Intestinal expression of the human apoA-I gene in transgenic mice is controlled by a DNA region 3' to the gene in the promoter of the adjacent convergently transcribed apoC-III gene

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Abstract The apoA-I gene in humans is principally expressed in liver and small intestine. Using transgenic mice, we previously showed that 256 bp of 5' flanking DNA was sufficient for liver expression, but as much as 5.5 kb of 5' and 4.0 kb of 3' DNA did not allow intestinal expression of the human apoA-I transgene. In the current study, a 10.5 kb DNA construction containing the apoA-I and the adjacent convergently transcribed apoC-III genes, which extends from 300 bp 5' to the apoA-I gene to 2.5 kb 5' to the apoC-III gene, produced high levels of apoA-I intestinal expression. A similar DNA construction ending 1.4 kb 5' to the apoC-III gene also allowed apoA-I intestinal expression. The DNA region from 0.2 to 1.4 kb 5' to the apoC-III gene was then cloned 1.7 kb 3' to the apoA-I gene in both orientations in the absence of apoC-III gene sequences. Intestinal apoA-I expression was also achieved with both of these constructions. In summary, these in vivo experiments suggest that the intestinal control region for the apoA-I gene is distinct from the liver control region, resides 3' to the gene in the promoter of the adjacent apoC-III gene, and has some properties of a tissue-specific enhancer.—**Walsh, A., N. Azrolan, K. Wang, A. Marcigliano, A. O'Connell, and J. L. Breslow.** Intestinal expression of the human apoA-I gene in transgenic mice is controlled by a DNA region 3' to the gene in the promoter of the adjacent convergently transcribed apoC-III gene. *J. Lipid Res.* 1993. **34:** 617–623.

Supplementary key words apolipoprotein A-I • apolipoprotein C-III • gene expression

ApoA-I is the major protein of high density lipoproteins (HDL). Plasma levels of apoA-I and HDL cholesterol correlate with each other and both correlate inversely with the incidence of coronary heart disease (1–3). There is evidence from both animal and human studies that apoA-I synthesis influences HDL cholesterol levels (4, 5). In mammalian species, apoA-I is synthesized principally in liver and small intestine. The gene for apoA-I is 1.8 kb in length and resides in an apolipoprotein gene cluster on chromosome 11q23. With reference to the orientation of the apoA-I gene, from 5' to 3' the genes in the cluster are apoA-I, apoC-III, and apoA-IV. The apoC-III gene is in the opposite orientation from the other two genes. Only 2.1 kb separate the apoA-I and the 3.1 kb-long apoC-III genes, and 4 kb separate the apoC-III and the 2.6 kb-long apoA-IV genes (6–8).

To better understand the regulation of apoA-I synthesis, we have used transgenic mice to identify the *cis*-acting DNA sequences controlling tissue-specific expression of the apoA-I gene. In our initial description of human apoA-I transgenic mice, animals were made with apoA-I genomic fragments containing different amounts of 5' and 3' flanking DNA (5). These studies revealed that as little as 256 bp of 5' flanking DNA was sufficient for liver expression, but as much as 5.5 kb of 5' and 4.0 kb of 3' flanking DNA did not allow intestinal expression of the transgene.

Transgenic mice have now been made with apoA-I genomic constructions containing more 3' flanking DNA. We report that high level intestinal expression can be achieved with a 1.2 kb DNA region from the apoC-III gene promoter which has some properties of an intestinal specific enhancer. Some of this work was previously reported in abstract form (9).

MATERIALS AND METHODS

DNA constructions

Results are reported for transgenic mouse lines made

Abbreviations: HDL, high density lipoprotein.

