The –700/–310 Fragment of the Apolipoprotein A-IV Gene Combined with the –890/–500 Apolipoprotein C-III Enhancer Is Sufficient to Direct a Pattern of Gene Expression Similar to That for the Endogenous Apolipoprotein A-IV Gene*

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Spatial gene expression in the intestine is mediated by specific regulatory sequences. The three genes of the apoA-I/C-III/A-IV cluster are expressed in the intestine following cecal and crypt-to-villus axes. Previous studies have shown that the –780/–520 enhancer region of the apoC-III gene directs the expression of the apoA gene in both small intestinal villi and crypts, implying that other unidentified elements are necessary for a normal intestinal pattern of apoA-I gene expression. In this study, we have characterized transgenic mice expressing the chloramphenicol acetyltransferase gene under the control of different regions of the apoC-III and apoA-IV promoters. We found that the –890/+24 apoA-III promoter directed the expression of the reporter gene in crypts and villi and did not follow a cecal and crypt-to-villus gradient of expression. In contrast, the –700/+10 apoA-IV promoter linked to the –500/–890 apoC-III enhancer directed the expression of the reporter gene in enterocytes with a pattern of expression similar to that of the endogenous apoA-IV gene. Furthermore, linkage of the –700/–310 apoA-IV distal promoter region to the –890/+24 apoC-III promoter was sufficient to restore the appropriate pattern of intestinal expression of the reporter gene. These findings demonstrate that the –700/–310 distal region of the apoA-IV promoter contains regulatory elements that, in combination with proximal promoter elements and the –500/–890 enhancer, are necessary and sufficient to restrict apoC-III and apoA-IV gene expression to villus enterocytes of the small intestine along the cecal and crypt-to-villus axis.

The mammalian small intestine is lined with a constantly renewing epithelium that is compartmentalized into a proliferative undifferentiated zone located in intestinal crypts and a nonproliferative differentiated zone located in the villi. The epithelium is composed of four specialized cell types that arise from stem cells located just above the base of the crypts (1). Enterocytes, mucus-producing goblet cells, and enteroendocrine cells differentiate as they migrate to the top of the villi, whereas Paneth cells differentiate and migrate to the base of the crypts. Each lineage completes its differentiation program through an orderly migration (2, 3). Enterocytes are the most abundant epithelial cells in the small intestine and express a variety of specific genes as they exit the crypt compartment. Despite the rapid renewal of the intestinal epithelium, numerous genes display a specific pattern of expression in enterocytes from the proximal to the distal intestine and from the crypt to the villus tip (4, 5).

Several studies performed with transgenic mice expressing a human gene or a reporter gene have established that spatial gene expression in the intestine is supported by specific regulatory sequences (6–11). Transcription of the intestinal fatty acid-binding protein (FABP-I) gene is strictly confined to the intestinal epithelium. The FABP-I promoter (–103/+28) is sufficient to direct transcription of the gene along the duodenum-colon axis. However, upstream sequences are needed to confine FABP-I expression to differentiated enterocytes of the villus. In particular, a 20-bp element located between nucleotides –263 and –244 of the promoter prevents FABP-I expression in crypt cells (6, 10). Liver and intestinal human apob gene expression is governed by distinct regulatory regions. Intestinal expression requires a very distant element located between 33 and 70 kb upstream from the apoB gene (11). Similarly, the –256/+22 proximal promoter of the human apoA-I gene is sufficient to direct its hepatic transcription. However, the sequences responsible for the intestinal expression reside 9 kb downstream from the human apoA-I gene (12).

The apoA-I gene is located on chromosome 11 in a cluster that also contains the apoC-III and apoA-IV genes. The human apoA-I gene is expressed at similar levels in the intestine and liver, whereas the human apoC-III gene is expressed predominantly in the liver and to a lesser extent in the intestine (13). The apoA-IV gene is mainly expressed in the intestine in humans and non-human primates. ApoA-IV is also expressed in the liver in mice (13, 14). The apoA-I and apoA-IV genes are transcribed in the same direction, whereas the apoC-III gene is transcribed in the opposite direction. The apoC-III/A-IV intergenic region therefore constitutes a common 6.6-kb 5’-flanking sequence for these two genes.

