Localization of a liver-specific enhancer in the apolipoprotein E/C-I/C-II gene locus

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Abstract The sequences necessary for liver-specific expression of the apolipoprotein (apo) E gene have been shown to reside 3' to the gene, within the apoE/C-I/C-II gene cluster, but have not been precisely characterized. Utilizing a transient transfection reporter gene assay based on the apoC-II promoter, we have localized a liver-specific enhancer to its approximate limit dimension of 154 base pairs. This enhancer directed liver-specific expression of an apoE gene construction in transgenic mice. A DNaseI protection assay revealed two footprints over an inverted repeat of a known transcriptionally active motif, TGACCT. DNaseI-sensitive sites were present in three of six repeats of a motif (consensus GCAAACA) which has been postulated to represent the recognition sequence of a hepatic transcriptional activity, HNF-5. This region of DNA may function as a liver-specific enhancer for the entire apoE/C-I/C-II gene cluster.—**Shachter, N. S., Y. Zhu, A. Walsh, J. L. Breslow, and J. D. Smith.** Localization of a liver-specific enhancer in the apolipoprotein E/C-I/C-II gene locus. *J. Lipid Res.* 1993. **34:** 1699–1707.

Supplementary key words apolipoprotein E • transgenic mice • tissue-specific gene expression

Apolipoproteins (apo) E, C-I, and C-II are components of a gene family whose members play a major role in the trafficking of plasma cholesterol and triglycerides (1). ApoE is expressed predominantly in the liver, but to some degree in virtually all tissues, with significant amounts made in brain, skin, spleen, kidney, and steroidogenic tissues (2–11). ApoE is a high affinity ligand for the low density lipoprotein (LDL) receptor and the LDL receptor-related protein (12), and is felt to mediate the hepatic clearance of lipolyzed very low density lipoproteins (VLDL) and chylomicrons (13). ApoE may also be involved in the removal of excess cholesterol from peripheral tissues and in the local redistribution of cholesterol (14). ApoC-I is also expressed primarily in the liver, with lesser amounts found in skin, spleen, and lung (15, 16). Expression of apoC-I, and apoE, is induced during monocyte differentiation into macrophages (15). The function of apoC-I is less well understood but has been shown to interfere with the apoE-mediated hepatic uptake of small chylomicrons (17). Human apoC-I-expressing transgenic mice were mildly hypertriglyceridemic, an effect that was opposed in mice that simultaneously expressed human apoE (16). ApoC-II expression has not been extensively studied. In the fetus it is expressed primarily in the liver (7). ApoC-II is an obligatory cofactor for lipoprotein lipase (18) and its hereditary absence causes a well-defined clinical syndrome which mimics lipoprotein lipase deficiency (19). ApoC-II also appears to interfere with particle uptake in models of chylomicron remnant clearance (17). The cloning of cDNA sequences for the human apoE, apoC-I, and apoC-II mRNAs was followed by the documentation of close association of all three genes on chromosome 19 (20–23). Subsequent genomic cloning of the locus revealed a 48 kb cluster consisting of apoE, apoC-I, apoC-I' (a pseudogene), and apoC-II (24).

We have previously characterized transcriptionally active elements in the apoE promoter and first intron by the technique of deletional analysis of transiently transfected reporter gene constructions (25). In initial work in transgenic mice, both our laboratory (11) and Simonet et al. (26) found that an apoE construction containing 5.7 kb of 5' and 1.9 kb of 3' flanking sequence was expressed at high levels in the kidney, but not in the liver. Simonet et al. (26) observed that a cosmid containing both the apoE and apoC-I genes, terminating 23 kb 3' to apoE, was expressed in the liver of transgenic mice. In a later study (16) they reported that a 5.7 kb BamHI fragment within this cosmid, spanning the 5' end of the apoC-I' pseudogene, directed liver-specific expression of apoC-I, raising the possibility of regulation of the entire locus by

Abbreviations: apo, apolipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; PCR, polymerase chain reaction; CAT, chloramphenicol acetyl transferase.

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a single liver-specific enhancer. The present study was undertaken to further define this enhancer. We now describe the localization of this liver-specific enhancer to a 154 bp fragment with documentation of activity in both transient transfection and transgenic mice. We have also analyzed sequence motifs and characterized protein binding regions within this enhancer.

