Identification of promoter sequences in the 5′ untranslated region of the baboon apolipoprotein[a] gene

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Abstract Like humans, baboons possess apolipoprotein[a] (apo[a]), a unique protein component of the atherogenic lipoprotein [a] (Lp[a]) particle. Baboon apo[a] also exhibits extensive variation with respect to size and serum levels. In this report, we have cloned the 5′ flanking region of the baboon apo[a] gene (I isoform) and performed promoter mapping studies to identify sequences that control apo[a] transcription. The sequence of the baboon apo[a] 5′ flanking region is similar to the human gene, and contains two Alu repeats that distinguish the apo[a] gene from plasminogen and other apo[a]-like genes. The transcription start site for the baboon apo[a] gene is located 85 bp upstream from the major start site for the human apo[a] gene. For promoter mapping studies, we constructed two sets of deletion clones (5′ to 3′ and 3′ to 5′) in luciferase reporter plasmids for transfection of hepatic cell lines (HepG2 and Huh7). These experiments showed that the 5′ untranslated region (5′ UTR) contains a positive promoter element with 85% identity to the consensus binding site for hepatocyte nuclear factor 1α (HNF-1α), and a negative element that is functional in HepG2 cells, but not Huh7 cells. Transfection assays with HeLa cells showed that the positive promoter element acts in an hepatocyte-specific manner. We also cloned the 5′ flanking region from a baboon carrying a null allele that produced no detectable hepatic transcripts or serum isoforms in vivo. Surprisingly, the 5′ flanking regions of the null allele possessed a promoter that was functional in transfection assays. We conclude that the baboon apo[a] gene 5′ UTR contains hepatocyte-specific promoter elements, but that other unknown sequences must influence apo[a] expression in vivo.—**Hixson, J. E., C. Jett, and S. Birnbaum.** Identification of promoter sequences in the 5′ untranslated region of the baboon apolipoprotein[a] gene. *J. Lipid Res.* 1996. **37:** 2324–2331.

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Apolipoprotein[a] (apo[a]) is a unique protein constituent of the atherogenic lipoprotein particle lipoprotein[a] (Lp[a]). Apo[a] is highly polymorphic with respect to size in human populations, due to differences in copy numbers of tandemly repeated kringle IV subunits (1). The gene for apo[a] is a member of a gene family on chromosome 6q26–q27 that also contains plasminogen and two shorter apo[a]-like genes (2, 3).

The apo[a] gene itself accounts for over 90% of the variation in serum Lp[a] levels in human populations, with size responsible for a large percentage of that variation (4, 5). Larger isoforms are generally associated with lower serum levels of Lp[a]. The remainder of the variation may be due to *cis*-acting sequences such as promoter elements that regulate apo[a] transcription. Recent studies have searched for effects of polymorphisms in the 5′ flanking region of the human apo[a] gene, and associations have been found between numbers of a TTTTA repeat and serum Lp[a] levels (6, 7). Using in vitro transfection assays in human hepatocarcinoma HepG2 cells, Zysow et al. (8) reported that a C/T polymorphism in the 5′ UTR may reduce apo[a] translational efficiencies by introducing an upstream ATG start codon. Wade, Lindahl, and Lawn (9) have mapped key promoter elements to the 5′ UTR region of the human apo[a] gene that act in an hepatocyte-specific manner. In contrast, Bopp et al. (10) reported that the proximal apo[a] 5′ flanking region only supports basal promoter activities that are not hepatocyte-specific.

Like humans, baboons possess Lp[a] that contains apo[a] (11), and the apo[a] gene is linked with the plasminogen gene (12). In addition, baboon apo[a] is highly polymorphic with respect to size (13), due to differences in sizes of hepatic transcripts (14). The size differences are presumably due to differences in numbers of kringle IV subunits, which are present in apo[a]

Abbreviations: apo[a], apolipoprotein[a]; Lp[a], lipoprotein[a]; kb, kilobase; PCR, polymerase chain reaction; RT, reverse transcriptase; 5′ UTR, 5′ untranslated region.

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cDNA from the rhesus monkey, a close relative of the baboon (15). Like humans, size heterogeneity of apo[a] isoforms influences Lp[a] levels in baboons. Recent studies of cultured primary baboon hepatocytes showed that larger apo[a] isoforms have reduced efficiency with respect to post-translational processing and secretion (16).

