Transgenic Mice Expressing Full-length Human Apolipoprotein B-100

FULL-LENGTH HUMAN APOLIPOPROTEIN B mRNA IS ESSENTIALLY NOT EDITED IN MOUSE INTESTINE OR LIVER

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Apolipoprotein (apo) B-100 mRNA is edited in the small intestine (in all mammals examined) and the liver (in mice and rats only) to produce apoB-48 mRNA. ApoB mRNA editing involves a C \(\rightarrow\) U conversion of the first base of the codon CAA for Gln-2153 in apoB-100, changing it to an in-frame stop codon (UAA). The edited mRNA encodes apoB-48, which is colinear with the N-terminal 48% of apoB-100. ApoB mRNA editing can be reconstituted in vitro using cellular extracts from one species to edit synthetic apoB mRNA sequences from a different species. Editing of transcripts from transfected genes also appears not to be species-specific. We have produced transgenic mice that express full-length human apoB-100 mRNA at high levels in the liver and small intestine. Human apoB-100 (a 550-kDa protein) but not apoB-48 (a 260-kDa protein) is detected in total plasma (at \(\sim\)22 mg/dl) and in very low density and low density lipoproteins. The endogenous mouse plasma apoB concentration is reduced in the transgenic animals. Thus, the transgenic mice can form an animal model for familial hyperapolipoproteinemia. In surprise, we found that the full-length human apoB-100 mRNA is resistant to editing in the liver and small intestine. ApoB-48 (edited) mRNA is present in the small intestine, responsible for full-length human apoB-100 mRNA editing by the mouse editing enzyme. Human apoB-100 mRNA competes poorly with human apoB-48 mRNA for interacting with the editing enzyme. This has implications for the sequence-specific mechanism of RNA editing. Further caution is needed in the interpretation of in vitro RNA-editing experiments.

Apolipoprotein (apo) B-100 is a major protein component in the plasma lipoproteins, very low density lipoproteins (VLDL), low density lipoproteins (LDL), and lipoprotein(a). It is a physiological ligand for the LDL receptor, and plasma apoB-100 levels are positively correlated with the development of atherosclerosis (Brunzell et al., 1984; Brown et al., 1990). The hyperapolipoproteinemia (hyperapolipoprotein B) syndrome, defined as elevated apoB levels in the presence of normal plasma cholesterol, is strongly correlated with coronary artery disease (Snyderman et al., 1980, 1982; Teng et al., 1986). ApoB-100 is known to bind to LDL receptors of 550 kDa and containing 4536 amino acids. A transgenic mouse with a deletion of the N-terminal 48% of apoB-100 (in mice and rats only) to produce apoB-48 mRNA is used as a model for familial hyperapolipoproteinemia (hyperapolipoproteinemia B) syndrome, defined as elevated apoB levels in the presence of normal plasma cholesterol, is strongly correlated with coronary artery disease (Snyderman et al., 1980, 1982; Teng et al., 1986). ApoB-100 is one of the largest proteins known, having a molecular mass of 550 kDa and containing 4536 amino acids. A transgenic mouse with a deletion of the N-terminal 48% of apoB-100 (in mice and rats only) to produce apoB-48 mRNA is used as a model for familial hyperapolipoproteinemia (hyperapolipoproteinemia B) syndrome, defined as elevated apoB levels in the presence of normal plasma cholesterol, is strongly correlated with coronary artery disease (Snyderman et al., 1980, 1982; Teng et al., 1986). ApoB-100 is one of the largest proteins known, having a molecular mass of 550 kDa and containing 4536 amino acids.

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1 The abbreviations used are: apo, apolipoprotein; PCR, polymerase chain reaction; FPLC, fast protein liquid chromatography; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; kb, kilobase pair(s); bp, base pair(s); VNTR, variable number of tandem repeats.
ims in cultured cells indicate that there is little species specificity in editing, and human apoB mRNA is a good substrate for editing enzymes from different mammalian species. Partial purification of the rat apoB editing enzyme reveals that the editing activity is sensitive to protease but unaffected by RNAse treatment (Driscoll et al., 1989; Chen et al., 1990; Driscoll and Casanova, 1990). It has an apparent molecular mass of 40–50 kDa, which is very similar in size to a rat liver nuclear protein that has been shown by cross-linking experiments to bind to synthetic human apoB mRNA in vitro (Lau et al., 1990; Greene et al., 1991). The partially purified rat enzyme also edits synthetic human apoB mRNA in vitro (Lau et al., 1990; Greene et al., 1991).