MATERIALS AND METHODS

Enhancer test constructions

The apoC-II-CAT expression vector was created from a cosmid comprising the human apoC-I, C-I', and C-II genes which was the generous gift from Marten Hofker (24). An 8.3 kb fragment extending from an NcoI site present 6 kb upstream of the apoC-II gene to a SacI site in the first intron was subcloned into pUC31. A 110 bp polymerase chain reaction (PCR) fragment was generated, which spanned the SacI site in the first intron and created a false HindIII site just prior to the translation initiation codon in the second exon, and was ligated into the above subclone. The NcoI site was then blunted with T4 polymerase and the total NcoI-HindIII insert was ligated into pKT (27), a previously described promoterless chloramphenicol acetyl transferase (CAT) vector, and entitled pC2KT. To facilitate the subcloning of enhancer fragments, a shuttle vector, pG9K2, was created by converting both the NotI and SfiI sites of pGEM9zf(−) (Promega) to KpnI sites. The 5.7 kb BamHI fragment spanning the 5' end of apoC-I' was cloned into the HindIII site of pG9K2. The entire 5.7 kb fragment or subcloned portions, produced by restriction digestion of an internal and polylinker site followed by blunt ending and self-ligation, could be excised with KpnI for ligation into the unique 5' KpnI site of pC2KT. After localization of the enhancer to 154 bp, site-directed mutagenesis was performed by digestion with StyI, treatment with T4 polymerase in the presence of all four deoxynucleotides and either self-ligation (mutation 1) or ligation of a PstI linker (mutation 2). Mutation 1 led to the duplication of bases 121–123 (CCT); mutation 2 led to a 16 bp insertion (CTGGCAGCTGCAGCTT) following base 123, both as determined by DNA sequence analysis. (The numbering of bases is as shown in Fig. 5). The minimal Adenovirus major late promoter-CAT expression vector, pXT, was created by inserting the 499 bp SalI fragment of bacteriophage λ into the EcoRI site of pCT (25). The apoE promoter-CAT expression vector contained apoE sequences from −1000 bp to +803 bp as previously described (25). Constructions were verified either by restriction enzyme mapping or sequencing by the dideoxynucleotide method. All plasmids were prepared by alkaline lysis and banded twice through cesium chloride-ethidium bromide gradients.

Cell culture, transfections, and reporter gene assays

HepG2 and HeLa cells were grown and transfected as previously described (25). Each transfection contained the CAT expression vector along with the β-galactosidase expressing vector pCH110 (28), which was used as an internal control for transfection efficiency. CAT and β-galactosidase assays were performed as previously described (25). The percent of the chloramphenicol substrate acetylated was determined by liquid scintillation counting after thin-layer chromatography. The CAT activity of each sample was corrected for transfection efficiency by dividing by its corresponding β-galactosidase activity.

Transgenic mice

Transgenic mice were created as reported previously (11). Integration of the human apoE gene was determined by Southern blotting of tail-derived DNA as previously described (11). RNA derived from various tissues were probed for the presence of human apoE mRNA using a previously described RNase protection assay (11).

ApoE protein assay

The level of human apoE in the plasma of transgenic mice was determined through the courtesy of Henry N. Ginsberg, Columbia College of Physicians and Surgeons. A previously reported apoE radioimmunoassay was used that showed no significant cross reactivity with normal mouse plasma (29).