Since the three genes are expressed at different levels in the liver and intestine, this gene cluster represents an interesting model to decipher the molecular mechanisms involved in the

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1 The abbreviations used are: FABP-I, intestinal fatty acid-binding protein; bp, base pair(s); kb, kilobase pair(s); CAT, chloramphenicol acetyltransferase; HNF4, hepatic nuclear factor 4.
determination of tissue-specific expression. The intestinal expression of the cluster is entirely restricted to enterocytes as they emerge from the crypt (15, 16) and decreases from the proximal to the distal small intestine (14). A preliminary report indicated that the apoC-III/A-IV intergenic region allows the intestinal expression of the apoA-IV gene in transgenic mice (17). More recently, Bisaha et al. (18) have demonstrated that the –890/–500 apoC-III enhancer is sufficient to direct the intestinal expression of the apoA-I gene. Based on these in vivo studies and previous in vitro promoter studies performed by us and others, we hypothesized that common regulatory sequences control the intestinal expression of the three genes of the cluster.

In this study, we generated transgenic mice expressing the CAT reporter gene under the control of specific regulatory sequences of the apoA-IV/C-III intergenic region. Analysis of these mouse lines showed that the –700/–310 apoA-IV promoter in combination with the –500/–890 apoC-III enhancer is sufficient for correct gene expression in the enterocytes along the proximal-to-distal and crypt-to-villus axes.

MATERIALS AND METHODS

Generation of Transgenic Mice—The different transgenes used in this study are shown in Fig. 1. The transgenes C3-CAT and eC3-A4-CAT were obtained by digestion of the pUCSH-CAT plasmid containing the –890/+24 5′-flanking region of the human apoC-III gene or the –700/+10 apoA-IV promoter region fused with the –500/–890 apoC-III enhancer region, respectively, with XbaI and BamHI (see Fig. 1A). These plasmids have previously been described (18, 19).

The transgene dA4-C3-CAT was obtained from a plasmid in which the human –890/+24 apoC-III promoter region upstream from the CAT reporter gene was linked in the opposite direction to the –700/–10 apoA-IV promoter region fused with the lacZ reporter gene. This vector was constructed as follows. The –700/–10 apoA-IV promoter region was amplified by polymerase chain reaction using nucleotide primers from –700 to –680 (coding strand) and +10 to +10 (noncoding strand) containing a SalI and a HindIII restriction site, respectively. The resulting apoA-IV fragment was cloned upstream from the lacZ gene fused to a nuclear localization sequence (20); the resulting apoA-IV promoter in the opposite direction. The dA4-C3-CAT transgene was excised from the plasmid by digestion with BamHI and SmalI at a site located at nucleotide –310 in the apoA-IV promoter. Transgenes were digested at a concentration of 4 ng/ml and microinjected into fertilized eggs from C57BL/6 × CBAJ females mated with males of the same strain using established procedures (21).

Characterization of Transgenic Mice—DNA was extracted from tails of 10–15-day-old pups and then analyzed by polymerase chain reaction amplification and subcloned in the pBluescript KS plasmid. Oligonucleotide primers corresponding to sequences –310 apoA-IV promoter (dIII) and 5′-CCACGTATACTCTCTCTCTC-3′ (coding strand) and 5′-GCTCTAGA-3′ (noncoding strand) were used for the amplification of apoC-III and apoA-IV sequences, respectively (24, 25). The two cDNAs were digested with XbaI and HindIII and ligated into the pBluescript KS vector that had previously been digested with XbaI and HindIII. A 265-bp fragment of the CAT gene was obtained from pUCSH-CAT by digestion with HindIII and EcoRI and cloned into the pBluescript SK vector.