The efficiency of apoB mRNA editing in vitro is variable and can be artificially changed from no editing to low level editing of the apoB mRNA substrate by adjusting the conditions for the reaction (Driscoll et al., 1989; Chen et al., 1990). Even under the best conditions, only a small fraction (~5%) of the in vitro apoB mRNA is edited compared with over 90% editing detected in the small intestine in vivo. In order to examine the editing phenomenon under natural in vivo conditions, we have constructed a full-length human apoB minigene for microinjection to produce transgenic mice. We have generated transgenic animals that efficiently express the 14-kb apoB mRNA in a tissue-specific manner. It is the first time that the full-length apoB is expressed in transgenic mice; attempts to do so in the past have frustrated investigators because of the length and complexity of the apoB gene. To our surprise, the transgenic human apoB mRNA consists almost entirely of the unedited (apoB-100) form in both liver and small intestine; in contrast, in the same animals, the endogenous mouse apoB mRNA contains the normal proportions (60 and 90%, respectively) of apoB-100 and apoB-48. This is in contrast to the situation in the human apoB-48 mouse, where the edited and unedited forms are present in about equal amounts. These two tissues. The circulating mouse apoB mRNA consists entirely of human apoB-100, making these transgenic mice an ideal model for hyperapolipoproteinemia.

**EXPERIMENTAL**

**Construction of Full-length ApoB Minigene**—The full-length apoB minigene construct is assembled from two parts: part 1 was isolated from a human cosmid library (Wei et al., 1985). The primers used were: for human apoB: 5'-ATCATAATTATCTTTAATATACTG-3'; 3' primer 5'-GGAATTCATTATCT-3' (3'; 3' primer 5'-GGAATTCATTATCT-3'). The two fragments were ligated together and subcloned in the HindIII/EcoRI sites of the plasmid pBR322 using artificial linkers with unique restriction sites Xmal and Sfil. The sequences across all ligation sites were checked by direct sequencing.

**Transgenic Mice Production**—The minigene construct was purified free of plasmid sequences and injected into the male pronucleus of fertilized mouse ova. Microinjection was performed in FVB mice. Transgenic animals were identified by Southern hybridization of tail DNA. A total of 240 eggs was injected, and 170 were transplanted into pseudopregnant ICR females. Among the 60 offspring, four showed integration into the host chromosomes. Three transmitted the transgene to their progeny.

**Immunoblot Analysis**—Plasma or lipoprotein samples were electrophoresed on 4–20% SDS-polyacrylamide gels. They were transferred onto nitrocellulose membrane (BA85, Schleicher & Schuell) electrophoretically. The membranes were then exposed to rabbit polyclonal or mouse monoclonal antibodies. The rabbit polyclonal antibody was directed against highly purified human LDL, which contained apoB-100 as the only protein component. The antibody does not recognize mouse apoB (Xiong et al., 1991). The monoclonal antibodies were generous gifts of Dr. Y. L. Marcel (Clinical Research Institute of Montreal) (for Bsl 72) and Dr. Linda Curtiss (Scipri Institute for Medical Research) (for MB19, MB5, and MB47). The antibodies were detected by 125I-protein A followed by autoradiography using Kodak XAR-5 x-ray films.

Mouse plasma apoB concentration was determined by quantitative Western blot analysis using an anti-rat apoB antibody that does not detect human apoB (Sparks et al., 1992). We present the relative concentration using the values in nontransgenic controls as 100%. FFPC Chromatography—FFPC chromatography was performed by the method of Jiao et al. (1990), using two Superose 6 columns (Pharmacia LKB Biotechnology Inc.) connected in series on a Beckman System Gold HPLC/FFPC system. Transgenic mice were fed regular mouse chow. They were fasted 16 h before being bled at 10 a.m. Plasma from a single mouse (250 ml) was applied to the system. The columns were eluted at a constant flow rate of 0.5 ml/min with 1 mM EDTA, 150 mM NaCl, and 0.02% Na2HPO4 (pH 8.2) at room temperature. Fifty fractions of 0.5 ml were collected, and a sample (150 ml) of each fraction was used to measure total cholesterol and triglyceride content.