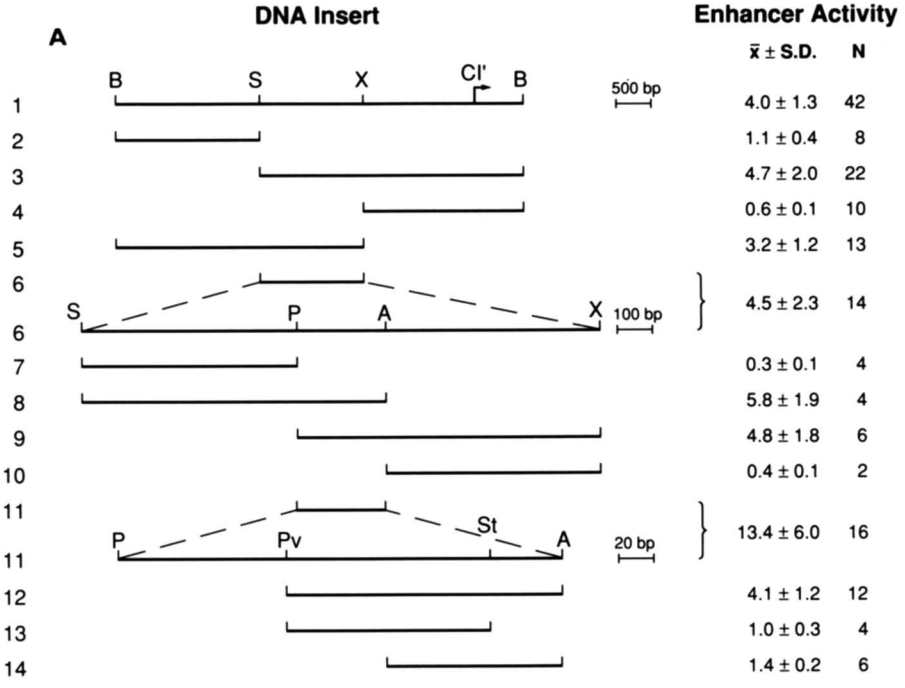
DNaseI protection assay

The 154 bp PvuII-ApaI liver-specific enhancer fragment was cloned with linkers into the HindIII site of pG9K2 and sequenced to determine its orientation. In order to specifically label the 3' end of the sense strand, the fragment was excised from the polylinker with XbaI and NsiI, and labeled by filling in the XbaI overhang with T4 DNA polymerase in the presence of [α-^{32}P]dCTP and the other three dNTPs. The antisense strand was labeled similarly using a SacI-Tth111I fragment in the presence of [α-^{32}P]dATP. The probe (50,000 cpm) was incubated in a final volume of 50 μl with either albumin, as a control, or HepG2 nuclear extract, prepared according to the method of Dignam, Lebowitz, and Roeder (30), in 15 mM HEPES, pH 7.9, 45 mM potassium chloride, 1 mM magnesium chloride, 1 mM dithiothreitol, 3% Ficol, and 25 μg/ml of poly dIdC. The mixture was incubated for 15 min at room temperature and treated with 5 μl of a DNaseI mix for 1 min at room temperature. The DNaseI mix contained 5 mM calcium chloride, 1 mM EDTA, and either 0.45 μg/ml or 30 μg/ml DNaseI for the albumin- and nuclear extract-treated probes, respectively. DNaseI digestion was halted by the addition of 10 μl of 10 mg/ml proteinase K, 5 μl of 10 mg/ml tRNA, and 10 μl of a solution containing 0.6 M sodium acetate, 1% sodium

dodecyl sulfate, 5 mM EDTA, and 100 μg/ml glycogen. This mixture was incubated at 65°C for 20 min, and ethanol-precipitated before running on a 6% polyacrylamide, 6 M urea sequencing gel. In order to define the position of the protected regions, a G + A sequence ladder (31) was prepared from each probe and run alongside the reaction products in the gel.

RESULTS

The goal of this study was the localization of a liver-specific enhancer that has been shown to reside within a 5.7 kb region spanning the 5' end of the apoC-I' pseudogene. We suspected that this region might mediate the hepatic expression of all three expressed apolipoprotein genes in the chromosome 19 cluster. In order to rapidly localize this enhancer, this 5.7 kb region was tested in the context of an apoC-II promoter–CAT expression vector, pC2KT, in a cell culture assay system. As diagrammed in **Fig. 1A,** we observed that the 5.7 kb BamHI fragment (insert 1), in the context of pC2KT, behaved as an enhancer, giving rise to a consistent fourfold increase in CAT activity in transiently transfected HepG2 cells, a human hepatoma cell line. Successive deletions of the 5.7 kb fragment were performed in a shuttle vector. These smaller fragments were tested, many in both orientations; and the effect remained consistent, orientation-independent, and always present only in one part of a divided fragment. The enhancer activity was further



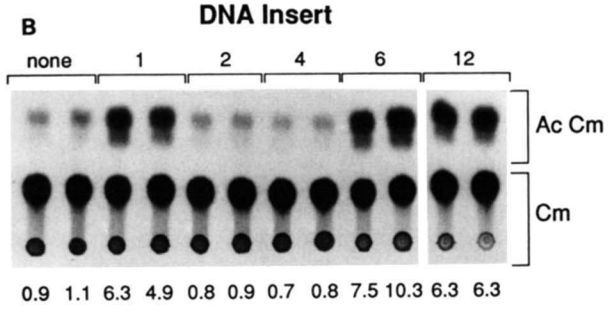


Fig. 1. Deletional analysis of the enhancer activity of the 5.7 kb BamHI fragment. A: A schematic representation of DNA fragments used as inserts in the pC2KT CAT expression vector and their enhancer activity versus pC2KT as measured in transiently transfected HepG2 cells. Data are presented as the mean ± standard deviation for the number of independent plates indicated corrected for transfection efficiency as stated in the Methods section. The CAT activity data for each transfection was normalized to the mean CAT activity of the enhancerless pC2KT expression vector. Construct 11 with 13-fold enhancer activity was subsequently found to have two copies of the 248 bp enhancer insert. The beginning of the apoC-I' pseudogene is denoted by the arrow labeled CI', and relevent restriction endonuclease sites are shown: B, BamHI; S, SphI; X, XbaI; P, PstI; A, ApaI, Pv, PvuII, St, StyI. B: A representative CAT assay of extracts from transiently transfected HepG2 cells. Each plasmid was transfected in duplicate plates, and extracts with equal units of β-galactosidase activity, used as an internal control for transfection efficiency, were assayed in each CAT reaction. The DNA insert refers to the numbering shown above in part A. The CAT activity data for each transfection was normalized to the mean CAT activity of the enhancerless pC2KT expression vector. Cm, chloramphenicol; Ac Cm, acetylated chloramphenicol.

