Regulatory Sequences of the Mouse Villin Gene That Efficiently Drive Transgenic Expression in Immature and Differentiated Epithelial Cells of Small and Large Intestines*

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Villin is an early marker of epithelial cells from the digestive and urogenital tracts. Indeed villin is expressed in the stem cells and the proliferative cells of the intestinal crypts. To investigate the underlying molecular mechanisms and particularly those responsible for the restricted tissue specificity, a large genomic region of the mouse villin gene has been analyzed. A 9-kilobase (kb) regulatory region of the mouse villin gene (harboring 3.5 kb upstream the transcription start site and 5.5 kb of the first intron) was able to promote transcription of the LacZ reporter gene in the small and large intestines of transgenic mice, in a transmissible manner, and thus efficiently directed subsequent β-galactosidase expression in epithelial cells along the entire crypt-villus axis. In the kidney, the transgene was also expressed in the epithelial cells of the proximal tubules but is likely sensitive to the site of integration. A construct lacking the first intron restricted β-galactosidase expression to the small intestine. Thus, the 9-kb genomic region contains the necessary cis-acting elements to recapitulate the tissue-specific expression pattern of the endogenous villin gene. Hence, these regulatory sequences can be used to target heterologous genes in immature and differentiated epithelial cells of the small and/or large intestinal mucosa.

Transgenic mice are routinely used to study the molecular and cellular basis of normal and pathological states in intestinal mucosa (1–5). The major limitation regarding the targeting of exogenous transgenes in this tissue is that the epithelium of the mouse intestinal mucosa is renewed every 2–5 days (6–8). The epithelial cells arise from multipotent stem cells functionally anchored at the base (more precisely in the lower third) of the proliferative compartment of the epithelium, the crypts of Lieberkühn. These crypts display a monoclonal organization because they are each derived from a single progenitor cell (9). Descendants of stem cells multiply in the middle portion of each crypt (10) and gradually differentiate into four principal cell types. In the small intestine, absorptive enterocytes (constituting >80% of the epithelial cells), mucus-producing goblet cells, and enteroendocrine cells migrate upward from the crypts to the apex of surrounding villi (whose colonic counterparts are hexagonal-shaped cuffs) (11), where they become apoptotic and are exfoliated into the gut lumen (12). In contrast, antimicrobial peptides secreting Paneth cells migrate to the bottom of the crypts, where they reside for about 20 days (13).

Given the remarkable protective effect of this epithelium, it is not surprising that most previous studies aiming to induce neoplastic transformation in intestinal mucosa of transgenic mice have failed (14, 15). In these reports, the use of promoter sequences that direct oncogenes in nonproliferating enterocytes located in the upper third of crypts produce only minor phenotypic abnormalities without tumorigenic consequences in the gut epithelium, suggesting that the residence time of these villus-associated cells may not be sufficient for the oncogenes to exert their effects. Furthermore this suggests that transgenic mouse models of neoplasia may require an efficient targeting of oncogenes in crypt stem cells or their immediate descendants.

Villin is a cytoskeletal protein that is mainly produced in epithelial cells that develop a brush border responsible for absorption as in the digestive apparatus (epithelial cells of the large and small intestines) and in the urogenital tract (epithelial cells of the kidney proximal tubules). Because it is expressed in the proliferative stem cells of the intestinal crypts (16, 17), it is believed to be an early marker for committed intestinal cells. The multiple levels of regulation control villin gene activity during mouse embryogenesis (18–20) and account for the strict pattern of tissue-specific expression observed in adults. Moreover, the expression of the villin gene in intestinal epithelial cells is conspicuously maintained in their corresponding carcinomas (21–24).