Both sense and antisense mouse apoA-IV and apoC-III RNA probes (size: 300 bp) were generated using T3 and T7 RNA polymerases, respectively (Promega). Sense and antisense CAT riboprobes were synthesized with T7 and T3 polymerases, respectively. All probes were labeled using 32P-UTP.

In Situ Hybridization—Adult mice were killed by cervical dislocation, and the entire small intestines were rapidly removed and divided into parts representing the proximal, middle, and distal regions of the small intestine. The samples were fixed in 2% paraformaldehyde in phosphate-buffered saline, pH 7.2, and embedded in paraffin. Sections (4 μm thick) were mounted on glass slides.

In situ hybridization was performed by a modification of the method of Sassoon and Rosenthal (26). Sections of small intestine were hybridized with 200,000 cpm of probes/slide at 42 °C overnight. Tissues were washed for 30 min in 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate, pH 7.0) and 10× dithiothreitol at 50 °C, two times for 20 min in 2× SSC and 50% formamide at 60 °C, and for 10 min in 1× SSC at 37 °C and then treated for 30 min with RNase A. Subsequent washes in 1× and 0.1× SSC at 37 °C were followed by dehydration in graded ethanol for total desiccation. The washed slides were dipped into Kodak NTB2 emulsion, stored in the dark at –20 °C for 8–16 days, and then developed.

RESULTS

Generation of Transgenic Mice—We generated transgenic mice expressing the CAT reporter gene under the control of either the human –890/+24 apoC-III promoter (C3-CAT) (Fig. 1B) or the human –700/+10 apoA-IV promoter fused to the –500/–890 apoC-III enhancer in the opposite direction to the apoA-IV promoter in accordance with the organization of the apoA-IV/C-III/A-IV gene cluster (eC3-A4-CAT) (Fig. 1B). The number of transgene copies incorporated into the genome of each transgenic founder was determined by Southern blot analysis in comparison with increasing amounts of the transgene diluted in nontransgenic DNA (Fig. 1C and Table I). CAT activity was determined in tissue extracts from adult transgenic mice of each line (Table I). In all cases, CAT activity was mainly detected in the liver and small intestine, although ectopic expression of the CAT gene was observed in the heart of C3-CAT transgenic mice. Despite differences in the level of expression of the transgene, tissular distribution of CAT activity was similar in the different lines produced with the same transgene. This distribution differed in different transgenes. In C3-CAT transgenic mice, the CAT activity in any part of the intestine did not exceed one-third of the liver activity measured. In contrast, in eC3-A4-CAT transgenic mice, the level of CAT activity in the proximal and middle regions of the small intestine was similar to that in the liver. From each construct, two mouse lines exhibiting the highest CAT activity were further characterized.
The two promoter segments are in their normal opposite direction. The dA4-C3-CAT contains the –890/–700 apoC-III enhancer. The two promoter segments are in their "normal" opposite direction. The dA4-C3-CAT contains the –890/–700 apoC-III promoter that were used to generate transgenic mice (21). The C3-CAT constructs driven by segments of the human apoC-III and/or apoA-IV proximal promoter are practically inactive in HepG2 and Caco-2 cells (19).

An apoA-I expression. The proximal promoter of apoA-IV (700 bp) is in vivo apoC-III suffice for transcription for each gene is shown by an arrow.

Comparison with known copy numbers of transgenic DNA (20 to 0.5) allowed the determination of the copy number in each line. Tail DNA from a normal mouse was used for a negative control (control). Three transgenic mouse lines were analyzed for the C3-CAT transgene (V, W, and Y), the eC3-A4-CAT transgene (Zf, Zg, and Ze) of apoA-IV and apoC-III mRNAs (Fig. 2B).