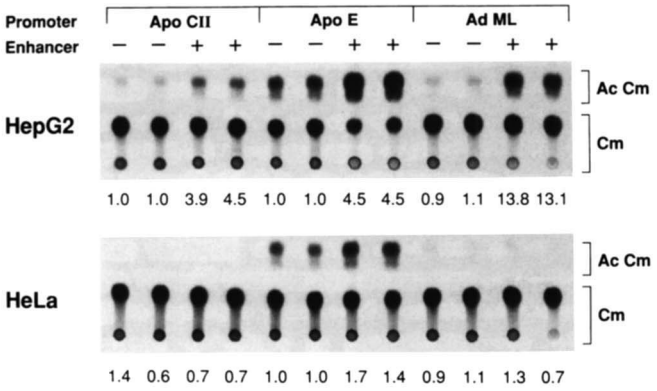
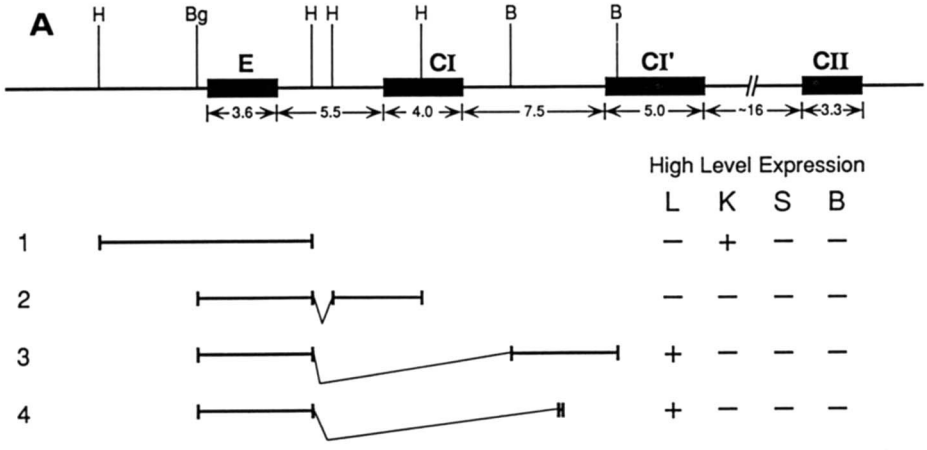


Fig. 2. Cell type-specificity of the 154 bp fragment enhancer in the context of three different promoters. CAT expression vectors driven by either the apoC-II, apoE, or Adenovirus major late (Ad ML) promoters were tested with or without the insertion of the 154 bp enhancer fragment (insert 12 of Fig. 1A) by transient transfection into both HepG2 and HeLa cells. Each plasmid was tested in duplicate plates. Equal volumes of extract were used for the CAT assay. The percent of chloramphenicol converted to the acetylated form was determined by scintillation counting and was subsequently normalized for the activity of the cotransfected β-galactosidase expression vector. The autoradiograph shown, therefore, does not reflect normalization for β-galactosidase activity. The normalized CAT activity numerical data for each promoter is shown. Values are relative to the mean CAT activity of the enhancerless promoter expression vector. Cm, chloramphenicol; Ac Cm, acetylated chloramphenicol.

localized to a 1.4 kb SphI-XbaI segment (insert 6) in the center of the BamHI fragment and subsequently to a 250 bp PstI-ApaI segment (insert 11) in the center of the 1.4 kb fragment. The 154 bp PvuII-ApaI fragment (insert 12) retained full activity while deletion of the 3' 30 bp at a Sty I site (insert 13, 124 bp) abolished activity. A 98 bp PCR product missing the 5' 56 bp (insert 14) also lacked enhancer activity. Therefore, enhancer activity required sequence elements found at both ends of the 154 bp fragment. The results of the CAT assays are presented quantitatively in Fig. 1A and a representative CAT assay is shown in Fig. 1B.