The specific expression pattern of villin suggests that it is an appropriate candidate for the characterization of regulatory sequences that could allow targeting of heterologous genes into a selected population of cells in the mouse digestive tract. With this goal in mind, the human villin gene has been isolated and characterized (25). A 2-kb1 5′-flanking region has been found to contain sufficient regulatory elements to promote tissue-specific expression of a reporter gene in intestinal and renal cell lines (26). In transgenic mice, this regulatory region is able to drive the expression of the human Ha-ras oncogene in the tissues in which the endogenous gene is actively transcribed. However, low levels of expression were observed that did not trigger malignant tissue appearance into the gut of these animals.2

These observations led us to further analyze an extended genomic region of the mouse villin gene with the goal of map-

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Villin Sequences Direct Intestinal Expression in Mice

6477

ping additional elements localized 5’ and/or 3’ and involved in promoting high levels of transgenic expression in the intestinal mucosa. Here we report the analysis of tissue-specific expression of the mouse villin gene using: (i) DNase I-hypersensitive sites assays, (ii) transient transfection assays, and (iii) transgenic mice.

**Proteinase & Gene Expression**

**Cell Culture and ex Vivo Transient Transfection—**Human colon carcinoma enterocyte-like CaCo2 cells, pig kidney proximal tubules derived LLC-PK1 cells, and distal tubules-derived Madin-Darby canine kidney cells were cultured as described (26). Cell DNA was isolated from 15 µL of Lipofectin reagent (Life Technologies, Inc.) with 5 µg each of β-galactosidase reporter plasmid construct and the control plasmid pRSVLuc, which contains the luciferase gene under the control of the Rous sarcoma virus promoter. Cells were harvested 48 h later, and cell extracts were assayed by chemiluminescent detection of both β-galactosidase (Galacto-Light, Tropix, Inc.) and luciferase (Luciferase Assay Kit, Tropix, Inc.) activities using a luminometer (Berthold).

**Plasmid Construction—**All constructs described were subcloned into the pBluescript II KS vector (Stratagene) with fragments isolated from the pA1 and pA2 plasmids cut with BglII fragment (480 bp) from the 3.5-kb region described above and in a plasmid containing the 3.5-kb region of the mouse villin gene (immediately 5’ to the 3.7-kb region). The pC1 and pA2 constructs were derived from the pA3 plasmid, respectively. Subcloning steps were confirmed by DNA sequencing.

**Transgenic Mice Generation—**The transgenes digested with Xhol-Ncol were injected into the pronuclei of the fertilized eggs of the B6/D2 mice in collaboration with the Service d’Expérimentation Animale et Transgénique, CNRS (Villejuif, France). Mice carrying transgenes were first identified by PCR screening of genomic DNA to confirm the presence of the gene and/or 3’ transgenic region described above from the Apol fragment (3.1 kb) and a BglII fragment (4.8 kb) derived from the 3.5-kb region described above from the Apol site in the pB3 plasmid, respectively. Subcloning steps were confirmed by DNA sequencing.

**Reverse Transcription-PCR Analysis—**Total RNA was isolated with SV Total RNA Isolation System (Promega). 20 ng of pd(N)6 random primer (Pharmacia) were hybridized with 2 µg of total RNA at 70°C for 10 min in distilled water. Reverse transcription with 200 units of Moloney murine leukemia virus reverse transcriptase (SuperScript II, Life Technologies, Inc.) was carried out at 37°C for 90 min in a 20-µl solution [First Strand Buffer (Life Technologies, Inc.), 10 mM dithiothreitol, 0.5 mM deoxynucleoside triphosphates, and 0.4 units/µl RNasin. 2 µl of the resulting cDNAs were amplified by PCR reaction in 50 µl for 40 cycles. Each cycle consisted of 60 s at 94°C, 60 s at 51°C (for transgene and villin) and 57°C (for TFIID), and 30 s at 72°C. For the transgene primers, 5’-CAACTCTCTGAATTCCTC-3’ coding strand and 5’-ATTCAGCTGTCGCCAACTGTGTT-3’ noncoding strand were used, generating a 250-bp product. For villin amplification 5’-CAAGTCG-3’ coding strand primer and 5’-GCAAGTCGCTG-3’ noncoding strand primer were used, generating a 473-bp product; for TFIID amplification 5’-CCAGGGACAACGTGTT-3’ coding strand primer and 5’-GCGTCATGCTACTGAGT-3’ noncoding strand primer were used, generating a 220-bp product. One-fifth of the PCR product was run on an ethidium bromide-containing agarose gel.