In this study, we cloned and sequenced the 5′ flanking region of the baboon apo[a] gene to search for sequences that regulate apo[a] gene transcription. To map such control elements, we constructed an overlapping set of deleted apo[a]/luciferase plasmids for transfection of cultured human hepatoma cells (HepG2 and Huh7) and a non-hepatic cell line (HeLa). In addition, we cloned the 5′ flanking region from a baboon carrying a null allele with no detectable hepatic apo[a] transcripts (or serum isoforms), and constructed a reporter plasmid to measure its promoter activities in transfected HepG2 and Huh7 cells.

MATERIALS AND METHODS

Cloning of the 5′ region of the apo[a] gene from wild-type and null alleles

Genomic DNA was isolated from leukocytes from a baboon homozygous for the I apo[a] isoform (serum Lp[a] level of 19.8 mg/dl), and subjected to partial digestion with Sau3A to obtain fragments 14–23 kb in length. These fragments were ligated with λGEM-11 arms (Promega; Madison, WI) for packaging and plating. Approximately 720,000 plaques were screened with a radiolabeled probe containing PCR-amplified baboon apo[a] exon 1 sequences. Five cross-hybridizing clones were obtained, and subjected to amplification using apo[a]-specific and plasminogen-specific primers as synthesized from human gene sequences. Two sets of apo[a]-specific primers used the same forward primer from the second Alu repeat in the 5′ flanking region of human apo[a] (Fig. 1, positions −727 to −706, CCCTC CTGGAAGGATTGATATC) and different reverse primers (−462 to −444, CAGGCAGTGCTGTACGAG; and +98 to +130, CACAGAATTCCATGGCATATGTATTT TTACTAC) (17–19). The plasminogen-specific primers used sequences to amplify 130 bp from exon 2, which is located approximately 5 kb downstream from exon 1 in the plasminogen gene (forward primer GTCAAGGA GAGCCTCTAGATGACTATGTG, reverse primer GGT GAATTCTTCGTCCTCCTCACATTTTGC) (20). One of the clones (λBabplg) amplified only with the plasminogen-specific primers, but not with the apo[a]-specific primers. Another clone [λBabapo[a]] amplified only with the apo[a]-specific primers, but not with the plasminogen-specific primers. Subsequent nucleotide sequencing of subclones derived from enzymatic deletion showed λBabapo[a] contained about 1.5 kb of the apo[a] gene 5′ flanking region (including the two diagnostic Alu repeats), and exon 1 (including 5′ UTR and coding sequences). The rest of λBabapo[a] contains a large intron similar to intron 1 (14 kb) in the human apo[a] gene, but does not contain kringle IV repeat sequences which begin with exon 2 in the human gene (18).

We cloned 1.5 kb of the 5′ region of the apo[a] gene from a baboon carrying a null allele that produces no detectable hepatic apo[a] transcripts or serum isoforms, and also from the control wildtype baboon (I isoform homozygote described above). Leukocyte DNA from these two baboons were amplified using apo[a]-specific primers with synthetic MluI sites and cloned into pAMP1 (Gibco BRL; Grand Island, NY) (forward primer −1424 to −1405, ACGCGTTGCGGAAAGATTG ATACTATGC; and reverse primer −6 to +16, ACGCGT CTTCCTTATGTTCCCTTATGGG). The downstream primer contained a mismatch (underlined) that inactivates the ATG codon for transfection assays as previously described (8). These clones were completely sequenced using flanking and internal primers.

Mapping the transcription start site for the baboon apo[a] gene

Hepatic mRNA from a baboon carrying the I isoform was used for primer extension with an oligonucleotide from the signal peptide and first kringle IV region of the apo[a] sequence (GACCACATGGCTTTGCTCAGG TGCTGCTGATTTCAG, ref. 19). Total RNA was isolated from homogenized liver tissue in the presence of RNase inhibitors as previously described (14), followed by addition of oligo-dT and column elution of mRNA (Invitrogen; San Diego, CA). For primer extension reactions, the primer was radiolabeled by T4 DNA kinase, mixed with hepatic mRNA (20 μg) and reverse transcriptase (800 units), and incubated for 50 min at 42°C. The reverse transcriptase products were electrophoresed on a denaturing 6% polyacrylamide gel adjacent to a DNA sequencing ladder and a molecular weight marker (HinfI cut phage ϕX174 DNA).

To verify results from primer extension experiments, we used four different primer sets for reverse transcriptase-mediated PCR (RT-PCR) of hepatic mRNA. All RT-PCR reactions used single-stranded cDNA made from reverse transcription of hepatic mRNA (20 μg) with the same reverse primer used for primer extension assays. This single-stranded cDNA was amplified using four different forward primers from positions −252 to −233 (upstream from predicted start site) (CAGCATG

GAGCAGCTGAGGG), −225 to −206 (GGCTTTCACC ACTTCCCAGC), −217 to −198 (CCACTTCCCAGCAT CTATTG), and −198 to −175 (GACATTCCACTCTCA AATATTTTG). These PCR reactions used 35 cycles of denaturation (94°C for 40 sec), annealing (58°C for 40 sec), and extension (72°C for 1 min).

