Regulation of the human biotin transporter \( hSMVT \) promoter by KLF-4 and AP-2: confirmation of promoter activity in vivo

Jack C. Reidling and Hamid M. Said
Veterans Affairs Medical Center, Long Beach; and University of California, Irvine, California

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Reidling JC, Said HM. Regulation of the human biotin transporter \( hSMVT \) promoter by KLF-4 and AP-2: confirmation of promoter activity in vivo. Am J Physiol Cell Physiol 292: C1305–C1312, 2007. First published November 29, 2006; doi:10.1152/ajpcell.00360.2006.—The mechanism of biotin uptake in human intestine has been well characterized and involves the human sodium-dependent multivitamin transporter (hSMVT), yet little is known about the molecular/transcriptional regulation of the system. Previous investigations cloned the 5’ regulatory region of the \( hSMVT \) gene and identified the minimal promoter. To expand these investigations, we compared activity of the \( hSMVT \) promoter in three human intestinal epithelial cell lines (NCM460, Caco-2, and HuTu-80) and contrasted a renal epithelial specific regulating promoter activity and confirmed activity of the cloned \( hSMVT \) promoter. To expand these investigations, we compared activity of the \( hSMVT \) promoter in vivo. In vitro studies demonstrated that all cell lines utilized the same minimal promoter region, and mutation of specific cis-regulatory elements [Kruppel-like factor 4 (KLF-4) and activator protein-2 (AP-2)] led to a decrease in promoter activity in all intestinal cell types but not in renal cells. Using electrophoretic mobility shift assays, we identified two specific DNA/protein complexes. Using oligonucleotide competition and antibody supershift analysis, we determined that KLF-4 and AP-2 were involved in forming the complexes. In HEK-293 cells, overexpressing KLF-4 increased the endogenous \( hSMVT \) message levels threefold and activated a cotransfected \( hSMVT \) promoter-reporter construct. In vivo studies using \( hSMVT \) promoter-luciferase transgenic mice established physiological relevance and showed the pattern of \( hSMVT \) promoter expression to be similar to endogenous mouse SMVT mRNA expression. The results demonstrate, for the first time, the importance of KLF-4 and AP-2 in regulating the activity of the \( hSMVT \) promoter in the intestine and provide direct in vivo confirmation of \( hSMVT \) promoter activity.

The essential micronutrient biotin acts as a coenzyme catalyzing fundamental steps in multiple pathways involved in fatty acid biosynthesis, gluconeogenesis, and catabolism of several branched-chain amino acids and odd-chain fatty acids (5, 7, 38). Insufficient biotin levels have been shown to lead to clinical aberrations, including neurological disorders, growth retardation, and dermal abnormalities (5, 7, 38, 39). Certain causes of biotin deficiency include inborn errors of biotin metabolism (5, 7, 38), long-term therapy with anticonvulsant agents (13, 15, 16), or parenteral nutrition (14, 17, 19). In addition, suboptimal biotin levels have been reported during pregnancy (20), in a substantial number of alcoholics (6, 12), in patients with inflammatory bowel diseases (4, 39), and in infants with seborrheic dermatitis and Leiner’s disease (18, 21). Designing effective strategies to optimize biotin body homeostasis during conditions of biotin deficiency and suboptimal levels are of physiological and nutritional importance and may benefit from insightful studies into the molecular controls that participate in regulating body biotin levels.

Humans cannot synthesize biotin but acquire the vitamin from exogenous sources by absorption in the intestine (42). The mechanism of biotin uptake has been studied using a variety of intestinal preparations (for review see Ref. 28 and references therein) and has been shown to occur via a Na+-dependent, carrier-mediated mechanism functionally located at the apical brush-border membrane domain of the polarized intestinal epithelial cells (26, 29, 32–35). Two other functionally unrelated nutrients, the water-soluble vitamin pantothenic acid and the metabolically important antioxidant lipoate (27, 31), also utilize the intestinal biotin uptake system and have led to the naming of the transporter as the sodium-dependent multivitamin transport system (SMVT). The SMVT system has been cloned from a number of species, including the rat, rabbit, human, and, more recently, mouse (Refs. 10, 22, 40; GenBank accession no. AY572835). The cloned cDNAs from these species all share significant identity at both the nucleotide and the amino acid levels and encode proteins predicted to have 12 transmembrane domains, with both the amino and carboxy terminals being on the cytoplasmic side of the membrane, and multiple potential N-glycosylation sites. Other studies have determined the contribution of the human SMVT system (hSMVT) toward aggregate carrier-mediated biotin uptake in human intestinal epithelial cells. This was accomplished using specific small interfering RNAs (siRNA) as an approach for gene silencing of the hSMVT gene (3). The results showed the hSMVT to be the main (if not the only) biotin uptake system in the human intestine.