In order to determine whether a cell-type-specific enhancing effect would be present in other promoter contexts, the enhancer activity of the 154 bp fragment was compared in the context of the apoC-II, apoE, and Adenovirus major late promoters in both HepG2 and HeLa cells. We observed an approximately fourfold effect of the 154 bp fragment in the apoC-II and apoE promoter contexts, and a 13-fold effect in the Adenovirus major late promoter in HepG2 transfections. In contrast, we observed a very slight effect in HeLa cells only in the apoE promoter context (**Fig. 2**). We observed a similar pattern and degree of cell line-specific enhancer activity for the parental 5.7 kb BamHI fragment (data not shown). We, therefore, concluded that the 154 bp fragment contains the entire sequence necessary for cell line-specific enhancer activity.



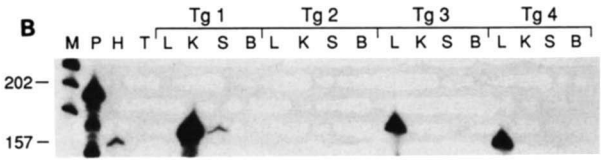


Fig. 3. Human apoE transgene constructions and their expression in various tissues of transgenic mice. A: A map of the human apolipoprotein gene cluster on chromosome 19 is shown along with intergenic distances in kb, and the restriction sites used in the transgene constructions. H, HindIII; Bg, BglII; B, BamHI. Under the map, lines 1 through 4 represent DNA inserts used to generate lines of transgenic mice, along with a summary of high level of expression of the human apoE transgene in liver (L), kidney (K), skin (S), and brain (B). B: An RNase protection gel assay detecting human apoE transcripts. Ten μg of total RNA derived from tissues of transgenic lines 1 through 4 (as defined above), along with a positive control, HepG2 RNA (H), and a negative control, yeast tRNA (T) were hybridized to a human apoE riboprobe as previously described (11). The probe alone (P) is 202 nucleotides, and the fragment protected by human apoE transcripts is 157 nucleotides. Lane M shows a labeled phiX DNA as a size marker.

We then sought to determine whether this 154 bp enhancer, defined in transient transfections, corresponded to the sequences necessary for liver-specific expression of the human apoE gene in transgenic mice. **Fig. 3A** shows the organization of the human chromosome 19 apolipoprotein gene cluster, along with four constructions used to generate human apoE transgenic mice. The expression of these constructions in liver, kidney, skin, and brain, as determined by a human apoE RNase protection assay, is summarized (Fig. 3A). A representative RNase protection gel is shown in Fig. 3B. ApoE transgenic construction 1 is an 11.1 kb HindIII fragment containing 5.7 kb of 5' and 1.9 kb of 3' flanking sequence. We have previously reported this construction as showing high levels of expression in kidney, but not in liver in three independent lines of transgenic mice (11). Construction 2 was created by truncating construction 1 at a −650 bp BglII site and joining it to a 5 kb HindIII fragment spanning the 5' end of the apoC-I gene. This construction exhibited little or no expression in any tissue tested in three independent lines. Replacement of the 5 kb fragment by the 5.7 kb BamHI fragment spanning the 5' end of the apoC-I' pseudogene (construction 3) conferred liver-specific expression of the apoE gene in two independent lines. The 154 bp fragment which exhibited enhancer activity in tissue culture, when linked to the same apoE gene fragment (construction 4), also gave a liver-specific expression pattern in two independent lines of transgenic mice. Human apoE mRNA levels in these construction 4 transgenics were very low or nondetectable in kidney, skin, and brain (Fig. 3B), as well as in heart, intestine, and spleen (data not shown). The plasma level of human apoE in one of the two lines made from construction 4 was 13 μg/ml, compared to an average value for normal human males of 46.7 ± 16.3 μg/ml (29). The 154 bp enhancer is therefore sufficient for liver-specific expression of the human apoE gene in transgenic mice.

Fig. 4 shows a DNaseI protection experiment using the 154 bp liver-specific enhancer fragment as a probe. After incubation with a HepG2 nuclear extract, we observed two DNaseI protected regions, or footprints, near the 3' end of the fragment, covering nucleotides 114 to 127 and 133 to 146. These two footprints were specifically competed by unlabeled DNA (data not shown). In addition to DNaseI protection by factors in the nuclear extract, we also observed sites that were rendered DNaseI-sensitive at positions 37, 88, and 95. The DNA sequence of the 154 bp liver-specific enhancer (**Fig. 5**) contains two copies in opposite orientation of the sequence CTGACCT, a known transcriptionally active motif that is found in the promoters of numerous apolipoprotein and other liver-expressed genes (**Table 1**). A partially overlapping inverted repeat, GACCTCT, was found at the 3' end of the 154 bp fragment. The sequence also contains six repeats (three perfect, two 6/7, and one 5/7) of a heptanucleotide