**Detection of β-Galactosidase Activity—**Cryosections (5 µm) from the tissues were fixed with 5% formaldehyde for 5 min and incubated in a staining solution that contained 0.4 mg of 5-bromo-4-chloro-3-indolyl-β-D-thiogalactopyranoside/ml, 4 mM potassium ferricyanide, 4 mM potassium ferrocyanide, 2 mM MgCl2 at 37°C for 8 h.

**RESULTS**

**Determination of the Transcription Start Site—**To determine the transcriptional start site of the mouse villin gene, total RNA was isolated from intestine and analyzed by primer extension assay using an oligonucleotide complementary to the mouse villin cDNA downstream of the ATG initiation codon. The efficiency of the reaction was confirmed by primer extension of the mouse intestinal fatty acid-binding protein gene (fabpi) from the same RNA preparation (27). Analysis of the fabpi extension product on a sequencing gel by comparison with a sequence ladder (Fig. 1A) revealed a strong signal band of a size of 51 bp as expected. The extension product of villin was 105 bp, indicating that the transcriptional start site (an adenine residue subsequently designed as nucleotide +1) was 57 nucleotides upstream of the translation initiation codon of the murine villin cDNA (Fig. 1B). Comparison of the genomic sequence encompassing 9 kb upstream from the ATG initiation codon with the cDNA sequence, positioned relative to ATG consensus sequences in the 9-kb genomic sequence (Fig. 1B), and determination of the transcription start site reveal that the mouse villin gene has one transcription start site that is separated from the ATG initiation codon by a 5.5-kb intronic region (Fig. 1C).

**DNase I-hypersensitive Sites in the Mouse Villin Gene—**To characterize the key regulatory regions involved in the specific control of villin expression, we have mapped the DNase I-hypersensitive sites (31) in the mouse villin gene (along a region...
An analysis was performed with mouse intestinal total RNA (30 μg) and with either the end-labeled villin oligonucleotide (generating a 105-nucleotide extension product) or the end-labeled mouse intestinal fatty acid-binding protein gene (fabb) oligonucleotide used as a positive control (generating an 81-nucleotide extension product). The sizes of the fragments obtained by primer extension are shown at the right. The unrelated sequence ladder that was run in the same gel is used as a size marker.

**Fig. 1.** Determination of the transcription start site of the mouse villin gene by primer extension. A, primer extension analysis was performed with mouse intestinal total RNA (30 μg) and with either the end-labeled villin oligonucleotide (generating a 105-nucleotide extension product) or the end-labeled mouse intestinal fatty acid-binding protein gene (fabb) oligonucleotide used as a positive control (generating an 81-nucleotide extension product). The sizes of the fragments obtained by primer extension are shown at the right. The unrelated sequence ladder that was run in the same gel is used as a size marker.