In contrast to our current knowledge of the mechanisms of the intestinal biotin uptake process and of the system involved, little is presently known about the molecular/transcriptional regulation of the SMVT system in the intestine. In preliminary investigations we cloned the human 5’-regulatory region of the \( hSMVT \) gene and determined the minimal region required for basal activity of the promoter (11). In the present study we sought to expand our knowledge in the area of transcriptional regulation of \( hSMVT \) expression in human intestinal epithelial cells. Thus we examined and compared the activity of the \( hSMVT \) promoter in three human intestinal and one renal epithelial cell line (NCM460, Caco-2, HuTu-80, and HEK-293, respectively), investigated the role of putative cis-elements in regulating promoter activity, and confirmed the activity of the cloned \( hSMVT \) promoter in vivo by using transgenic mice to...
establish physiological relevance. The results showed that the same hSMVT minimal promoter region is used in the human epithelial cell lines examined. The results also demonstrated that mutations of Kruppel-like factor 4 (KLF-4) or activator protein-2 (AP-2) sites in the minimal promoter region lead to a decrease in hSMVT promoter activity in all of the tested intestinal cell lines, but not in renal HEK-293 cells, and that the nuclear factors KLF-4 and AP-2 participate in forming DNA/protein complexes with the hSMVT promoter in intestinal cells. In support of our findings, HEK-293 cells transfected with a plasmid expressing KLF-4 increased the endogenous hSMVT message levels threefold and activated a cotransfected hSMVT minimal promoter-reporter construct. Furthermore, the results demonstrated the physiological relevance of our cloned hSMVT promoter in vivo in transgenic mice and showed that the hSMVT promoter-luciferase construct had a pattern of expression similar to that of the endogenous mouse SMVT.

**MATERIALS AND METHODS**

**Materials.** Restriction enzymes were purchased from New England Biolabs (Beverly, MA). DNA polymerase was purchased from Clontech (Palo Alto, CA). Routine biochemicals were all of molecular biology quality and were purchased from Fisher Scientific (Tustin, CA). Cell culture reagents were purchased from Sigma Chemical (St. Louis, MO) and Life Technologies (Rockville, MD). Fetal bovine serum (FBS) was obtained from Omega Scientific (Tarzana, CA). DNA oligonucleotide primers were purchased from Sigma Genosys (Woodlands, TX).

**Cell culture, transfection, and luciferase assay.** Generation of promoter-luciferase constructs was described previously (11). Four micrograms of each promoter-luciferase construct were transfected separately into either a human normal colonic (NCM460), colonic adenocarcinomic (Caco-2), duodenal carcinogenic (HuTu-80), or renal embryonic (HEK-293) cell line using the Lipofectamine 2000 reagent (Life Technologies) according to the manufacturer’s procedure. Our recent experience has shown that this transfection reagent yields more consistent transfection results for Caco-2 cells. Cell lines were maintained using previously published methods (11, 31). To normalize for transfection efficiency, the cells were cotransfected with 100 ng of pRL-TK (Promega, Madison, WI) plasmid along with the minimal promoter-luciferase construct. Cotransfection with KLF-4 was performed using 2 μg of a vector containing the human cDNA clone (Origene, Rockville, MD). For all transfections, total cell lysate was prepared from 2- to 5-day postconfluent monolayers of Caco-2 cells by using a standard published method (1). Five to ten micrograms of nuclear extract were incubated for 20 min at room temperature with [γ-32P]ATP end-labeled DNA fragments in a binding buffer consisting of 20 mM Tris-HCl (pH 7.5), 2 mM MgCl2, 1 mM EDTA, 50 mM NaCl, 0.1% Triton X, 6 μg of bovine serum albumin, 2 μg of poly(dI-dC), 0.5 mM dithiothreitol, and 10% glycerol. Competition analysis was performed with a 100-fold molar excess of commercial competitor consensus oligonucleotides (Santa Cruz Biotechnology, Santa Cruz, CA) or a 100-fold molar excess of unlabeled hSMVT DNA fragment (−4830 to −4732). For electrophoretic mobility shift assays (EMSA), the nuclear extract was pretreated with nonspecific IgG, KLF-4-, AP-2-, or erythroid Kruppel-like factor (EKL)-specific antibodies (Santa Cruz Biotechnology) for 15 min in binding buffer before the addition of labeled DNA. DNA/protein complexes were separated on 6% nondenaturing polyacrylamide gels in 1× Tris-acetate-EDTA (pH 8.5) at 30–35 mA. The gels were dried and exposed to film for autoradiography.