Construction of deletion clones for promoter mapping

Two sets of apo[a] deletion clones (5′ to 3′ and 3′ to 5′) were constructed from the I allele using a reporter plasmid containing the luciferase gene. A clone spanning the region from the second Alu repeat to the start codon (−727 to −6) was constructed by amplification of λBabapo[a] and subcloning into pGL2 containing the luciferase reporter gene (Promega; Madison, WI) (forward primer −727 to −706, CCCTCCTGGAAGGA TTGATATC; and reverse primer −29 to −6, GACTGG CCAGCAGTGCCCAGAAAG). The 3′ to 5′ deletions were constructed by PCR amplification of the −727 apo[a]/pGL2 clone using the same forward primer containing a MluI site (−727 to −706, CCCTCCTGGA CGCGTGATATC), and different reverse primers containing synthetic XhoI sites (−41 to −24, GCCTCGAG AAAGTGTGTCCCCATC; −61 to −44, GCCTCGAGAA TGTTGTTGACTTAC; −81 to −64, GCCTCGAGAGTA AACGCATCCAC; and −135 to −118, GCCTCGAGACC CATGCCACTGCC). These PCR fragments were cloned into the MluI/XhoI site of the pGL2 promoter plasmid. The 5′ to 3′ deletion clones were constructed using S1 nuclease/Exonuclease III digestion under conditions recommended by the supplier (Promega; Madison, WI).

Transfection of baboon apo[a]/luciferase constructs into hepatic cell lines

Each of the full length and deleted apo[a]/luciferase plasmids was transfected into HepG2 and Huh7 cell lines, and cell extracts were measured for luciferase activity. Cell lines were grown to near confluency (70%–80%) in DMEM:HamsF12 media (Cell-Gro; Herndon, VA) supplemented with L-glutamine and 10% fetal bovine serum. For transfections, cells were plated in 60-mm dishes (2.0×10^6 cells for HepG2 and 1.5×10^6 for Huh7 cells), grown for 24 h, and then incubated (4 h) with 10 μg purified plasmid DNA, 30 μg lipofectin (Gibco BRL; Grand Island, NY), and 5 μg β-galactosidase plasmid (pSV-β-gal; ProMega, Madison, WI). The transfected cells were washed, and then grown in complete medium for an additional 72 h. To prepare extracts, harvested cells were treated with lysis buffer (Promega; Madison, WI), centrifuged, and supernatants were assayed for luciferase and β-galactosidase using a luminometer (Turner Designs Model 20E; Sunnyvale, CA). Luciferase measurements were normalized by β-galactosidase measurements to adjust for differences in efficiencies of transfection. Each transfection assay was performed in duplicate, and included control transfections with no DNA and with luciferase reporter plasmids (pGL2 plasmid containing the SV40 early promoter). Sets of transfections experiments were repeated two to five times for apo[a] constructs. Luciferase activities for each deletion clone or null allele were derived as a percentage of those of the wild type apo[a] allele in each set of transfection assays.

RESULTS

Sequence of the 5′ flanking region of the baboon apo[a] gene

Leukocyte DNA from a baboon homozygous for the I isoform was used to construct a genomic library for screening with an apo[a] exon 1 probe, and several cross-hybridizing clones were isolated. To distinguish clones containing apo[a] from those containing plasminogen or other apo[a]-like genes, we synthesized primer sets from sequences that were only present in the human apo[a] gene (17–19). These primers amplified sequences in one of the cross-hybridizing clones (λBabapo[a]). We also synthesized a set of plasminogen-specific primers from exon 2 that is not found in the human apo[a] gene (20). These primers did not amplify λBabapo[a] sequences, but did amplify another clone (λBabplg). The nucleotide sequence of approximately 1.5 kb of the λBabapo[a] insert was determined from the set of deletion clones that was constructed for promoter mapping.

In addition to the cloning of λBabapo[a], we used PCR to clone the 5′ flanking region of the apo[a] gene from the same baboon homozygous for the I isoform. Apo[a]-specific primers were used to amplify a region from the beginning of the first Alu repeat to the coding sequence in exon 1 (17, 19). Amplification of leukocyte DNA yielded a single 1.5 kb PCR fragment, which was subsequently cloned into a plasmid vector for sequencing with flanking and internal primers.