**Sequential Ultracentrifugation Fugitation**—Plasma samples were fractionated by ultracentrifugation flotation essentially as described (Schuckman and Pupponie, 1986) based on a method previously used for separating mouse lipoproteins (Beauf et al., 1983). Plasma samples were adjusted to the appropriate densities using potassium bromide solutions. Samples were layered at 30 °C, 50,000 rpm in a Ti 70.1 rotor. VLDL (d < 1.006 g/ml) for 18 h, and HDL (d > 1.063 g/ml) for 48 h. Further purification of the VLDL was performed by repeat ultracentrifugation. Plasma lipoprotein fractions were washed twice with 10% trichloroacetic acid. Total RNA was isolated using the method of Chirgwin et al. (1979). It was dissolved in water and transferred to 0.1 M NaCl. The RNA was methylated with methyl group incorporation into DNA (or cDNA) using α-[32P]dCTP (100 mCi/mM, Amersham). The samples were annealed to the VLDL, LDL, and HDL fractions was performed by repeat ultracentrifugation.

**Assay of Proportion of Edited ApoB mRNA**—The proportion of edited apoB mRNA was determined by RNAse protection assay (Zegers et al., 1990). The assay was performed with RNA from 150 mice, each of which had apoB-100 as the only protein component. The antibody does not recognize mouse apoB (Xiong et al., 1991). The monoclonal antibodies were generous gifts of Dr. Y. L. Marcel (Clinical Research Institute of Montreal) (for Bsl 72) and Dr. Linda Curtiss (Scipri Institute for Medical Research) (for MB19, MB5, and MB47). The antibodies were detected by 125I-protein A followed by autoradiography using Kodak XAR-5 x-ray films.

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GATCAAATTATACG-3'; and 5'-TACTGATCAATTGTATCG-3', respectively. Hybridization was performed in 6 x SSC, 5 x Denhardt's solution, 0.1% SDS, 3.5 μg/ml salmon sperm DNA at 48 °C for B-Stop or 50 °C for B-Gln for 12-24 h using 5'-end-labeled B-Stop or B-Gln probes. Washings were performed in 6 x SSC, 0.1% SDS at room temperature for 20 min, followed by 2 x SSC, 0.1% SDS at 50 °C for 1.5 min (for B-Stop) or 52 °C for 2 min (for B-Gln). Blots were exposed to Kodak X-Omat AR film at -70 °C for 18 h. All blots were first hybridized to the B-Stop probe, stripped, and rehybridized to the B-Gln probe. This PCR cloning/colony hybridization technique was used for analyzing the B-Stop/B-Gln ratio in total RNA isolated from rat liver and intestine described below. Sequencing of over 200 randomly selected clones showed a 100% concordance between oligonucleotide hybridization results and direct sequence analysis.

Cloning and Partial Sequencing of Human ApoB mRNA Expressed in Transgenic Mice—We amplified the human sequences from total transgenic mouse intestinal and liver RNA by PCR using the following primers: 5' primer: 5'-ATGACCATCAGGATGCACATA-3'; 3' primer: 5'-ATTCCTGTCGCCCCAGGCTCAGG-3'. The 3-kb amplification product was subcloned into the plasmid pBR322. Multiple clones were sequenced in the double-stranded form by the dideoxynucleotide chain termination technique. The completed sequence covered nucleotides 6009-7522.

RESULTS

Transgenic Mice Express Human ApoB in a Tissue-specific Manner—The design of the human apoB gene construct is shown in Fig. 1. It spans 22 kb and is assembled from five cloned cassettes, three from genomic clones and two from cDNA clones. It contains 4.5 kb of the 5'-flanking region and 0.5 kb in the 3'-flanking region ending 69 bp downstream to the hypervariable A-T-rich repeat (Boerwinkle et al., 1989). The minigene contains 7 introns (natural introns 22, 23, 24, 27, 28). The 8 exons correspond to natural apoB exons and 4 artificial fusion exons (encompassing exons 26-29). Transgenic animals were generated by transfection of ES cells. Four independent transgenic lines each contained the transgene.

Northern blot analysis of apoB mRNA from transgenic mouse tissues and nontransgenic littermate controls. A 14-kb human apoB mRNA was identified in the liver and intestine of the transgenic mice (Fig. 2). It was identical in size to human apoB mRNA isolated from HepG2 cells, indicating that the introns were accurately spliced in the mouse. Under the experimental conditions, the human apoB cDNA probe did not cross-hybridize to mouse apoB mRNA, and the 14-kb band was not present in nontransgenic littermate control tissues. Conversely, use of a rat apoB cDNA probe detected endogenous mouse apoB mRNA but not the human apoB mRNA isolated from HepG2 cells (Fig. 2). The human apoB mRNA signal was consistently more intense than the mouse signals, indicating that the concentration of the human mRNA is higher than that of the mouse mRNA.