**Generation of transgenic mice.** We utilized the expertise of the transgenic mouse facility at the University of California Irvine (UCI-TMF) to provide us with founders carrying hSMVT promoter-luciferase constructs. The procedure utilizes the method of pronuclear DNA injection and is described briefly below. A 2,167-bp construct containing the 5′-regulatory region of the hSMVT gene (−5846 to −3679) fused to the luciferase reporter gene, described previously (11), was excised with the enzymes KpnI and SalI, gel purified, and provided to the UCI-TMF. Eggs from superovulating females were injected and implanted into foster mothers. Seventy-eight pups were born from implanted mothers 19–20 days after implantation, and tail tips were cut ∼14 days after birth and sent to us for genomic DNA extraction. We genotyped the pups by performing a PCR with specific primers for the hSMVT promoter (forward 5′-CTACTGAGTACGGAGCCTGGATCGC-3′ and luciferase gene (reverse 5′-GATTTAAAAATGCCTCTTCATGTG-3′) that would yield a 408-bp product. We reconfirmed these results by performing another PCR with the same forward primer designed from the promoter sequence and a different reverse primer from the luciferase gene sequence. Identical results were obtained (data not shown). The transgenic mice were mated with wild-type C57B1/6J littermates to ensure the transgene was passed to the F1 progeny and to establish transgenic hSMVT promoter-luciferase mouse colonies. The Institutional Animal Care and Use Committee (IACUC) at Long Beach Veterans Affairs as well as the IACUC at UCI approved the experimental procedures used for mice in this study.

**Tissue isolation, RNA, and luciferase analysis for transgenic mice.** Mice were euthanized, and specific tissues were immediately removed and split into either ice-cold TRIzol (Invitrogen) for RNA isolation or ice-cold passive lysis buffer (Promega) for luciferase assays. The
tissue was immediately lysed on ice using a PowerGen125 (Fisher, Pittsburgh, PA) hand blender and frozen at −80°C. RNA was isolated by following the manufacturer’s procedure (Invitrogen). The RNA was DNase treated, first-strand cDNA was made from 5 μg of the isolated total RNA primed with oligo(dT) using an Invitrogen SuperScript III synthesis system (Invitrogen), and quantitative real-time PCR (qPCR) was performed using the mouse SMVT-specific primers 5’-GGTGGGATCGGTGGTGTGGTGGGTT-3’ and 5’-CTTAGGTGATGGGCTCTTC-3’. The qPCR consisted of a 15-ns 95°C melt followed by 40 cycles of 95°C melt for 30 s, 58°C annealing for 30 s, and 72°C extension and data collection for 1 min. Melt curve analysis with plasmid DNA was performed for the generation of standard curves, and negative controls without RT were used with every reaction. To compare the relative relationship between mouse SMVT in various tissues, we used a calculation method provided by the iCycler manufacturer (Bio-Rad, Hercules, CA). The approach determines the relative relationship among various samples by first determining the threshold cycle (the number of cycles in the PCR that were required to achieve a specific level of product) for our gene of interest and a housekeeping gene in each sample (β-actin). All samples are then normalized to the housekeeping gene, and the lowest expressing sample gene is set at one. Luciferase assays were performed according to the manufacturer’s procedures and using a Turner Design TD-20/20 luminometer. Luciferase assays were normalized to total protein for each sample measured using the DC protein assay kit (Bio-Rad).

Statistical analysis. All statistical analysis in the present study utilized one-way ANOVA of the data points for each group, followed by range tests to compare which groups were significantly different. P values are provided for each study, with values of P < 0.05 considered statistically significant. Samples for qPCR were prepared from three separate individual animals. Each sample was then analyzed in at least three independent experiments.