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with different DNA constructions identified by letters A to G and diagrammed in Figs. 1 and 3A. The original DNA was isolated from a human genomic library constructed in λphage (λ apoA-I-8) (10). As previously reported, constructions A, B, and C were derived from an 11.5 kb DNA fragment extending from a false EcoRI site 5.5 kb 5' to the apoA-I gene to a BamHI site 4.0 kb 3' to the gene and cloned into the pBR 328 vector, called p31. Construction C is the entire insert of p31. Construction B is an 8.2 kb XmnI fragment extending from 4.5 kb 5' to 1.9 kb 3' to the gene. Construction A is a 2.2 kb SmaI fragment extending from 256 bp 5' to 80 bp 3' to the gene.

Construction D was made as follows. Plasmid p31 was digested with NcoI and BamHI releasing a fragment from 300 bp 5' to 4 kb 3' to the apoA-I gene, which was ligated with SalI linkers and cloned into SalI cut pUC19. Digestion of this plasmid with SalI and XbaI produced a 3.8 kb fragment from 300 bp 5' to the XbaI site 1.7 kb 3' to the apoA-I gene in the A-I/C-III intergenic region. Next, λ apoA-I-8 was digested with KpnI to release a 6.2 kb fragment which included the entire apoC-III gene and extended from the KpnI site in the A-I/C-III intergenic region to 2.5 kb 5' of the apoC-III gene. This fragment was ligated with SalI linkers, and cloned into SalI cut pUC19. Digestion of this plasmid with XbaI and SalI produced a 6.7 kb fragment. The 3.8 kb fragment derived from p31 was ligated with this 6.7 kb fragment in SalI cut pUC19. The resulting plasmid pHA-I/C-III contained the apoA-I and apoC-III genes on a 10.5 kb SalI fragment extending from 0.3 kb 5' of the apoA-I gene to 2.5 kb 5' of the apoC-III gene.

Construction E was made by partial digestion of plasmid pHA-I/C-III. The enzyme EcoRI was used to release a 9.5 kb DNA fragment extending from a polylinker site now 5' to the apoA-I gene to an EcoRI site 1.4 kb 5' to the apoC-III gene.

Construction F was made by cutting plasmid pHA-I/C-III with XbaI and KpnI. This releases a 6.7 kb DNA fragment with 1.1 kb of A-I/C-III intergenic sequence, the 3.1 kb apoC-III gene and 2.5 kb of the apoC-III gene promoter. Construction F was also cloned into pUC19 and called pH-C-III-11.

Two G-type constructions were made which consisted of the apoA-I gene with apo-C-III promoter sequences inserted 3' to the apoA-I gene in either orientation. As described above for construction D, the 3.8 kb fragment which included the apoA-I gene from the NcoI site 300 bp 5' to the XbaI site 1.7 kb 3' to the gene, was cloned into pUC19 and called pH-A-I-14. The apoC-III promoter region from −0.2 to −1.4 kb was prepared by digestion of pH-C-III-11 (see construction F) first with EcoRI and then with PstI. The 5' protruding ends were filled in with Klenow fragment and ligated with BamHI linkers. pH-A-I-14 was then linearized at the BamHI site in the polylinker 3' to the apoA-I gene and the 1.2 kb PstI apoC-III promoter fragment ligated in either orientation producing plasmids pAI-7 and pAI-14. These differed only in the orientation of the apoC-III promoter. The human genomic inserts were removed by EcoRI and SalI digestion.

Production of transgenic mice

The human DNA fragments described above were gel purified and microinjected into fertilized eggs from superovulated (C57BL/6J × CBA/J)F1 females that had been mated to males of the same genetic background, as previously described (5). Founder animals were bred to (C57BL/6J × CBA/J)F1 animals and transgenic lines were established.