**Fig. 2.** A schematic representation of the organization of the 5′-flanking region of the murine villin gene. The open box represents the untranslated exon, and the shaded box represents the first coding exon. The sizes of the exon and the intron are indicated. Extending 9 kb upstream and 4.4 kb downstream from the translation initiation codon, as represented in Fig. 2A. The chromatin form of the mouse villin gene in different tissues (intestine, kidney, liver, and spleen) was submitted to limited DNase I digestion and subsequently digested with the appropriate restriction enzymes. Using BglII digestion and a 0.5-kb probe homologous 5′ of the 7.5-kb BglII fragment (Fig. 2B), two sets of DNase I incubation-related fragments were detected, migrating at 5.5 and 2.7 kb, and corresponding to hypersensitive sites designated as HS I (located at approximately +5.5 kb downstream from the transcription start (+1) site, just upstream from the ATG initiation codon) and HS II (located at approximately +3.5 kb downstream from the (+1) site), respectively. HS I was observed in nuclei isolated from intestine, kidney, and liver, whereas HS II was only present in intestinal tissue. No specific hypersensitive sites were detected in nuclei isolated from spleen. The presence and location of these hypersensitive bands were confirmed by hybridization with the 0.8-kb probe (Fig. 2A) homologous to the 3′ end of the 7.5-kb BglII fragment (data not shown). Using BamHI digestion and the 0.5-kb probe (Fig. 2C), five sets of DNase I-treated nuclear-related fragments were detected, migrating at 3.4, 4.3, and 4.7 kb and approximately 10 and 15 kb, corresponding to the hypersensitive sites HS II, HS III (located at −0.5 kb upstream from the (+1) site), HS IV (located at −1 kb upstream from the (+1) site), HS V (located at −10 kb upstream from the (+1) site), and HS VI (located at −15 kb upstream from the (+1) site), respectively. HS III was observed in nuclei isolated from both intestine and kidney, whereas HS IV was only present in intestinal tissue as HS II. The hypersensitive sites HS V and HS VI were only present in liver tissue (in which villin is weakly expressed) and were located far upstream from the transcription start site in regions (i) that have not been subcloned and (ii) that could belong to an adjacent gene; for these reasons, these hepatic-specific hypersensitive sites were not analyzed further. As for BglII digestion, no specific hypersensitive sites were detected in nuclei isolated from spleen. Using other independent restriction digestions (EcoRI and HindIII) and the 0.5, 0.8, and 1.25 kb probes (as represented in Fig. 2A), similar results were obtained (data not shown).

In conclusion, four major distinct DNase I-hypersensitive sites (HS I to HS IV) were shown to be present in the region extending from −1 kb to +5.5 kb in respect to the transcription start site (Fig. 3A) of the mouse villin gene. These sites were detected in intestine (HS I to HS IV), kidney (HS I and HS III), and liver (HS I), tissues in which villin is expressed, but they were not found in spleen, a tissue that does not produce villin. These findings correlate with the tissue-specific control of villin gene expression and suggest that the putative critical regulatory elements lie within these regions. HS II and HS IV were only detected in intestine and are probably associated with tissue-specific transcription factor-binding sites involved in the positive control of villin gene intestinal expression.

**Analysis of Promoter Activity by Transient Expression**—To test the effects of the segments containing the DNase I-hypersensitive sites (Fig. 3A) on transcriptional activity and to define more precisely the element(s) controlling villin gene expression in the intestine, segments were subcloned upstream of a promoterless LacZ plasmid (Fig. 3B). The construct pA1 contained all the subcloned regions downstream from the ATG initiation codon, encompassing the four DNase I-hypersensitive sites (HS I to HS IV) described above and the 5.5-kb intronic sequence, intron 1. Plasmids pA2 and pA3 were identical to pA1 except for the presence of intestine-specific hypersensitive site HS II and intron 1, respectively. Plasmid pB1 and plasmid pC1 were similar to plasmid pA1 but lacked the regions extending from −480 bp to −3.5 kb and −100 bp to −3.5 kb according to the transcription start site, respectively. Plasmid pC2 was identical to pA2 but lacked the region extending from −100 bp to −3.5 kb. Plasmids pB3 and pC3 were identical to pB1 and pC1 except for the presence of intron 1, respectively. The plasmid pD1 was identical to pA1 except for the presence of the transcription start site and the region extending upstream from this site. The plasmid pBasic, which does not contain a promoter or enhancer, and a pControl plasmid, which possesses the SV40 promoter, were also tested in each experiment.