RESULTS

Identification of hSMVT minimal promoter and activity in human intestinal epithelial cell lines NCM460, Caco-2, and HuTu-80 and in human renal epithelial cell line HEK-293. In a previous preliminary study we cloned the 5′-regulatory region of the hSMVT gene and identified two distinct promoters: promoter 1 (P1), included in sequence between −5846 and −4400, and promoter 2 (P2), included in the sequence between −4417 and −3679 (using the A in the initiator ATG as position +1) (11). In this investigation we sought to examine and compare the activity of the hSMVT promoter and determine the minimal region required for its basal activity in three human-derived intestinal and one renal epithelial cell line(s), namely, NCM460, Caco-2, HuTu-80, and HEK-293. Simultaneous transfection of these cells was performed using identical transfection methods and equal amounts of promoter-luciferase constructs. The results showed that all three intestinal epithelial cell lines had similar promoter activity for hSMVT P1 and P2 and that P1 is the more active promoter (Fig. 1A). Renal cells had lower promoter activity compared with intestinal cells, but again, P1 was the more active promoter (Fig. 1A). In addition, all cell lines appeared to utilize the same minimal promoter region for P1 (−4830 to −4603) and for P2 (−4417 to −3679) (Fig. 1, A and B).

Role of putative cis-regulatory elements on activity of the hSMVT minimal promoter: mutational analysis. Given that the hSMVT P1 is more active than P2 in all the human epithelial cell lines examined, we focused our subsequent investigations on the minimal region of this promoter. First, we performed a computational analysis of the minimal P1 to identify potential cis-regulatory elements using MatInspector version 2.2 (8). Our analysis led to the identification of several putative cis-regulatory sites that include three KLF-4 (gut) sites, an AP-2 site, a stimulating protein-1 (SP-1) site, and an EKLF site (Fig. 1B). To directly examine the role of these putative cis-regulatory sites, we mutated (see Fig. 1B for sites) independently the three KLF-4 sites (located at −4810, −4780, and −4760), the AP-2 site (located at −4773), and the EKLF site (located at −4746) and then examined the effect of these mutations on the activity of the minimal P1 promoter in all four cell types (see MATERIALS AND METHODS; note the KLF-4 site at −4780 overlaps with the SP-1 site, and thus the introduced mutation disrupts both sites). Our results showed that mutating the KLF-4 or the AP-2 sites decreased the promoter activity by ~50% compared with activity of the control (unmutated) minimal hSMVT promoter in intestinal epithelial cells but did not affect promoter activity in renal cells. For comparison, mutating the EKLF site (located at −4746) or introducing a mutation at a random site (at −4798) did not change the promoter activity (Fig. 2). Interestingly, when we performed simultaneous mutations in the KLF-4 and/or AP-2 sites, no further decrease in promoter activity was observed (data not shown).

Identification of a DNA/protein profile for the hSMVT promoter using EMSA. To further confirm the importance of the cis-elements found by mutational analysis to effect promoter activity and identify factors that interact with these elements, we performed EMSA with a specific labeled DNA probe containing the 98-bp region (−4830 to −4732) in the minimal hSMVT promoter by using nuclear extract isolated from Caco-2 cells. The typical mobility shift pattern that we observed for the DNA/protein complexes includes two major bands (Fig. 3, lane 2 with 2 bands indicated by numbers and arrows) that are competed away in the presence of unlabeled, 100-fold molar excess (−4830 to −4732) minimal hSMVT promoter DNA, thus showing specificity of the interaction (Fig. 3, lane 3).

Competition analysis of observed hSMVT DNA/protein interactions using EMSA. The specificity of the DNA/protein interactions was further established using EMSA and competition analysis with consensus oligonucleotides to specific cis-regulatory elements. When we added a 100-fold molar excess of unlabeled oligonucleotides specific for the consensus AP-2 site to the binding mixture, we observed a decrease in intensity of DNA/protein complexes 1 and 2 (Fig. 3, lane 4). However, oligonucleotides corresponding to consensus binding sites for SP-1 did not change the mobility shift pattern (Fig. 3, lane 5). Please note that KLF-4-specific oligonucleotides are not currently commercially available.

Confirmation of regulatory factor binding to cis-elements using EMSA and supershift analysis. To assess the identity of the proteins involved in the EMSA pattern, we used supershift analysis with specific antibodies to cis-regulatory elements. The addition of an antibody to either KLF-4 or AP-2α in separate reaction mixtures caused the appearance of new supershifted DNA/protein complexes (Fig. 4, lanes 2 and 3, respectively). The addition of an equal amount of either an antibody to SP-1 or nonspecific IgG did not cause a supershifted pattern (Fig. 4, lanes 4 and 5, respectively).