DNA analysis

Tail tips were excised from 3- to 4-week-old mice and DNA was extracted (5). The copy number of the human apoA-I and apoC-III transgenes in each line of mice was estimated by quantitative Southern blotting analysis (11). Transgenic mouse genomic DNA was digested with PstI. Hybridization under standard conditions with a 0.7 kb SacI–PstI human apoA-I genomic probe (from the SacI site at 1449 bp in the fourth exon of the apoA-I gene to 300 bp 3' to the gene) and a 0.5 kb human apoC-III cDNA probe (pC-III607 (12)) produced bands of 2.2 kb for apoA-I and 4.1 kb for apoC-III as expected. Copy number was estimated by comparing the signal produced from comparable amounts of transgenic mouse DNA and human white blood cell DNA (two copies) by densitometric scanning of the autoradiograms. Different amounts of DNA and exposure times were used to remain in the linear range of the assay. Control mouse DNA did not interfere with this assay.

RNA analysis

Total RNA was extracted from the murine tissue using the single step guanidinium thiocyanate–phenol–chloroform method (13). The integrity of the RNA and the absence of DNA contamination for each sample was verified by denaturing formaldehyde/agarose gel electrophoresis (14). Intestinal RNA was prepared from the proximal third portion of the small intestine.

Riboprobes were used to detect gene expression. The human apoA-I riboprobe protected an mRNA sequence 230 nt in length. It was isolated from apoA-I cDNA clone pAI-113 (nt 390–620) (15) by digestion with BamHI/BSSHII, and the 230 bp fragment was cloned into a BamHI/SmaI digested pGemI vector. The human apoC-III riboprobe protected an mRNA sequence 147 nt in length. It was isolated from pCIII-607 and has been previously described (16). The mouse apoA-I riboprobe protected an mRNA sequence 188 nt in length. It was isolated from a mouse apoA-I cDNA clone p1804 (17). The p1804 was digested with PstI and the cDNA fragment

shortened by Bal 31 digestion. The ends were filled in with T4 DNA polymerase and cloned into a SmaI digested pGemI vector. The mouse apoA-I sequence corresponded to nt 634–822 (18). The mouse apoC-III riboprobe protected an mRNA sequence 160 nt in length. It was isolated from a mouse apoC-III genomic clone. This clone was digested with SmaI and AvaII to release a 269 bp fragment which was cloned into pGemI. The mouse apoC-III sequences corresponded to nt 2017–2286 (18). The orientation and sequence of the inserts in the pGemI vectors were determined by sequencing using both the T7 and Sp6 primers. The pGemI plasmids containing the inserts were linearized by restriction enzyme digestion 5' to the insert and riboprobes corresponding to the antisense DNA strand were synthesized. The human apoA-I, mouse apoA-I, and mouse apoC-III riboprobes required T7 RNA polymerase, whereas the human apoC-III riboprobe required Sp6 RNA polymerase. The riboprobes were labeled with [^{32}P]UTP. As previously described, riboprobes (5×10^6 cpm) were hybridized in solution with either 20 μg of yeast tRNA or 5 μg of control or transgenic mouse organ RNA for 4 h at 63°C. The samples were then digested with RNase "A" and "T1" and analyzed by either electrophoresis (8 M urea, 8% polyacrylamide gel) and autoradiography or a quantitative filter counting assay (15). As the insert sequences had less than 60% homology, mouse RNA did not react with the human riboprobes to give significant protection nor did human RNA react with the mouse riboprobes. In the filter counting assay, the background for each riboprobe was less than 0.4% of total counts.

Apolipoprotein quantitation

Human apoA-I in the plasma of transgenic mice was quantified by ELISA using a polyclonal goat antibody to human apoA-I (generously supplied by Dr. Peter Herbert) as previously described (19) or by immunoturbidimetric analysis with a commercial antibody (Incstar Corp, Stillwater, MN) using a Roche-Cobas Fara II micronephelometer (Roche Diagnostic Systems). Human apoC-III levels were determined by rocket electroimmunoassay using a polyclonal goat antibody to human apoC-III (Daiici Pure Chemicals, Tokyo, Japan) as previously described (11). In the assays used, there was no cross-reactivity observed with the endogenous mouse apolipoproteins.