Transient transfections with these recombinant plasmids were performed in CaCo2 and LLCPK1 cell lines, which express villin, and in Madin-Darby canine kidney epithelial cells, in which no villin expression is detected. Transcription from the villin promoter was measured by assaying β-galactosidase activity in extracts made from the transfected cells, and the results were expressed as fold induction over that of the promoterless vector, pBasic (Fig. 3C). High levels of β-galactosidase activity in the pControl transfected cell lines (CaCo2 cells, 50-fold over that of pBasic; LLCPK1 cells, 98-fold) demonstrated the presence of efficient general transcription/translational mechanisms in these cells (data not shown). Very low levels of β-galactosidase activity in pD1 both transfected cells compared with pBasic transfected cells showed that the transcription start site was necessary for an efficient specific transcription of the reporter gene and that nonspecific transcription was not initiated elsewhere in the villin regulatory sequences. The construct pA1 expressed the β-galactosidase gene at the highest level in CaCo2 cells (8-fold over pBasic) as compared with LLCPK1 cells (1.5-fold over pBasic), suggesting that the four DNase I-hypersensitive sites together with the first intron are necessary to efficiently promote transcription in cells of intestinal origin. Deletion of the fragment containing the intestinal-specific hypersensitive site HS II (pA2) dramatically
Villin Sequences Direct Intestinal Expression in Mice

Fig. 2. DNase I hypersensitivity in the mouse villin gene. A, a partial restriction map diagram of the mouse villin gene regions subcloned (−3.5 to +9.9 kb in respect to the transcription start site, indicated by an arrowhead). BamHI (B), BglII (Bg), EcoRI (E), and HindIII (H) restriction sites, ATG initiation codon, and the probes used to map the hypersensitive sites (0.5, 0.8, and 1.25 kb) are shown. B and C, intestine, kidney, liver, and spleen nuclei were digested with increasing amounts of DNase I at 0 °C for 10 min (0, 20, 40, 80, and 160 units). 10 μg of purified genomic DNA was digested with BglII (panel B) and BamHI (panel C), electrophoresed, and transferred to a nylon membrane. Hypersensitive sites were revealed by probing with a 32P-labeled fragment of 0.5 kb. Positions of coelectrophoresed molecular mass markers are indicated at the left, and the hypersensitive bands are marked by arrows at the right. The maps represented at the bottom of panels B and C show the position of restriction sites, the deduced DNase I-hypersensitive sites (indicated by arrows), and the 0.5-kb probe used.

decreased β-galactosidase expression in CaCo2 cells (2-fold over pBasic) to about 25% of that of pA1, demonstrating that a major element that confers intestinal activity was confined within this fragment. Similar results were obtained when the region upstream from the transcription start site (encompassing HS III and HS IV) was almost wholly deleted with or without HS II (pC1 and pC2, respectively). The deletion of the intronic region alone (pA3) or in combination with deleted sequences upstream from the transcription start site (pB3 and pC3 extend only from −480 and −100 bp, respectively) affected to a lesser extent β-galactosidase expression in the same intestinal cells (5.5-fold over pBasic), with a decrease to only about 65% of that of pA1, demonstrating that the regulatory elements that lay within 100 bp were sufficient to promote transcription in cultured cells. However, the level of β-galactosidase activity increased strongly when the plasmids pA3, pB3, and pC3 were transfected in LLCPK1 cells (10-, 44-, and 45-fold over pBasic, respectively), showing that the absence of the first intron, in combination with the lack of intestine-specific HS IV, was able to promote transcription in a kidney cell line. This would suggest that negative elements that confer repression in kidney transcription are confined in these elements.

To test specificity, the villin promoter-related constructs were transfected in Madin-Darby canine kidney cells, which do not express villin. After transfection, these cells showed only base-line levels of β-galactosidase activity when compared with pBasic-related activity (data not shown), demonstrating that the villin regulatory sequences were unable to promote efficient transcription in nonexpressing villin cells and that consequently the expression of the reporter gene in CaCo2 and LLCPK1 cells is specifically dependent upon these regulatory sequences. Taken together, these results from transient transfection of cultured cells demonstrate that (i) the mouse villin genomic sequence, extending from −3.5 to +5.5 kb, specifically directs an efficient level expression of the β-galactosidase reporter gene in intestine-derived cells, (ii) this level is dramatically reduced when the intronic intestine-specific hypersensitive site HS II or the region upstream from the (+1) site is deleted, (iii) lack of the entire first intron seems to partially restore the intestine-related ability to promote transcription, and (iv) lack of the entire first intron in combination with intestine-specific hypersensitive site HS IV is correlated with a strong increase of ability in promoting transcription in intestine-derived cells.