Transgenic expression along the crypt-to-villus axis in the intestine was analyzed by in situ hybridization (Fig. 3). To demonstrate the specificity of the signal, an antisense and a sense CAT riboprobe were first hybridized to sections of non-transgenic and transgenic jejunum, respectively, as negative controls (Fig. 3, a and b). Using dark-field microscopy, a few scattered grains representing the background signal were seen with both probes. The CAT reporter gene driven by the human –890/+24 apoC-III promoter was expressed markedly in the crypt cells and lightly in the villus epithelial cells of the transgenic mice (Fig. 3d). The endogenous mouse apoC-III gene was expressed similarly in the crypt and villus epithelial cells (Fig. 3c).

In contrast to C3-CAT transgenic mice, eC3-A4-CAT transgenic mice exhibited a pattern of CAT mRNA expression in the crypt-to-villus unit that was strikingly similar to that of the endogenous mouse apoA-IV mRNA (Fig. 3, e and f). These results suggest that the –700/+10 apoA-IV promoter contains a regulatory region that, in combination with the –500/–890 apoC-III enhancer, restricts the expression of the reporter gene to the villus, thus reproducing in the transgenic mice the crypt-to-villus gradient of expression that is observed for the endogenous apoA-IV gene.

The ApoA-IV Distal Promoter Confers a Cephalocaudal and Crypt-to-Villus Expression Gradient to C3-CAT Transgenes—To determine which region of the apoA-IV promoter was responsible for the expression pattern along both the cephalocaudal and crypt-to-villus axes, we generated additional mouse lines expressing the reporter CAT transgene under the control of the –890/+24 apoC-III promoter fused to the –310/–700 apoA-IV distal promoter region (Fig. 1B). Three founders were identified by polymerase chain reaction and were analyzed by Southern blotting (Fig. 1C).

As in C3-CAT transgenic mice, CAT activity was much higher in the liver than in the intestine of dA4-C3-CAT mice. However, in the latter, we observed a decreasing gradient of CAT activity from the proximal to the distal region of the intestine (Fig. 4). This pattern of expression differed from that of C3-CAT transgenic mice (Fig. 2). Thus, the addition of the –310/–700 apoA-IV promoter region to the –890/+24 apoC-III promoter restored the cephalocaudal pattern of expression observed for the endogenous apoC-III gene.

The expression of the transgene in the crypt-to-villus unit was visualized by histochemical staining of the nuclei of CAT-expressing cells in the small intestine. No staining was observed in control mice (Fig. 5, a and a') or in the lamina propria of transgenic mice (Fig. 5). CAT histochemistry revealed a pattern of expression similar to that observed previously by in situ hybridization (Fig. 3). To demonstrate the specificity of the signal, an antisense and a sense CAT riboprobe were first hybridized to sections of non-transgenic and transgenic jejunum, respectively, as negative controls (Fig. 3, a and b). Using dark-field microscopy, a few scattered grains representing the background signal were seen with both probes. The CAT reporter gene driven by the human –890/+24 apoC-III promoter was expressed markedly in the crypt cells and lightly in the villus epithelial cells of the transgenic mice (Fig. 3d). The endogenous mouse apoC-III gene was expressed similarly in the crypt and villus epithelial cells (Fig. 3c).

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situ hybridization. Crypt and villus nuclei were stained in C3-CAT transgenic samples (Fig. 5, b and b'), whereas staining was restricted to the villus in eC3-A4-CAT transgenic mice (c and c') as well as in dA4-C3-CAT transgenic samples (d and d'). Thus, the addition of the −310′−700 apoA-IV distal promoter region restricted the expression of the dA4-C3-CAT transgene to the villus in a pattern similar to that of endogenous apoA-IV, but not of endogenous apoC-III.