RESULTS

Transgenic mouse lines were established with the human apoA-I gene and different lengths of 5' and 3' flanking sequence as diagrammed in **Fig. 1** and Fig. 3A. The copy number of the transgene, the plasma level of the encoded human apolipoprotein(s), and the liver and intestinal human apolipoprotein mRNA expression pattern for each line are summarized in **Table 1.**

We previously reported that transgenic mouse lines created with DNA fragments A, B, and C expressed human apoA-I mRNA in liver, but not intestine. The previous analysis was done by slot blotting tissue RNA and probing with a relatively specific human 3' apoA-I genomic probe. In the current study, we used a human apoA-I riboprobe in a solution hybridization/RNase protection assay. This assay is much more sensitive and specific than the previous one. As can be seen in **Fig. 2,** upper panel, RNA from the human hepatoma cell line HepG2, which is known to make apoA-I, protected a riboprobe fragment of 230 nt, as expected. Yeast tRNA or RNA from tissues from control mice showed no protection. Liver RNA from transgenic mouse liver made with DNA fragments A, B, and C, protected the 230 nt fragment,

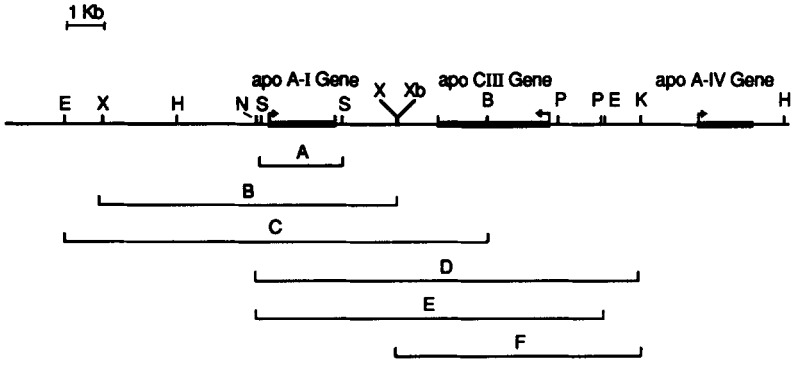


Fig. 1. Restriction map of human apolipoproteins A-I and C-III gene constructions used for microinjection. A restriction map is shown of a cloned portion of the human genome containing the 1.8 kb apoA-I gene, the 3.1 kb apoC-III gene, and the 2.6 kb apoA-IV gene (thick bars). The direction of transcription for each gene is shown by the arrows. The six pieces of DNA used for microinjection, and identified by letters A-F, contain the following gene fragments: A (2.2 kb) extends between SmaI (S) sites 256 bp 5' to the apoA-I gene and 80 bp 3' to the gene; B (8.2 kb) extends between XmnI (X) sites 4.5 kb 5' to the apoA-I gene and 1.5 kb 3' to the gene; C (11.5 kb) extends between a false EcoRI (R*) site 5.5 kb 5' to the apoA-I gene and a BamHI (B) site 4.0 kb 3' to the gene; D (10.5 kb) extends between an NcoI (N) site 0.3 kb 5' to the apoA-I gene and a KpnI (K) site 8.4 kb 3' to the gene; E (9.5 kb) extends between an NcoI (N) site 0.3 kb 5' to the apoA-I gene and an EcoRI (R) site 7.4 kb 3' to the gene; F (6.7 kb) extends between the XbaI (Xb) site, in the intergenic region of apoA-I and apoC-III, and the KpnI (K) site 2.5 kb 5' to the apoC-III gene.