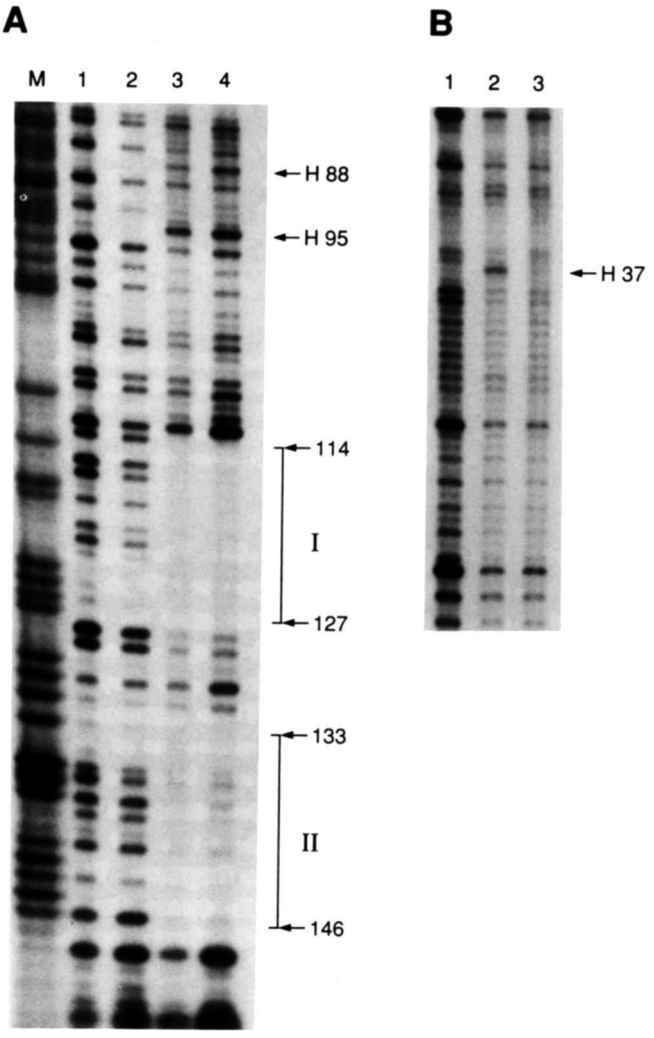


Fig. 4. Gel autoradiography of DNase I protection assay. Panel A shows the sense strand of the 154 bp liver-specific enhancer which was labeled on its 3' end as described in Methods. Lane M is the G + A sequence ladder; lanes 1 and 2 were incubated with 30 μg of bovine serum albumin, as controls, before DNase I digestion; and lanes 3 and 4 were incubated with 30 μl of a HepG2 nuclear extract. The brackets with Roman numerals delineate two protected footprints, shown along with their nucleotide positions. H represents sites rendered DNase I sensitive by incubation with the HepG2 nuclear extract. Panel B shows the antisense strand labeled on its 3' end. Lane 1 shows the albumin control; lane 2 HepG2 nuclear extract; and lane 3, HepG2 nuclear extract plus excess unlabeled specific DNA fragment. The extract-mediated DNaseI-sensitive site at position 37 is competed away by the inclusion of unlabeled probe fragment.

sequence, GCAAACA, which matches the recognition sequence of a proposed liver-specific transcriptional activity, HNF-5 (38) (**Table 2**). Two of the sites rendered DNaseI-sensitive by incubation with nuclear extract were found at the third A of adjacent perfect copies of the GCAAACA motif at positions 88 and 95. The third sensitive site was located on the antisense strand at position 37 within a 6/7 HNF-5 consensus homology. The position of the DNaseI-sensitive sites within the heptanucleotides is consistent

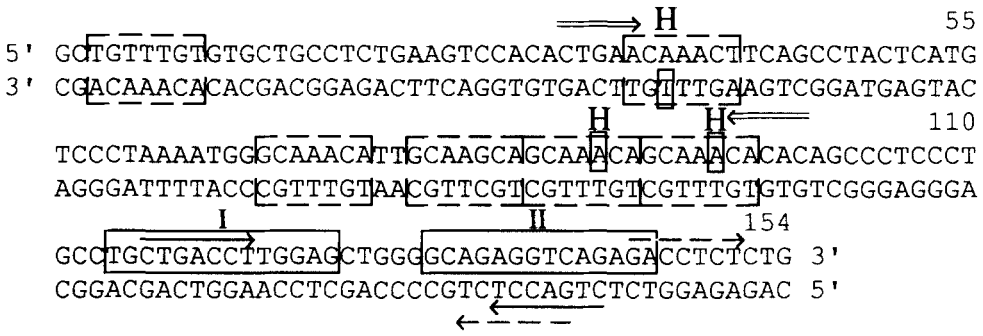


Fig. 5. Annotated sequence of the 154 bp liver-specific enhancer fragment. The HepG2 nuclear extract-mediated DNaseI footprints and hypersensitive sites are shown in the solid boxes labeled with Roman numerals and H, respectively, on the strand as shown in Fig. 4. The GCAAACA motif, or related elements, are shown in dashed line boxes. The single arrow shows the position of the perfect inverted repeat CTGACCT; the dashed arrow, the inverted repeat AGAGGTC; and the double arrow, the inverted repeat ACTGAA.