Analysis of Transgene Expression in Mice—Because the −3.5 to +5.5-kb region of the mouse villin contained the enteroctyte-like-specific promoter/enhancer activity in transient transfection assays, we examined the ability of this region to drive intestine-specific expression of the β-galactosidase reporter gene in transgenic mice. Five founder animals that contained the pA1 construct as a transgene were obtained. The founder mice were analyzed for mRNA reporter gene expression in several adult tissues by reverse transcription-PCR analysis. From the same cDNA samples, products encoding β-galactosidase, villin, and TFIID were analyzed. The PCR assays enabled the detection of spliced transcribed mRNA, excluding that from genomic DNA itself, by means of an exon connection.
Villin Sequences Direct Intestinal Expression in Mice

FIG. 3. Transient transfection analysis of the mouse villin promoter. A, above a partial restriction map diagram of the mouse villin gene from 9 kb with respect to the translation initiation codon. Apal (A), BamH1 (B), BglII (Bg), BstEII (Bl), DsrI (D), NcoI (N), and XhoI (X) restriction sites are shown. The schematic representation at the bottom of panel A shows the location of the four hypersensitive sites (I–IV) as well as the 5.5-kb intron (represented by a hatched rectangle) separating the transcription start site (indicated by an arrowhead) and the translation initiation codon. B, diagrams of the various constructs generated by deletion. Different portions of the 5′-flanking region of the mouse villin gene were fused with the Escherichia coli β-galactosidase gene containing the nuclear localization signal (nls). C, β-galactosidase activities resulting from transient transfections into CaCo2 colon cells (shaded bars) or LLCPR1 kidney cells (open bars) with the reporter constructs generated (represented in panel B). Basal activity resulting from the promoterless pBasic plasmid was set arbitrarily at 1. Values indicate the averages of at least three independent transfections.

strategy by combination of a 5′-PCR primer from within the mouse villin promoter sequence just upstream of the splice donor site and the 3′-primers from within the β-galactosidase gene or the villin gene. For each founder, no reporter gene expression was detected in the tissues in which villin mRNAs were not detected using the PCR assay (Fig. 4). For all founder mice, the reporter gene transcription was detected along the crypt-villus axis, thus confirming that the expression of the transgene in the stem cells of the crypt, of both small intestine (Fig. 5C) and colon (Fig. 5D) epithelium, was observed, as expected because the β-galactosidase gene contains a nuclear localization sequence signal (nls). Basal activity resulting from the promoterless pBasic plasmid was set arbitrarily at 1. Values indicate the averages of at least three independent transfections.

FIG. 4. Expression pattern of the transgene. Transgene-specific (β-Gal) transcripts were detected by reverse transcription-PCR in a ethidium bromide-containing agarose gel. Above each lane are the different tissues tested and the controls. +, kidney mRNA from a mouse in which the β-galactosidase was inserted at the villin locus (32); –, distilled H2O as a template. Reverse transcription-PCR were also performed on mRNAs of the endogenous villin gene and the ubiquitous TFIID gene.

in which the transgene was detected by reverse transcription-PCR, the staining was only observed in the epithelial cells of the small intestine (data not shown). The founder animals were able to transmit the transgene to their offspring with a similar pattern of β-galactosidase expression (data not shown). In our attempt to direct an efficient expression of the reporter gene in the intestinal epithelium with shorter regulatory sequences, plasmids pA3, pB3, and pC3 were used to generate transgenic mice, because these constructs display efficient levels of β-galactosidase activity in intestine-derived CaCo2 cells. The presence of the transgene assessed by β-galactosidase staining procedure was observed in three of the four independent lines of pA3 transgenic mice generated. These three lines expressed the reporter gene only in the small intestine (both the immature and differentiated epithelial cells along the crypt-villus axis), and all these mice failed to express the transgene in the other tissues tested, particularly noteworthy is the lack of expression in the colon and the kidney (data not shown).