TABLE 1. Plasma concentration and mRNA expression of the human apoA-I and C-III gene in transgenic mice

DNA Construct	Tg Line	Copy Number[a]	Concentration		mRNA Expression			
					ApoA-I		ApoC-III	
			Human ApoA-I	Human ApoC-III	L	I	L	I
			mg/dl, mean ± SD					
A (2.2 kb)	427	>50	339 ± 69 (n = 10)		+	−		
B (8.2 kb)	139	3–5	2.5 ± 2.1 (n = 5)		+	−		
	145	1–2	0.07 ± 0.03 (n = 5)		+	−		
	149	>20	94 ± 27 (n = 7)		+	−		
C (11.5 kb)	179	>50	245 ± 86 (n = 31)		+	−		
D (10.5 kb)	2365	>20	93 ± 17 (n = 5)	52 ± 24	+	+	+	+
	3386	3–5	143 ± 25 (n = 11)	4 ± 0.3	+	−	+	−
	3395[b]	8	94 (n = 1)	n.d.[e]	+	+	+	+
	3703	[c]	62 ± 22 (n = 4)	48 ± 12	+	+	+	+
	3704	[c]	58 ± 17 (n = 4)	62 ± 9	+	+	+	+
	4368	[d]		2 ± 1 (n = 6)	−	−	+	+
	4510	>20	227 ± 28 (n = 5)	22 ± 10	+	+	+	+
E (9.5 kb)	4867	20–25	198 ± 21 (n = 4)	53 ± 20	+	+	+	+
	4868	20–25	207 ± 62 (n = 6)	24 ± 11	+	+	+	+
F (6.7 kb)	2721	100		54 ± 11 (n = 6)			+	+
	2674	1–2		3 ± 0.3 (n = 14)			+	+
	3640	3–5		18 ± 12 (n = 6)			+	+
	3641	>20		15 ± 7 (n = 4)			+	+
	3707	3–5		46 ± 22 (n = 5)			+	+
G (5.4 kb)	A-7	1–2	60 (n = 1)		+	+		
	A-14	1–2	293 ± 61 (n = 8)		+	+		

[a]Quantitative Southern blots of tail tissue DNA were scanned with a densitometer and the relative copy number was calculated as described under Experimental Procedures.
[b]This founder mouse died without producing viable progeny.
[c]The copy numbers for apoA-I and apoC-III were different: 3–5 copies (A-I); 20–25 copies (C-III).
[d]Copy number for apoA-I was <1.
[e]Not determined.

whereas RNA from intestine and other tissue (for example, brain) did not. As a control, the same RNA samples were analyzed with a mouse apoA-I riboprobe. As shown in Fig. 2, lower panel, endogenous apoA-I gene expression was found in both liver and intestine at approximately equal levels.

These data strongly suggested that DNA fragments A, B, and C did not contain the apoA-I intestinal control region. To search for this region, transgenic lines were made with construction D, which extended from 300 bp 5' to the apoA-I gene through the apoA-I gene, the A-I/C-III intergenic region and the apoC-III gene, to 2.5 kb 5' to the apoC-III gene. As shown in Table 1, seven independent founder transgenic mice or their progeny were studied. In five of the seven, human apoA-I was expressed in both liver and intestine. As an example, RNA from