with HNF-5 binding, as described (38). The position 37 site was bracketed by inverted repeats of CTGAA.

A StyI site within footprint I was used for the site-directed mutagenesis of the 154 bp liver-specific enhancer. Two mutations were made; mutations 1 and 2 led to 3 bp and 16 bp insertions, respectively. These mutations were tested for enhancer activity in the context of pC2KT by transient transfection into HepG2 cells. Mutations 1 and 2 showed 2.2 ± 0.1 and 0.9 ± 0.1 fold enhancer activity, respectively, eliminating most of the 4.1 ± 1.2 fold enhancer activity of the native 154 bp fragment. The site-directed mutagenesis data support the notion that factors binding to the footprint I region are involved in mediating enhancer activity.

DISCUSSION

In the present study we have localized the liver-specific enhancer in the apoE/C-I/C-II gene cluster using transient transfection and transgenic technology. A 5.7 kb region of DNA spanning the 5' end of the apoC-I' pseudogene, previously shown to be responsible for the liver-specific expression of apoC-I in transgenic mice (16), was shown to function in tissue culture as a liver cell line-specific enhancer in the context of the apoC-II promoter. The discovery of enhancer activity in a tissue culture system allowed us to perform a rapid deletional analysis of the 5.7 kb region and localize the enhancer to its limit size of about 154 bp. The 154 bp region most likely functions as a general hepatocyte-specific enhancer. The enhancer effect was evident in the context of apoC-II, apoE, and Adenovirus major late promoters in transient transfections of HepG2 cells but not of HeLa cells, models for hepatic and nonhepatic tissues, respectively. We have confirmed the liver-specific enhancer activity of this 154 bp region by demonstrating its mediation of the liver-specific expression in transgenic mice of a short apoE fragment, which is otherwise not expressed in liver (16). The human apoE gene construction containing the 154 bp enhancer led to full liver-specific expression in transgenic mice, without detectable renal expression, identical to that produced by the 5.7 kb parent region.

TABLE 1. Liver enhancer footprint I sequence homology in selected hepatically expressed genes

Gene	Location	Sequence	Reference
Liver enhancer	114	TGCTGACCTTG	current
ApoC-II	- 157	ACGTGACCTTG	32
ApoC-III	- 86	AGGTGACCTTT	32
ApoB	- 73	TTTGGACCTTT	33
ApoA-I	- 135	AGCTGATCCTT	32
ApoA-I	- 214	ACTGAACCCTT	34
ApoA-II	- 737	CCTTCAACCTT	35
Acyl CoA ox.	- 572	ACGTGACCTTT	36
ApoA-IV	- 150	TTGTGACCCTG	37
α1-AT	- 85	GGGTGACCTTG	34

Species is human unless otherwise indicated. Location for liver enhancer refers to sequence presented in Fig. 5. All other locations are relative to the transcriptional start site. α1-AT, α1-antitrypsin; Acyl CoA ox., rat acyl-CoA oxidase (peroxisomal proliferator responsive enhancer, footprint A).

TABLE 2. HNF-5 site sequence homology in selected hepatically expressed genes

Gene	Location	Sequence	Reference
TAT, HNF-5 consensus		$^{G}_{A}$CAAA$^{C}_{T}$A	38
TAT	- 5354	AGCAAATA	38
Liver enhancer	+ 83	AGCAAACAG	current
ApoB	- 103	AGCAAACAG	39
α1-AT	- 128	AGCAAACAG	40
Albumin	- 8.75 kb	AGCAAATA	41

Species is human unless otherwise indicated; TAT, rat tyrosine aminotransferase, the antisense strand sequence is shown; albumin, mouse albumin, the antisense strand of the enhancer region is shown.

