinations of transcriptional regulatory elements present in the Fabpl<sub>BGH</sub> transgenes described above can now be used to deliver other proteins to different lineages and locations within the intestine. In particular, Fabp1<sup>4+</sup> at –132 can be exploited to deliver gene products to the entire colonic epithelium of transgenic animals. This provides an opportunity to design gain-of-function or loss-of-function experiments that test the effects of various factors on colonic epithelial biology or that create mouse models of human colonic pathology.

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