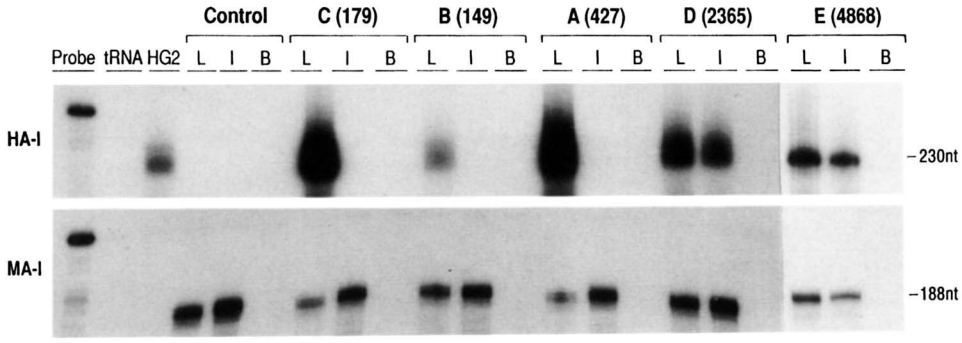
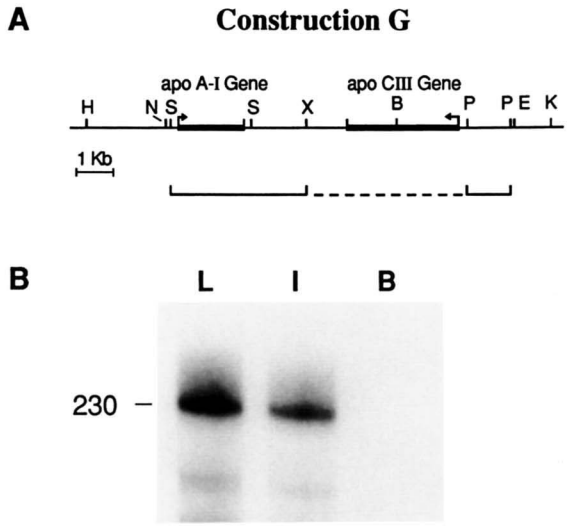


Fig. 2. RNase protection of human and mouse apoA-I mRNA. ApoA-I mRNA-specific riboprobes (5 × 10^6 cpm) were hybridized to yeast (tRNA), HepG2 cell RNA (HG2), or RNA isolated from liver (L), small intestine (I), and brain (B) of either control mice or transgenic mice produced with constructions A–E. Hybridization with a 255 nt human apoA-I riboprobe (upper panel) resulted in a protected fragment of 230 nt. Hybridization with a 210 nt mouse apoA-I riboprobe (lower panel) produced a 188 nt protected fragment.



C **RNase Protection Counting Assay**

Mouse	Probe	pg/μg total RNA	
		Liver	Intestine
6963 (A-7)	hA-I	165±15	119±12
6960 (A-14)	hA-I	496±18	473±36

Fig. 3. Construction G transgenic mice. A. Restriction map of human apolipoproteins A-I and C-III gene fragments used to make construction G. The region between PstI (P) sites at −0.2 kb and −1.4 kb 5' to the human apoC-III gene was ligated 3' to the 3.8 kb fragment of the human apoA-I gene extending from the NcoI (N) site 300 bp 5' to the XbaI (Xb) 1.7 kb 3' to the gene. B. RNase protection of human apoA-I mRNA in transgenic line A-14 produced with construction G. A human apoA-I mRNA-specific riboprobe was hybridized to 5 μg of RNA isolated from liver (L), small intestine (I), and brain (B) of transgenic mice produced with construction G. The 255 nt human apoA-I riboprobe protected a fragment 230 nt in length. C. RNase protection filter-counting assay. Apolipoprotein A-I mRNA was measured in total RNA extracted from transgenic lines A-7 and A-14, representing both orientations of construction G (see Methods). Each value was calculated from the respective hybridization standard curve, protected probe length, and the mRNA size (15). Each value represents the mean ± SD of six independent measurements read on the same standard curve generated under sample assay conditions and performed simultaneously as sample hybridization.

both liver and intestine of line 2365 is shown to protect the correct size fragment of the human apoA-I riboprobe in the upper panel of Fig. 2. In transgenic line 3386, human apoA-I was expressed in liver but not intestine and in transgenic line 4368, apoA-I was not expressed in either tissue. Although we do not have an obvious explanation for the discrepancies, overall these results strongly suggest that construction D contains the DNA region controlling human apoA-I intestinal expression.