These results demonstrate that (i) the 3.5-kb regulatory region upstream from the transcription start site of the mouse villin gene is necessary and sufficient to sustain expression strictly in small intestine of transgenic mice, (ii) the first intron of the mouse villin gene is required for colon and kidney expression in transgenic mice. Concerning the pB3 and pC3
Villin Sequences Direct Intestinal Expression in Mice

In the kidney, for only one animal of five analyzed, mouse villin gene expression was restricted to epithelial cells of the whole intestine of transgenic mice. Thus, distinct and separable regulatory elements in the mouse villin gene may direct transgene expression along the cephalocaudal axis of the gut: the regulatory elements required for transgene expression in the small intestine might be localized in the 3.5-kb region (i.e. the HS II site) upstream from the transcription start site, whereas those necessary for the colonic expression might be localized in the first intron (i.e. the HS II site).

The inability of shorter regulatory sequences of the mouse villin gene to direct correct expression of the reporter gene in the whole intestine of transgenic mice might also be explained by spatial rearrangement of chromatin structure due to the lack of the entire first intron. In fact, the results described here are reminiscent of those of the adenosine deaminase gene (34) and the aldolase B gene (35), in which elements located in the first intron are required for transgene expression in vivo, because they may contain cis-acting tissue-specific enhancer elements and/or elements involved in promoting decondensation of the chromatin structure, allowing the accessibility for transcription factors and RNA polymerase. Further investigations will be required to elucidate the precise role of the first intronic region of the mouse villin gene.

To explain the discrepancy seen in the ability of the mouse villin gene regulatory elements to promote transcription of the reporter gene in cell cultures versus transgenic animals, we may argue that the regulation of gene expression in the intestinal epithelium occurs as cells differentiate and migrate along the crypt-villus axis. This process depends on the contacts that these cells maintain with other neighboring cells on one hand and with the extracellular matrix on the other hand (36). Thus, an ex vivo system as the intestine-derived CaCo2 cell line used in our study, is limited by its weak ability to recapitulate the temporal and spatial complexities of this epithelium and emphasizes the importance of using in vivo models to define a function for specific regulatory sequences (37, 38).

Previous studies carried out in transgenic mice to map transcriptional regulatory elements responsible for intestinal expression have been performed using cis-acting sequences of genes expressed in villus-associated-enterocytes of small intesti-
Villin Sequences Direct Intestinal Expression in Mice

In some of these cases, precocious activation in the crypts in combination with extended expression in the colon occurs in an inappropriate manner. Thus, to our knowledge, the 9-kb regulatory region of the mouse villin gene represents the only characterized cis-acting sequences reported today that allow the expression of a heterologous gene in small intestine and colon epithelial cells of transgenic mice reproducing with great fidelity the tissue-specific and cell-specific pattern of expression when compared with that of the endogenous gene itself. In addition, the mice lines that drive a transgenic expression exclusively restricted to the intestinal mucosa could already be studied after selection of those that will not display expression into the kidney because of the positional effects.

The ability to target genes of interest in transgenic mice following the villin-restricted pattern of expression and particularly in the crypt stem cells should lead to the development of targeted genes in animal models. Experimental mouse models reproducing several steps of human colorectal carcinogenesis (a possible genetic pathway has been proposed by Fearon and Vogelstein (41)) could for instance be obtained by efficiently targeting the associated oncogenes or mutated tumor suppressor genes to colonocytes using the villin regulatory region. Another use could lie in the establishment of new cell lines derived from the digestive tract by targeting a thermosensitive SV40 T antigen to the crypt-resident progenitors of intestinal cells, as used in other systems (42–44).

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