Transgenic Human ApoB-100 Is Present in Total Plasma, Exclusively in the VLDL and LDL Fractions—The plasma cholesterol and triglyceride were not different between transgenic and nontransgenic controls (Table I). When transgenic mouse plasma was characterized by immunoblot analysis using an anti-human apoB polyclonal antiserum, we detected a band of molecular mass ~ 550 kDa identical in size to human plasma apoB-100 (Fig. 3a).

To test whether the transgenic apoB is secreted in association with lipoprotein fractions, we fractionated the transgenic mouse lipoproteins by FPLC (Jiao et al., 1990). This technique gives good resolution of the mouse plasma lipoproteins into very low density lipoprotein (VLDL), low density lipoprotein (LDL) fractions, and monomeric apolipoprotein profile between these two fractions (Fig. 3b). There is no difference in the plasma triglyceride concentration between the two types of animals and no apoB bands were run on 4-20% polyacrylamide gels, showing that there is no apoB in the dense LDL (Fig. 3c) but the apoB band was also visible in the nontransgenic sample (see below).

Fig. 1. The full-length human apoB minigene construct. The natural human apoB gene structure is shown at the top. Thick black bars represent exons, and thin lines represent introns and 5' and 3' flanking sequences. An open box at the 3' end of the construct represents the variable number of tandem repeats (VNTR) region characterized by Boerwinkle et al. (1989). The construct is made up of five cassettes: I, genomic clone containing 4.5 kb in the 5' flanking region, and the first 3 exons and introns, and part of exon 4; II and III, two cDNA clones incorporating exon sequences from exon 4 to exon 25; IV, genomic clone encompassing part of exon 25, intron 25, exon 26, intron 26, and part of exon 27; V, genomic clone containing part of exon 27, intron 27, exon 28, intron 28, exon 29, 3'-flanking region ending 69 bp 3' to the VNTR.
Electrophoresed on apoR mate. RNAs from various tissues were transferred to nitrocellulose membrane. Two different cDNA probes were used for hybridization. The size standards were an RNA ladder of the marked sizes in kb.

**Fig. 2.** Northern blot analysis of apoB mRNA from a transgenic mouse and its nontransgenic littermate. RNAs from various tissues were electrophoresed on 1% agarose gel and transferred to nitrocellulose membrane. Two different cDNA probes were used for hybridization. The size standards were an RNA ladder of the marked sizes in kb.

**TABLE I**

| Lipoprotein Fract. | Total Protein (mg/dl) |
|--------------------|-----------------------|
| Triglyceride       | 114.0 (87.98)         |
| Transgenic         | 108.0                 |
| Control            | 86.53                 |
| Cholesterol        | 114.0 (87.98)         |
| Transgenic         | 108.0                 |
| Control            | 86.53                 |
| Transgenic LDL     | 9.12 (89.61)          |
| Transgenic VLDL    | 14.96 (8.45)          |
| Transgenic HDL     | 10.19 (32.25)         |
| Control LDL        | 5.69 (40.51)          |
| Control VLDL       | 2.66 (7.15)           |
| Control HDL        | 9.43 (2.27)           |
| Transgenic VLDL    | 5.69 (40.51)          |
| Control VLDL       | 2.66 (7.15)           |
| Control HDL        | 9.43 (2.27)           |

Full-length Human ApoR mRNA Is Essentially Not Edited in Transgenic Mouse Intestine or Liver. Immunoassays using anti-human apoR antisera, were used to detect any protein products the size of apoB-48. This is in contrast to the easily detectable mouse apoB-48 since both mouse liver and intestine are known to efficiently edit apoB mRNA and produce large amounts of apoB-48. Occasionally, in the transgenic animals, small amounts of immunoreactive material were detected that were slightly smaller than apoB-100. These likely represent minor proteolytic products of apoB-100. They were always much larger than apoB-48 in size. The absence of circulating human apoB-48 could be explained either by a rapid turnover of secreted apoB-48 or the lack of apoB-48 production by the transgenic animals because of inefficient editing of human apoB mRNA by the mouse.

In order to check whether the human apoB mRNA is edited or not, we performed a primer-extension assay on apoB mRNAs isolated from transgenic mouse liver and intestine. This assay gives the approximate proportion of edited mRNA.