### Table 1

| Transgene | Mouse line | Copy No. | Liver (pmol product formed/mg tissue (wet weight)/min) | Intestine (pmol product formed/mg tissue (wet weight)/min) | Kidney | Heart | Lung | Brain | Muscle |
|-----------|------------|----------|----------------------------------------------------------|----------------------------------------------------------|--------|-------|------|-------|--------|
| C3-CAT    | V          | 2        | 420 ± 300<sup>a</sup> | 67 ± 10 | 150 ± 120 | 51 ± 12 | 113 ± 57 | 48 ± 3 | 60 ± 49 | 48 ± 3 |
|           | W          | >10      | 6217 ± 1171 | 2388 ± 737 | 1705 ± 401 | 1705 ± 50 | 51 ± 12 | 113 ± 57 | 0      | 48 ± 42 | ND<sup>b</sup> |
|           | Y          | 2–5      | 62 ± 22 | 0.43 | 0 | 0 | 0.7 ± 0.7 | 0.5 ± 0.8 | 0.2 ± 0.3 | ND<sup>b</sup> |
| eC3-A4-CAT| Zf         | 2        | 1820 ± 660 | 2574 ± 71 | 1251 ± 415 | <90 | 35 ± 45 | 0 | 0 | 70 ± 40 |
|           | Zc         | >20      | 17,055 | 23,588 | 12,566 | 104 | 42 | 128 | 0 | 0 | 29 |
|           | Zg         | 2–5      | 5.5 ± 1.5 | 0.3 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 |

<sup>a</sup> Results are presented as means ± S.D. of at least three mice analyzed.

<sup>b</sup> ND, not determined.

**Fig. 2.** Comparison of the distribution of endogenous mouse apoC-III and apoA-IV mRNAs with CAT activity along the intestinal cephalocaudal axis of transgenic and nontransgenic mice. A, distribution of CAT activity along the small intestine of the different transgenic mouse lines (C3-CAT and eC3-A4-CAT). The CAT activity of each intestinal segment (proximal, middle, and distal) was calculated relative to the total amount of CAT activity present in the small intestine of each transgenic mouse line. B, distribution of mouse apoA-IV and apoC-III mRNAs in the small intestine. Total RNA was prepared from each fragment, and 15 µg of total cellular RNA were examined by Northern blot analysis. Values are expressed as percent ratio of duodenal RNA.
results suggest that the −310/−700 apoA-IV distal promoter region is sufficient to restrict gene expression to villus enterocytes along the cephalocaudal axis.

**DISCUSSION**

A preliminary report has shown that the entire intergenic region between the apoC-III and apoA-IV genes directs a pattern of expression of the transgene similar to that of endogenous apoA-IV (17). This expression was abolished with shorter constructs lacking the apoC-III promoter region. Bisaha et al. (16) showed that the apoC-III enhancer, located at nucleotide −520 upstream from the transcription initiation site of the apoC-III gene, is sufficient to direct the intestinal expression of the apoA-I gene, the third gene of the apoA-I/C-III/A-IV cluster, but not to restrict its expression to the villus. This prompted us to decipher the regulatory regions responsible for the accurate expression of apoA-IV by combining the apoC-III enhancer and the apoA-IV promoter. In our present study, we found that a combination of the −890/−500 apoC-III enhancer and the −700/−310 apoA-IV distal promoter allowed the intestinal expression of the apoA-IV gene in transgenic mice, specifically in the villus enterocytes, with a cephalocaudal gradient. Taken together, these results demonstrate that the appropriate lineage-specific crypt-to-villus and cephalocaudal patterns of human apoA-IV expression in transgenic mouse intestine require both the apoC-III enhancer and the apoA-IV distal promoter.