Figure 1 shows the sequence of the 1.5 kb 5′ flanking region of the baboon apo[a] gene, which was identical between λBabapo[a] and the PCR-derived clone. This region contains two tandemly associated Alu repeats in the same orientation, a feature that is unique to the 5′ flanking region of the human apo[a] gene relative to plasminogen and the other apo[a]-like genes (19). This region also contains exon 1 of the baboon apo[a] gene and a small portion of intron 1. **Figure 2** shows the per-

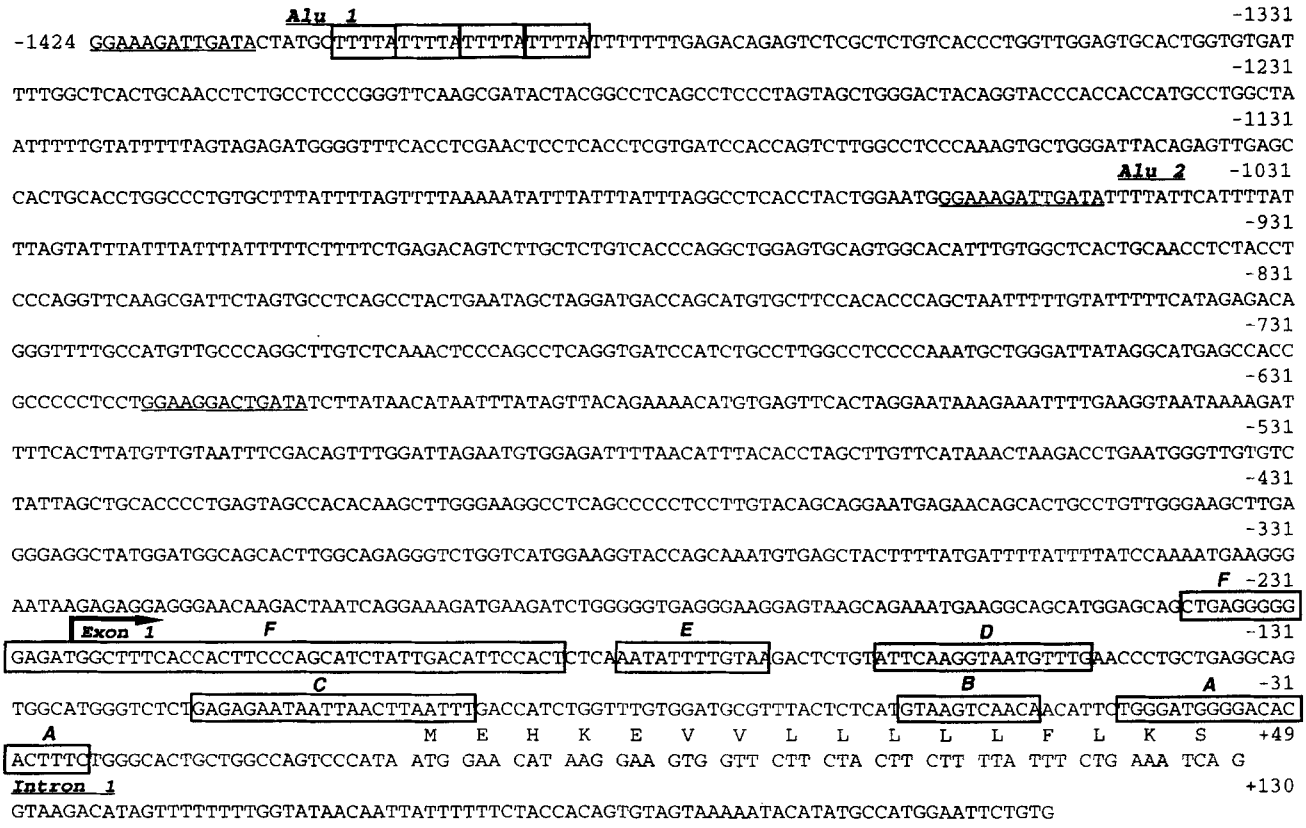

Fig. 1. Sequence of the 5′ flanking region of the baboon apo[a] gene. The sequence of the baboon apo[a] gene 5′ flanking region is numbered relative to the ATG translational start codon; and the positions of Alu repeats, Exon 1, and Intron 1 are indicated above the sequence in italics. The start site of transcription is shown by an arrow above the sequence. The boxed sequences show TTTTA repeats, and the labeled boxes (A–F) show DNaseI footprints (9). Underlined sequences show the short direct repeats flanking the Alu repeats.

cent identity between human and baboon apo[a] sequences for each of these regions.