Additional transgenic mouse lines were then created with smaller DNA fragments than the one used in construction D to further localize the apoA-I gene intestinal control region. Construction E, which extended from 300 bp 5' to the apoA-I gene to 1.4 kb 5' to the apoC-III gene was used to create two new independent lines. As shown in Table 1 and Fig. 2, upper panel, both of these expressed human apoA-I in liver and intestine. These results suggested that the intestinal control region of the apoA-I gene resided between the 5' half of the apoC-III gene and 1.4 kb 5' to the apoC-III gene. To eliminate the possibility that a DNA region within the apoC-III gene was responsible for apoA-I intestinal expression, transgenic mice were then made with construction G. For this construction, the region from 0.2 to 1.4 kb 5' to the apoC-III gene was placed in a position 3' to the apoA-I gene and eliminated the entire apoC-III gene. As shown in Table 1 and **Fig. 3,** transgenic lines made with this region in each orientation with respect to the apoA-I gene expressed human apoA-I in both liver and intestine. These results indicate that the region responsible for intestinal expression of the human apoA-I gene is 3' to the gene between 0.2 to 1.4 kb 5' to the apoC-III gene.

DNA fragments D, E, and F (Fig. 1) contained the entire human apoC-III gene. Transgenic lines created with these fragments expressed human apoC-III in plasma (Table 1). RNase protection assays indicated that each DNA fragment was capable of expressing the human apoC-III gene in both liver and intestine, as shown in **Fig. 4.** It appears that both the human apoC-III transgene and the endogenous gene are expressed at a higher level in liver as compared to intestine. These experiments suggest that the intestinal control region of the apoC-III gene is either within the gene or in the proximal 1.4 kb 5' to the gene.

DISCUSSION

In mammalian tissues, the apoA-I gene is expressed principally in liver and small intestine. To identify the *cis*-acting DNA regions controlling tissue specific expression of this gene, we have taken an in vivo approach utilizing transgenic mice. In a previous study using this method, we have shown that as little as 256 of 5' flanking sequence directs liver expression of the apoA-I gene, but 5.5 kb of 5' and 4.0 kb of 3' DNA did not allow intestinal expression. In the current study, transgenic mice were made with DNA constructions containing additional 3' sequences. Intestinal expression was achieved in transgenic mice by a DNA region from 0.2 to 1.4 kb 5' to the adjacent convergently transcribed apoC-III gene. This region has some of the properties of a tissue-specific enhancer, as it was able to direct intestinal expression when placed 3' to the apoA-I gene in the absence of apoC-III gene DNA in either orientation. We conclude from these studies that the *cis*-acting DNA sequences required for liver and intestinal expression of the apoA-I gene are physically distinct.

The apoA-I gene resides in an apolipoprotein gene cluster on chromosome 11q23 along with the apoC-III and apoA-IV genes. Although we did not set out to study