Our findings indicate that CAT activity in the intestine and liver in mice expressing the eC3-A4-CAT transgene reproduces the correct pattern of expression of endogenous mouse apoA-IV rather than that of human apoA-IV, which is predominantly expressed in the intestine. Lauer et al. (17) reported no expression of the human apoA-IV gene in the liver of transgenic mice expressing human apoA-IV genomic sequences containing 5′-flanking regions 0.3–7.7 kb long and a 3′-flanking region 1.5 kb long. These results, taken conjunction with our own, suggest that a hepatic silencer may reside downstream from nucleotide +24 of the human apoA-IV gene. It is possible that either a silencer in the human apoA-IV promoter or differences in the nuclear activities between humans and rodents may account for this difference in tissue-specific expression of the apoA-IV gene. Similarly, the weak ectopic expression of the C3-CAT and eC3-A4-CAT transgenes in tissues other than those of the liver and intestine may reflect the lack of tissue-specific silencers in the −890/+/24 apoC-III and −700/+10 apoA-IV promoter regions.

In vitro transfection assays in the Caco-2 cell line showed that the transcription of the apoA-IV gene is controlled by hepatocyte nuclear factor 4 (HNF4), which binds to its proximal promoter region and requires the presence of other transcription factors that recognize elements of the apoC-III enhancer (19, 29). Similarly, the transcription of apoA-I and apoC-III genes is driven through a synergy between HNF4 and others factors that bind the apoC-III enhancer (16, 30–34). HNF4 binds the hormone response elements located in the three proximal promoters and in the apoC-III enhancer. Bisaha et al. (16) have shown that a region of the apoC-III enhancer containing the HNF4-binding site is insufficient to drive in vivo the intestinal expression of the apoA-I gene. Nevertheless, this factor could actively participate in the determination of intestinal apoA-IV-C-III/A-IV gene expression. A HNF4-binding site has also been described in the FABP-I proximal enhancer, which is essential for intestinal expression (35). Furthermore, the involvement of HNF4 in the onset of intestinal functions has been demonstrated by the interruption of intestine development under extinction of the HNF4 homolog in *Drosophila* (36).

The eC3-A4-CAT transgene, which retains the −890/−500

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**Fig. 3.** Distribution of CAT mRNA and mouse apoC-III and apoA-IV mRNAs along the crypt-to-villus axis of the small intestine of adult mice. A sense or antisense CAT riboprobe and an antisense riboprobe for mouse apoC-III or apoA-IV were hybridized to a jejunal section of the intestine. *In situ* hybridization dark-field images are presented. *a*, shown is a control of jejunal sections from a nontransgenic mouse hybridized with an antisense CAT riboprobe. *b*, the specificity of the antisense CAT probe signal was assessed in the same experimental series by hybridization of jejunal sections of transgenic mouse with a sense CAT riboprobe. Only scattered grains are present in control sections *c* and *d*. *b*, shown are jejunal sections from a nontransgenic mouse hybridized with an antisense mouse apoC-III riboprobe. ApoC-III mRNA expression can be observed in villus-associated epithelial cells, and a specific signal is also seen in crypt-associated epithelial cells. *c*, shown are jejunal sections from a C3-CAT transgenic mouse hybridized with an antisense CAT riboprobe. CAT mRNA expression was strongest in epithelial cells of the crypt and at the base of the villus and decreased toward the villus tip. *d*, shown are jejunal sections from a nontransgenic mouse hybridized with an antisense mouse apoA-IV riboprobe. ApoA-IV mRNA was present from the villus base to the upper villus epithelial cells; apoA-IV mRNA was not observed in crypt epithelial cells. *f*, shown are jejunal sections from an eC3-A4-CAT transgenic mouse hybridized with an antisense CAT riboprobe. CAT mRNA expression was restricted to the villus-associated epithelial cells.

**Fig. 4.** Detection of CAT activity in the liver and in segments of the small intestine of dA4-C3-CAT transgenic mice. Samples from homogenates of the liver and different parts of the small intestine were assayed for CAT activity as described under "Materials and Methods." The presence of the −310/−700 apoA-IV promoter sequence establishes an appropriate cephalocaudal pattern of gene expression.
apoC-III enhancer and −700/+10 apoA-IV promoter regions, was able to direct a pattern of CAT activity among intestinal segments that resembled both the pattern of endogenous mouse apoA-IV and that observed in rats and chickens (14, 37). Furthermore, the expression of the reporter gene was restricted to villus cells in a manner similar to the expression pattern of the endogenous mouse apoA-IV gene and also to that observed in rats (38). These results suggest that the apoA-IV promoter contains an element prohibiting the transcription of the reporter gene in crypt epithelial cells and in the distal part of the small intestine.