Mapping of the transcription start site of the baboon apo[a] gene

We determined the start site for transcription of the baboon apo[a] gene by primer extension using baboon hepatic mRNA and an oligonucleotide from the signal peptide and the first kringle IV region of the apo[a] gene (19). The reverse transcription product was 301 bp (**Fig. 3**), corresponding to a transcription site at position −226 (Fig. 1). This site is 85 bp upstream from that of the human apo[a] gene at position −141. To verify the primer extension results, we used RT-PCR of baboon hepatic mRNA using the same reverse primer with four different forward primers located upstream from

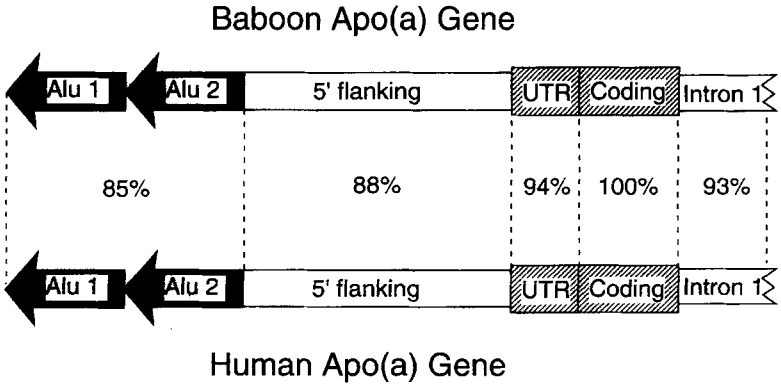

Fig. 2. Sequence comparisons between baboon and human apo[a] gene 5′ flanking regions. In this schematic comparison of baboon and human apo[a] genes, the filled arrows show the position and orientation of two Alu repeats, the open boxes show the 5′ flanking region and intron 1, and the hatched boxes show the 5′ UTR and coding sequences of exon 1. The percent identities between the baboon and human apo[a] sequences (17, 19) are given between the maps.

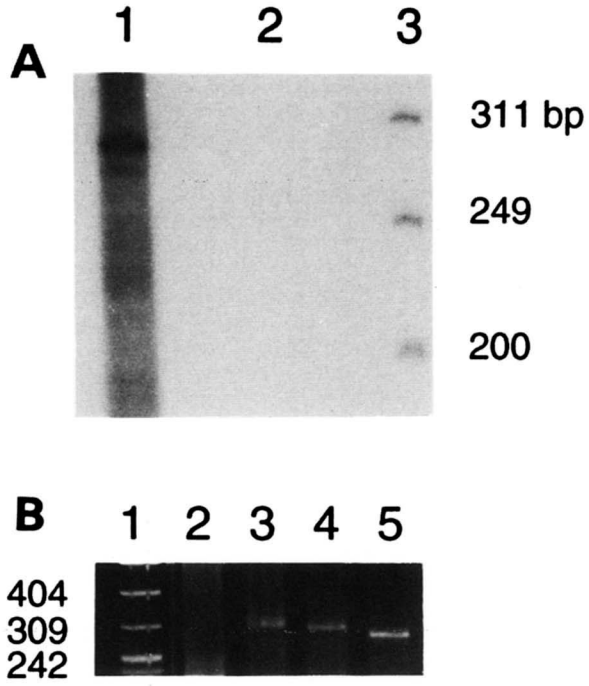

Fig. 3. Mapping the transcription start site of the baboon apo[a] gene. Panel A shows the products of primer extension with baboon hepatic mRNA using a radiolabeled reverse primer from the signal peptide and the first kringle IV of baboon apo[a] (lane 1). Lane 2 shows a control experiment in which no mRNA was added to the primer extension reaction, and lane 3 contains HinfI cut phage ϕX174 DNA as a size marker (sizes shown to the right in base pairs). Panel B shows RT-PCR products using the same reverse primer and forward primers located upstream (lane 2) and downstream (lanes 3–5) from the transcription initiation site as predicted from primer extension. Lane 1 contains HpaII-digested pBR322 DNA as a size marker (sizes shown to the left in base pairs).

the predicted start site (position −252 to −233) or downstream from the start site (−225 to −206, −217 to −198, −198 to −175). Figure 3 shows that the upstream primer did not amplify baboon apo[a] sequences, while all sets of downstream primers produced PCR products with sizes predicted from the baboon apo[a] sequence (300 bp, 292 bp, and 273 bp, respectively).