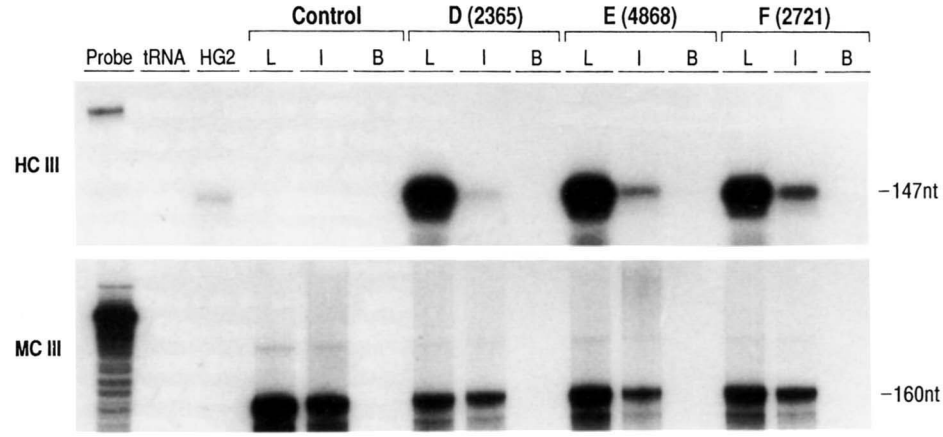


Fig. 4. RNase protection of human and mouse apoC-III mRNA. ApoC-III mRNA-specific riboprobes (5×10^6 cpm) were hybridized to yeast tRNA (tRNA), HepG2 cell RNA (HG2), or RNA isolated from liver (L), small intestine (I), and brain (B) of either control mice or transgenic mice produced with constructions D, E, and F. Hybridization with a 220 nt human apoC-III riboprobe (upper panel) resulted in a protected fragment of 147 nt. Hybridization with a 269 nt mouse apoC-III riboprobe (lower panel) produced a 160 nt protected fragment.

the *cis*-acting regions controlling tissue specific expression of the apoC-III gene, all of the constructions we used that contained the entire apoC-III gene expressed apoC-III mRNA in both liver and intestine. One of these, construction F, contained no apoA-I sequences. Thus, it appears that the apoA-I and apoC-III genes have distinct liver controlling elements. However, with the constructions we used, it is possible that the apoA-I and apoC-III genes share an intestinal control region. In a recent abstract, Lauer et al. (20) have suggested that a region similar to the one we have described controlling intestinal expression of apoA-I also directs the intestinal expression of the apoA-IV and apoC-III genes. Together these studies suggest that a single DNA element in the middle of the apolipoprotein A-I/C-III/A-IV gene complex controls intestinal expression of all three genes. This cluster of genes may have stayed tightly linked through evolution to preserve intestinal expression of these genes. If this is true, it suggests an important but as yet undefined function for intestinal expression of these genes. There is precedent for apolipoprotein genes sharing a tissue specific control region. Three apolipoprotein genes E, C-I, and C-II reside in a complex on chromosome 19q13. Evidence has been presented that a liver control element resides 3' to the adjacent apoE and C-I genes (21, 22). The intestinal control region for this complex has not yet been identified.

The identification of a gene's tissue specific control region is typically accomplished by transfecting promoter-reporter gene constructions into differentiated cells in culture. Attempts to identify the apoA-I gene intestinal element have been made by this method by transfecting various apoA-I promoter-CAT constructions into a human colon carcinoma cell line, Caco-2. In one study, a region of activity was identified between −2052 and −192 bp 5' to the apoA-I gene (23). The transgenic mouse lines made with DNA fragments B and C include this region, yet do not express the apoA-I gene in the intestine. It is still possible that a region 5' to the apoA-I gene modulates its intestinal expression, either in the basal state or in response to a physiological stimulus. Our experiments suggest that the in vivo approach is necessary to define tissue specific elements for some genes and should be used to confirm the function of such elements for other genes that have been identified by in vitro methods. The actual intestinal control element in the region from 0.2 to 1.4 kb 5' to the apoC-III gene has yet to be identified. Surprisingly little is known about DNA regions that control intestinal expression of apolipoprotein or other genes. Sweetser et al. (24) have studied the intestinal fatty acid binding protein (I-FABP) gene which is expressed specifically in the intestine. In a comparison of the 5' regions of the rat and human I-FABP genes, they identified a conserved 14 bp consensus element composed of two direct 7 bp repeats (5'-TGAACTTTGAACTT-3'). Several imperfect copies were present in each gene promoter as well as two copies in the promoter of a related gene, rat CRBP, which is also expressed in the intestine. In a follow-up study, the authors showed that I-FABP gene promoter–human growth hormone constructions containing the 14 bp consensus sequences were expressed in intestine in transgenic mice (25). However, these experiments did not exclude the possibility that another DNA sequence in the promoter region is actually responsible for intestinal expression of the I-FABP gene. Analysis of the region −0.2 to −1.4 kb 5' to the apoC-III gene reveals only a 9/14 bp match from −1226 to −1234 which may or may not be significant. Further studies with transgenes containing smaller fragments of this region will be necessary to identify the actual intestinal control element.

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