As already discussed, the apoC-III promoter region was not sufficient to restrict intestinal expression along the crypt-to-villus and cephalocaudal gradients. The addition of the −700/−310 apoA-IV distal promoter to the apoC-III enhancer allowed the expression of the reporter gene to mimic the cephalocaudal gradient displayed by endogenous mouse apoC-III. Furthermore, the −700/−310 apoA-IV promoter region retained the elements sufficient to restrict expression to villus-associated enterocytes. These findings indicate that the −310/−700 apoA-IV promoter region confers two suppressor functions, one prohibiting gene expression in the distal small intestine and the other prohibiting gene expression in crypt epithelial cells.

The spatial patterns of gene expression in the intestine involve both positive and negative elements. This has also been reported for the rat FABP-I gene, the rat liver fatty acid-binding protein gene, and the sucrase-isomaltase gene (6, 7, 10). Simon et al. (10) identified a 20-nucleotide element in the

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**FIG. 5. Pattern of CAT activity along the intestinal crypt-to-villus axis of the three different transgenic mice.** Histochemical staining for CAT activity with no counterstaining was performed as described under “Materials and Methods.” Sections were photographed with phase-contrast (a–d) and with bright-field (a’–d’). Nontransgenic proximal intestine was incubated in the complete staining mixture (a and a’). No staining was observed in either crypt (asterisks) or villus (arrows) epithelial cells. In transgenic sections, only nuclei were stained (visible as a black deposit). This staining appears to be specific since it was observed only in the transgenic mice (compare a and a’ with the other panels). In the proximal section of the intestine from C3-CAT transgenic mice (b and b’), CAT staining was observed in both crypt and villus epithelial cells. The patterns of CAT staining along the crypt-to-villus axis of adult mice expressing the eC3-A4-CAT (c and c’) and dA4-C3-CAT (d and d’) constructs are indistinguishable and are restricted to villus epithelial cells.
FABP-I promoter that modulates the intestinal cellular and spatial expression of the FABP-I gene. This element acts as a suppressor of gene expression in the distal small intestine/colon, as a suppressor of gene activation in the crypt, and as a suppressor of gene expression in the Paneth cell lineage (10). No sequence in the −700/−310 apoA-IV distal promoter significantly matched this 20-bp element. Thus, it is reasonable to hypothesize that in both apoA-IV and FABP-I, a distinct element controls a similar appropriate pattern of gene expression. Whether these two different elements bind similar or different repressors of gene expression in crypts and the distal part of the intestine remains to be established.

FIG. 6. Double histochemical staining analysis of the villus-associated epithelial cells. CAT-stained sections of the proximal intestine of the dA4-C3-CAT transgenic lines were further stained by the Grimalius silver method to visualize the enteroendocrine cells and with periodic acid-Schiff base to visualize the goblet cells. With the Grimalius silver method, enteroendocrine cells appear with a diffuse brown cytoplasmic staining (a and b, open arrows), and nuclei of CAT-expressing cells are dark (a and b). With periodic acid-Schiff staining, mucins in the goblet cells are stained pink (c, arrowheads), and nuclei of CAT-expressing cells are stained brown (c). Only the villus epithelial cells are stained histochemically with CAT (a–c). Nuclei of the enteroendocrine cells appear to be negative within CAT staining (a and b). Periodic acid-Schiff-stained goblet cells do not appear to express the CAT reporter gene (c). Cell nuclei are designated by closed arrows and are negative for CAT staining.
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