Promoter mapping in the 5′ flanking region of the baboon apo[a] gene

In order to map promoter sequences in the apo[a] gene 5′ flanking region, we used PCR to amplify 720 bp of the λBabapo[a] clone downstream from the Alu repeats to immediately upstream from the ATG start codon in exon 1 (positions −727 to −6, Fig. 1). This fragment was cloned upstream from the luciferase gene in a reporter plasmid that did not contain any other enhancer or promoter sequences. We also constructed a set of overlapping clones with deleted sequences from 5′ to 3′, using enzymatic digestion of the −727 apo[a] clone. To construct a set of deletion clones from 3′ to 5′, we used a forward primer to amplify sequences from the −727 apo[a] clone and reverse primers from different sequences in the 5′ UTR region. **Figure 4** shows maps and nucleotide endpoints for each of the 5′ to 3′ and 3′ to 5′ deletion clones.

All of the full-length and deleted apo[a]/luciferase reporter clones were transfected into human hepatoma cell lines HepG2 and Huh7, and cell extracts were assayed for luciferase activity. As a control, a luciferase reporter plasmid containing the SV40 early promoter was also used for transfection assays. Figure 4 shows the promoter activities from each clone in HepG2 and Huh7 cells. The activities for the deletion clones are given as a percentage of the activity of the −727 apo[a] clone (set at 100%). Overall, transcriptional activities were approximately 10% of the control plasmid containing the SV40 promoter, and activities were 10-fold higher in Huh7 cells than in HepG2 cells. However, in most cases, the percent activities of the deletion clones relative to the −727 apo[a] clone were similar in the two cell lines.

The only two clones that showed striking differences in promoter activities from the −727 apo[a] clone contained deletions in the baboon apo[a] 5′ UTR region. In vitro studies of the human apo[a] gene have identified three control elements in the 5′ UTR that interact with transcription factors, and are protected from DNase 1 digestion by HepG2 extracts (called footprints) (9). These footprint sequences are also present in the baboon apo[a] 5′ UTR (Fig. 1). The 3′ deletion clone that deleted only the A footprint (Δ3′-44) had consistently higher activities (296%) in HepG2 cells than the −727 apo[a] clone, but not in Huh7 cells (Fig. 4). The Δ3′-64 clone that removed the B footprint had normal promoter activities. The Δ3′-118 clone that removed footprint C had consistently reduced promoter activities relative to the −727 apo[a] bp clone (18% in Huh7 cells, 30% in HepG2 cells). To determine whether the footprint C promoter activities were specific to liver cells, we also transfected the −727 apo[a] clone, the Δ3′-118 clone, and the control plasmid into HeLa cells. Unlike the HepG2 and Huh7 cells, we found no difference in the promoter activities between the active −727 apo[a] clone and the inactive Δ3′-118 clone in transfected HeLa cells.

Promoter analysis of the 5′ flanking region from an apo[a] null allele

After identification of promoter elements in the 5′ UTR, we examined the promoter activity of a null apo[a] allele. We isolated genomic DNA from a baboon in which we could not detect apo[a] hepatic transcripts (by Northern blot) or serum apo[a] isoforms (by West-