Increased hSMVT RNA levels and activation of promoter activity by overexpression of the human KLF-4 transcription factor in HEK-293 cells. Previous studies have suggested that KLF-4 is expressed at low levels in renal tissue (36); we
utilized the renal epithelial cell line HEK-293 to determine whether overexpression of the transcription factor could specifically activate hSMVT RNA expression and promoter activity. First, we transfected either a construct containing the human KLF-4 cDNA or an empty vector into HEK-293 cells, isolated the RNA, and then performed semiquantitative PCR and found that an increase in KLF-4 message levels led to a threefold increase in hSMVT message levels (Fig. 5A). For comparison, we also analyzed the KLF-4 and hSMVT message levels in intestinal epithelial Caco-2 cells and found that the endogenous KLF-4 message levels in Caco-2 cells were similar to the levels of the overexpressed KLF-4 in HEK-293 cells (Fig. 5A). When the KLF-4-containing vector was cotransfected with the minimal hSMVT promoter-luciferase construct, an increase in promoter activity occurred that was not observed with controls (Fig. 5B).
In vivo confirmation of hSMVT promoter activity in transgenic mice. To confirm the activity of the cloned hSMVT promoter in an in vivo setting, i.e., to establish physiological relevance of the in vitro findings, we generated transgenic mice expressing the full-length (H11002:5846 to H11002:3679) hSMVT promoter fused to the luciferase reporter and then examined promoter activity in the intestine and other mouse tissues (the UCI-TMF generated these mice). First, we employed PCR on isolated potential transgenic mouse genomic DNA to identify that four founder mice were carrying the hSMVT promoter-luciferase transgene (Fig. 6A). Next, we examined luciferase activity and mouse endogenous mRNA levels in different tissues of offspring from the founder hSMVT promoter-luciferase transgenic mice. As shown in Fig. 6B, the hSMVT promoter appears to be active in different mouse tissues. Interestingly, the pattern of expression of the hSMVT promoter-luciferase construct was found to parallel the pattern of expression of the mouse endogenous SMVT (Fig. 6, B and C). In the intestinal tract, the highest level of hSMVT promoter-luciferase activity was found to be in the duodenum and the colon, followed by lower levels in the jejunum and ileum (Fig. 6B). Expression of the hSMVT promoter-luciferase construct in other mouse tissues was found to be in the following order: heart > brain > kidney > liver.

DISCUSSION

Although the mechanism of biotin uptake in the human intestine has been well characterized in recent years, little is currently known about the molecular/transcriptional regulation of the system involved, i.e., the hSMVT. To expand our knowledge in the area, we sought in the present investigation to examine and compare the functionality of the full-length hSMVT promoter in three well-characterized human intestinal epithelial cell lines and a renal epithelial cell line: NCM460, Caco-2, HuTu-80, and HEK-293, respectively. We also investigated the possible role of putative cis-regulatory elements in promoter activity and confirmed the functionality of the full-length promoter in an in vivo setting of transgenic mice. Results of our in vitro investigations showed the activity of the hSMVT promoter to be similar in all the three human intestinal epithelial cell lines tested but lower in the renal cell line. In addition, P1 was found to be the more active hSMVT promoter (compared with P2) in all the cell lines examined. Furthermore, all tested human epithelial cells appeared to utilize the same minimal region (H11002:4830 to H11002:4603) for their basal promoter activity. These findings suggest that the observed activity of the hSMVT promoter in these cell lines is a reflection of the origin of these cells and raises the possibility that the same may apply in native human intestinal or renal epithelial cells.