We have also performed a DNaseI protection assay and found two footprints at the 3' end of the 154 bp enhancer, with each containing a copy (in opposite orientation) of a sequence, CTGACCT. Site-directed mutagenesis within footprint I was found to markedly diminish enhancer activity supporting a functional role for this element. Deletion of the 3' 30 bp comprising footprint II and part of footprint I abolished enhancer activity. However, the region of these footprints was insufficient for enhancer activity as demonstrated by the elimination of activity by deletion of 56 bp at the 5' end of the 154 bp fragment (Fig. 1A, insert 14). Factors interacting with the 5' end of the enhancer did not produce a footprint under the conditions of the assay. Three sites were, however, rendered DNaseI-sensitive after incubation with nuclear extract, two at the same position within adjacent copies of GCAAACA and the third in the 5' part of the enhancer within a 5/7 sequence identity (Fig. 5).

The TGACCT element, as shown in Table 1, has been found in transcriptionally active regions of numerous apolipoprotein (32) and other hepatically expressed genes (37). The extended motif found within footprint I, TGACCTTGG, is also found 145 bp 5' to the apoC-II gene (32). The core motif is similar to a family of elements binding a variety of transcription factors within the steroid hormone receptor superfamily (42). A number of transcription factors have been shown to interact specifically with this motif and regulate apolipoprotein gene expression, as summarized in a recent review (33). For example, nuclear factor AF-1, which binds this motif, has been shown to be necessary for high levels of expression of apoC-III gene transcription (32). Other factors that bind this motif, ARP-1 and EAR-3, members of a recently described steroid hormone receptor subfamily, and factor HNF-4, have been shown to interact with this motif in the apoA-I and apoC-III promoters (43). These factors and transcription factor EAR-2 have also been shown to interact with this motif within the apoB and apoA-II promoters (35). The apoA-I element has been shown to respond selectively to RXRα but not to other members of the steroid receptor superfamily (44). The enhancer footprint I is similar to footprint A of the acyl-CoA oxidase peroxisome proliferator-responsive enhancer (36). This raises the possibility that a characterized transcription factor, the peroxisome proliferator-activated receptor, may interact with the enhancer as part of the coordinate regulation of genes involved in triglyceride metabolism (45).

The GCAAACA motif has also been found in a transcriptionally active segment of the apoB promoter adjacent to a TGACCT-type element (39). Multiple repeats of a similar element (consensus G/A CAAA C/T A) have been noted to be present in liver-specific footprints of two remote glucocorticoid responsive units of the liver-expressed gene tyrosine aminotransferase (38). A cell type-specific activity binding to this motif has been characterized by DNaseI footprint analysis and termed HNF-5 (38). HNF-5 binding produces a characteristic asymmetric pattern of DNaseI cleavage (38). The sensitive sites at bp 88 and 95 are found within the HNF-5 consensus sequence and are consistent with the HNF-5 DNaseI cleavage pattern. The sensitive site at bp 37 is found within a 6/7 match to the HNF-5 consensus on the antisense strand and exhibits the expected cleavage site for this orientation. A number of liver expressing genes containing the HNF-5 motif in regulatory regions are listed in Table 2. A similar sequence is found in a DNaseI footprint within the mouse albumin enhancer but could be competed by an HNF-3 binding oligonucleotide (41).

In summary, we have shown that the 154 bp fragment functions as a tissue-specific enhancer in cultured cells, displaying positive transcriptional activity at a distance from the apoC-II promoter and in either orientation, as well as activity in a heterologous viral promoter. We have also shown that this 154 bp fragment is sufficient for liver-specific expression of the apoE transgene. This 154 bp enhancer is localized within, and may be responsible for the activity of, the 5.7 kb region shown by Simonet et al. (16) to be necessary for the liver-specific expression of the adjacent apoC-I gene in transgenic mice. We speculate that this 154 bp region may mediate the liver-specific expression of all three expressing apolipoprotein genes in the human chromosome 19 cluster. Examples of liver enhancers functioning throughout a multigene locus are limited. It appears that a single DNA region may mediate the intestinal expression of the chromosome 11 apolipoprotein gene cluster (apoA-I/C-III/A-IV) (46–48). ApoE, C-I, and C-II are components of VLDL and play a major role in triglyceride metabolism. It has been speculated that the consequent need for coordinate regulation has been the evolutionary impetus for the maintenance of this gene cluster (49). Further studies will be needed to establish whether the 154 bp enhancer mediates the liver-specific expression of the other two apolipoprotein genes in this cluster, and to elaborate the mechanism and factors involved.

Note added in proof: After the submission of this manuscript, Simonet, et al. published the localization of a liver-specific enhancer to a 764 bp region, 5' to the apoC-I' pseudogene. The 154 bp enhancer fragment that we have characterized lies within this 764 bp region. Simonet, W. S., N. Bucay, S. J. Lauer, and J. M. Taylor. 1993. *J. Biol. Chem.* **268:** 8221–8229.

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