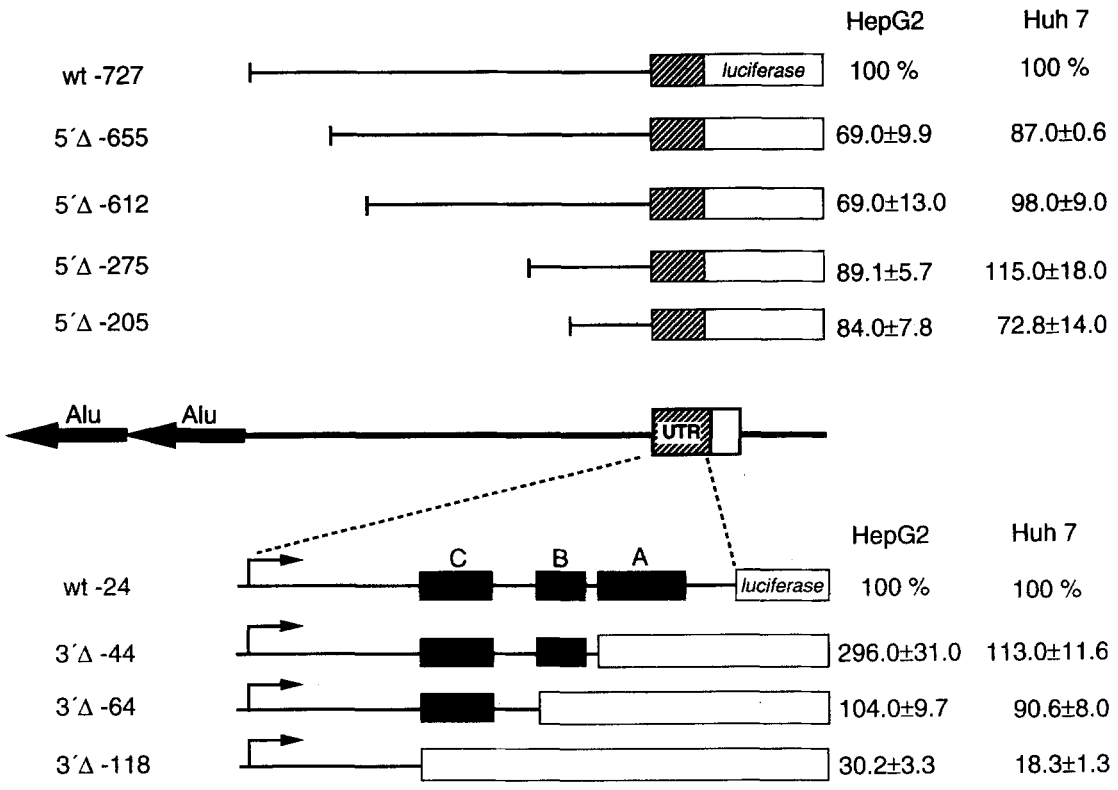

Fig. 4. Relative transcriptional activities of deleted baboon apo[a]/luciferase plasmids in transfected HepG2 and Huh7 cells. The map in the middle shows the position of Alu repeats in the 5′ flanking region and exon 1 of the baboon apo[a] gene. Above the map, the endpoints of 5′ to 3′ deleted apo[a]/luciferase clones are given on the left, and relative transcriptional activities in transfected HepG2 and Huh7 cells are given on the right. The means and standard errors for transcriptional activities of apo[a] deletion clones are presented as percentages of luciferase activities of the wildtype-727 apo[a] clone. Below the map, the endpoints and relative transcriptional activities of the 3′ to 5′ deletion apo[a]/luciferase clones are given. The filled boxes (A–C) show the DNaseI footprints in the 5′ UTR as determined for the human apo[a] gene (9).

ern blot) (13, 14). We then used PCR to amplify the 1.5 kb 5′ UTR fragment for cloning into luciferase reporter plasmids. As described above, we also used these primers to amplify and clone the 1.5 kb fragment from the same homozygous baboon (I isoform) that was used to isolate the genomic clone λBabapo[a]. The 1.5 kb fragment from the null allele contained an active promoter, although the promoter activities were reduced in both HepG2 (60.8% ± 5.9%) and Huh7 cells (65.6% ± 6.3%) relative to the wild-type I allele. We determined the complete nucleotide sequence of the 1.5 kb fragment from the null allele, and found a single nucleotide substitution (T→C) at position −194 in the 5′ UTR.

DISCUSSION

The 5′ flanking region of the baboon apo[a] gene is very similar to the human gene, including two Alu repeats that distinguish the human apo[a] gene from plasminogen and other related genes (19). Baboon and human gene sequences are 85% identical in the Alu repeats and 88% identical in the rest of the 5′ flanking sequences. Exon 1 is more highly conserved, with 94% identity in the 5′ UTR and 100% identity in coding sequences (Fig. 2) (17, 19). As predicted by the emergence of the duplicated apo[a] gene prior to the divergence of baboon and human lineages, the 5′ flanking region of the human apo[a] gene is more similar to the baboon gene than to the human plasminogen gene (77%) (17). The transcription start site of the baboon apo[a] gene is different from the human gene. We used primer extension and RT-PCR to map the start site to position −226, 85 bp upstream from the start site for the human gene (Fig. 3). The study that mapped the human gene start site also reported additional minor sites located further upstream (18). Perhaps the baboon apo[a] start site corresponds to one of these minor upstream sites in the human gene.

Transfection experiments with deleted baboon apo[a] sequences show that proximal promoter ele-

ments are not in their usual locations upstream from the transcriptional start site, but rather in the 5′ UTR of the first exon. This unexpected observation was also reported from promoter mapping experiments that found key regulatory elements in the 5′ UTR of the human apo[a] gene (9). Deletion of the baboon apo[a] gene 5′ UTR to position −118 (clone Δ3′-118, Fig. 4) consistently reduced transcriptional activities by 70% and 82% in transfected HepG2 and Huh7 cells, respectively. This deletion removes footprint C containing the sequence GAGAATAATTAAC (positions −113 to −101, Fig. 1), which is 85% identical to the HNF-1α consensus sequence (GTTAATNATTAAC) (9). This positive promoter element acts in a hepatocyte-specific manner, given that no differences were detected among these constructs in transfected HeLa cells which do not contain HNF-1α (21). These results were similar to those from Wade et al. (9), who found that deletion and point mutations in footprint C caused consistent loss of promoter activities in transfected HepG2 cells. They also performed gel mobility shift assays that showed binding of HNF-1α in HepG2 cell extracts with a synthetic oligonucleotide containing the putative HNF-1α binding site in the human apo[a] gene (9).