Subjecting the identified minimal region of the hSMVT P1 required for basal activity to MatInspector analysis revealed the existence of a number of potential cis-regulatory elements, including multiple KLF-4 sites, an AP-2 site, a SP-1 site, and an EKLF site. To directly test for a possible role of these cis-elements in regulating the activity of the hSMVT promoter,
we performed mutational analysis of these sites. The results showed that when the KLF-4 and AP-2 sites in the hSMVT minimal promoter were mutated, a loss in promoter activity occurred in intestinal epithelial cells but not in renal cells. The observed decrease in hSMVT promoter activity was not seen when a SP-1, EKLF, or a random site was mutated. The observations suggest a specific role for KLF-4 and AP-2 in hSMVT promoter activation in the intestine. In addition, we utilized EMSA to establish a pattern of hSMVT promoter DNA/protein complex formation and, using oligonucleotide competition and supershift analysis, determined that the transcription factors KLF-4 and AP-2 participate in forming the complexes, thus bringing us to the conclusion that they directly interact with the hSMVT promoter. Our results show that KLF-4 and AP-2 play an important role in regulating the activity of the hSMVT promoter. Although we were able to show KLF-4 and AP-2 directly interacting with the hSMVT minimal promoter region, we must mention that we do not know if other factors contribute to the two major DNA/protein complexes formed in the EMSA. In fact, our results from mutational analysis also suggest that multiple KLF-4 factor binding occurs, and each of the three sites may contribute to minimal activity. The role of multiple KLF-4 sites and whether or not other factors bind the minimal promoter region require further investigation. To support our findings that KLF-4 plays a role in the activation of the hSMVT promoter, we utilized HEK-293 cells transfected with a plasmid expressing KLF-4. We chose HEK-293 cells because previous work had suggested that renal tissue has low levels of KLF-4 (36) and our current studies of hSMVT promoter activity and mutational analysis had suggested that endogenous KLF-4 in HEK-293 cells may not be at levels that activate the promoter. We found elevated endogenous hSMVT message levels and activation of a co-transfected hSMVT minimal promoter-reporter construct in HEK-293 cells overexpressing KLF-4, thus supporting our findings. We also found that the level of KLF-4 overexpressed in the renal cell line was similar to the endogenous level observed in the Caco-2 intestinal epithelial cell line. It is interesting that the pattern of expression of the SMVT and KLF-4 along the crypt-villous axis of the intestinal tract is similar, being higher in the villous compared with the crypt area (9, 43), and together with our current results, this suggests the possibility that KLF-4 may play a role in this expression pattern for SMVT.

Fig. 4. EMSA of the hSMVT minimal promoter with antibodies specific to transcriptional regulatory factors. Gel shift assays were conducted with a labeled −4830 to −4732 fragment of the hSMVT promoter, Caco-2 cell nuclear extract, and no antibodies (lane 1) or specific antibodies to KLF-4 (lane 2), AP-2α (lane 3), or SP-1 (lane 4). As a control, a reaction also was included with a nonspecific IgG (lane 5). Arrows and numbers at left indicate the 2 specific DNA/protein complexes; an arrow also indicates the supershifted bands.

Fig. 3. EMSA of the hSMVT minimal promoter and competition analysis. Gel shift assays were conducted with Caco-2 cell nuclear extracts and a labeled 98-bp region (−4830 to −4732) of the minimal hSMVT promoter as described in MATERIALS AND METHODS. The 2 DNA/protein complexes are numbered with arrows, and the free probe is indicated at bottom. Gel shift assays were conducted with labeled hSMVT promoter and 0 nuclear extract (lane 1) plus nuclear extract (lane 2) with a 100-fold molar excess of unlabeled promoter (−4830 to −4732) (lane 3) or with a 100-fold molar excess of unlabeled consensus oligonucleotides for AP-2 (lane 4) or SP-1 (lane 5).
The role of the P1 promoter may be in providing intestinal epithelial cells with basal levels of hSMVT for biotin transport under biotin sufficient conditions. KLF-4 and AP-2 may provide cells with the ability to increase or decrease promoter activity and thus alter levels of hSMVT when biotin levels are fluctuating. In support of this idea is our recent finding confirming that human intestinal biotin uptake is adaptively regulated and providing novel evidence that the upregulation is not mediated via changes in hSMVT RNA stability, but rather is due to transcriptional regulatory mechanism(s) that likely involve KLF-4 sites in the hSMVT promoter (23).

Our in vivo results using transgenic mice established the functional relevance of our in vitro data and showed significant activity of the hSMVT promoter. This is not surprising, since human and mouse SMVT promoters appear to have common features, including similar putative cis-regulatory sites (2 potential KLF-4 sites in the mouse 300 bp 5’ of exon 1) were found using the MatInspector program; the mouse promoter was determined from the sequence available in the database: NCBI accession no. AC109608). Furthermore, previous studies have established that a variety of human gene promoters are active in vivo using transgenic mice (2, 24, 37). Interestingly, the pattern of expression of the hSMVT promoter-luciferase transgene was found to be similar to that of the mouse endogenous SMVT message, especially in the different regions of the intestinal tract. The findings on the expression of the hSMVT promoter in the intestinal tract are in line with our previous functional observations showing a higher biotin uptake in the human duodenum compared with the jejunum and the ileum (35). The hSMVT expression pattern in the human intestine has not been described; however, our results for the hSMVT promoter transgene in mice correlate well with published data for moderate expression levels in the human kidney and liver and detectable levels in other tissues (40).

In summary, our data demonstrate, for the first time, the importance of KLF-4 and AP-2 in regulating the activity of the hSMVT promoter and provide direct in vivo confirmation of hSMVT promoter activity.

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