In addition to this positive element, our experiments identified a negative promoter element in the 5′ UTR of the baboon apo[a] gene. Deletion of footprint A stimulates apo[a] transcriptional activities by 3-fold in HepG2 cells. This result was different from Wade et al. (9) who found that introduction of point mutations in footprint B, but not footprint A, caused increased promoter activities. This different result is not likely due to species-specific sequence differences, as footprint B in the baboon apo[a] gene is 100% identical to the human gene and footprint A contains only a single nucleotide substitution (T→G at position −38). It may be that point mutations in these footprints have different effects on promoter activities compared wth deletions. For example, deletion of footprint C in the human gene had considerably less effect on promoter activities than sequence substitutions in the same footprint (9).

Transfection analyses of 5′ to 3′ deletion clones revealed some small effects of sequences in the 5′ flanking region on transcriptional activities. Deletion of the region from −727 to −655 resulted in 30% reduction in transcriptional activity in HepG2 cells. Therefore, this region may contain a weak positive promoter element that is functional in HepG2, but not in Huh7 cells. Transfection analyses of 5′ to 3′ deletion clones of the human apo[a] gene using HepG2 cells also identified a weak positive element between −632 to −560 (corresponds to −744 to −701 of the baboon gene, Fig. 1) in the 5′ flanking region (9).

After identifying promoter elements in the baboon apo[a] gene, we cloned 1.5 kb from the 5′ flanking region from a baboon carrying a null allele that produced no detectable hepatic apo[a] transcripts or serum apo[a] isoforms. This null allele possessed an active promoter, but showed reduced activities in both HepG2 (61%) and Huh7 (66%) cell lines relative to the wild-type (I) apo[a] allele. We identified a single nucleotide difference in the 5′ UTR of the null allele, a T→C substitution at position −194. This substitution is located in footprint F (9), and may reduce promoter activities by altering interactions with hepatic proteins involved in apo[a] transcription. Nevertheless, the transfection assays showed that this null allele is not completely due to the T→C substitution in the 5′ UTR, but also likely due to changes outside the 1.5 kb fragment. It should be noted that Bopp et al. (10) also found that 1.5 kb of the 5′ flanking region from two human null alleles possessed unaltered in vitro transcriptional activities in transfected HepG2 cells.

In studies of human subjects, a simple repeat polymorphism $(TTTTA)_n$ in the first Alu sequence has been associated with differences in Lp[a] levels (6, 7). The baboon apo[a] 5′ flanking region contains the TTTTA repeat (boxed sequences, Fig. 1), but we did not find any polymorphisms in copy numbers among the sequenced alleles. In humans, extensive variation in copy numbers of this repeat (6 to 11) has been observed, while the baboon alleles only contain four copies. Presumably, this small number of TTTTA repeats in the baboon gene is not sufficient to undergo molecular processes resulting in extensive variation in copy numbers. A C/T polymorphism in the human apo[a] gene (corresponding to position −49, Fig. 1) has been shown to reduce translational efficiency by introduction of an upstream ATG that may interfere with translation from the normal start codon (8). In the baboon genes that were sequenced in this study, position −49 invariably contained a C. This substitution is much rarer than the hypervariable $(TTTTA)_n$ polymorphism in humans, and the present studies cannot exclude the possibility of a similar substitution in baboons. However the next nucleotide is an A (−48) rather than a G, so a T substitution would not introduce an upstream start codon in the baboon gene.

In conclusion, our results demonstrate the presence of an hepatocyte-specific promoter element in the 5′ UTR of the baboon apo[a] gene. Our results appear to contradict those from Bopp et al. (10), who concluded that this promoter region only operates at a basal level without tissue specificity. However, our finding that the 5′ UTR from a null allele possesses an active promoter in vitro shows that other, as yet unknown, promoter elements must also be important for apo[a] transcription. Supporting this conclusion are recent studies that failed

to detect significant differences in in vitro transcriptional activities of apo[a] alleles marked by specific polymorphisms that have been associated with differences in Lp[a] levels in human populations (6, 10). An alternate explanation for the apparent lack of correspondence between in vivo and in vitro allelic transcriptional activities may be the cultured cell lines used for transfection assays, which may lack particular factors involved in apo[a] gene expression. The definitive identification of apo[a] transcriptional control sequences will likely emerge from further experiments with cloned apo[a] alleles.

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