As an alternate method of checking the sequence of apoB mRNA in the editing site, we directly sequenced PCR amplification products of human apoB mRNA isolated from transgenic mouse and small intestine (Fig. 5b). Using this technique, we found that, like HepG2 apoB mRNA, the transgenic apoB mRNA contains almost exclusively apoB-48 sequences; furthermore, the small intestinal apoB mRNA from the intestine contained more edited than unedited mRNA and that from the liver also contained substantial amounts of unedited mRNA.

Full-length Human ApoB mRNA Is Not Edited in Transgenic Mouse Intestine or Liver. As shown in Fig. 5a, like apoB mRNA in the human hepatoma cell line HepG2, the transgenic apoB mRNA isolated from either mouse liver or intestine contains essentially the unedited species by this assay. In contrast, human small intestinal apoB mRNA contains almost exclusively apoB-48 sequences; furthermore, the endogenous mouse apoB mRNA from the intestine contained more edited than unedited mRNA and that from the liver also contained substantial amounts of unedited mRNA.

Both the primer-extension and the direct sequencing tech-
Transgenic Mice Expressing Full-length ApoB-100

Fig. 3
Transgenic Mice Expressing Full-length ApoB-100

FIG. 3. Immunoblot analysis of transgenic human apoB protein. a, immunoblot analysis of total mouse and human plasma. The data from two different mouse lines are shown. The different lanes are as shown. Molecular mass standards are 200 kDa; myosin; 97.4 kDa, phosphorylase b; 69 kDa, serum bovine albumin; 46 kDa, ovalbumin; 30 kDa, carbonic anhydrase; 21.5 kDa, trypsin inhibitor; 14.3 kDa, lysozyme. H, human; Tg M, transgenic mouse; Sth, nontransgenic littermate. Animals were fed regular mouse chow. They were fasted for 16 h before being bled at 10 a.m. Plasma from a single mouse (250 μl) was applied to the FPLC system comprised of two Superose 6 columns connected in series. For conditions of column, see “Experimental Procedures.” The slight difference in profile is not reproducible. c, immunoblot analysis of FPLC fractions from transgenic mouse plasma. Immunoblot was performed on FPLC fractions run on 4–20% SDS-polyacrylamide gels. Rabbit anti-human apoB serum that does not cross-react with mouse apoB (Xiong et al., 1991) was used for detection. 125I-Protein A was used for detecting the first antibody. The figure represents an autoradiogram using Kodak XAR-5 x-ray film. An immunoblot of the FPLC fractions from transgenic mouse plasma did not reveal any radioactive bands (data not shown; see Fig. 4 in Xiong et al., 1991). M, molecular weight markers; C, plasma from nontransgenic control; H, human plasma. The molecular mass markers were 200 kDa; myosin; 97.4 kDa, phosphorylase b; 69 kDa, bovine serum albumin; 46 kDa, ovalbumin; 30 kDa, carbonic anhydrase; 21.5 kDa, trypsin inhibitor; 14.3 kDa, lysozyme. d, immunoblot analysis of transgenic mouse plasma lipoproteins prepared by sequential ultracentrifugal flotation. Lipoproteins were isolated by sequential ultracentrifugal flotation as described under “Experimental Procedures.” Separation started from 2 ml fractions of pooled plasma obtained from six fasted transgenic and control mice, respectively. Immunoblot analysis was performed using polyclonal anti-apoB sera. BF, bottom (lipoprotein-free) fraction. The gel was exposed to Kodak XAR-5 x-ray film for 6 h. Inset, over-exposure (17 h) of the human plasma and transgenic mouse VLDL lanes. Arrows indicate the position of migration of apoB-100. Molecular mass standards were the same as in panel c.

FIG. 4. Immunoblot analysis of transgenic mouse apoB using monoclonal antibodies. a, location of epitopes of monoclonal antibodies against human apoB-100. N, N terminus; C, C terminus. The bar at the lower left is equivalent to 1000 amino acid residues. The epitope locations are drawn to scale. b, immunoblots using these antibodies. Transgenic and nontransgenic LDL were fractionated in 4–20% SDS-polyacrylamide gels. They were transferred onto nitrocellulose membrane (BA85, Schleicher & Schuell) electrophoretically. They were exposed to monoclonal antibodies MB19, MB3, MB47, and Bs7 individually. The antibodies were detected by [125I]-protein A, and the membranes were exposed to Kodak XAR-5 x-ray film. An immunohlot of the FPLC fractions from nontransgenic mouse plasma is shown. Immunoblot analysis of FPLC fractions from transgenic mice was performed using polyclonal anti-apoB sera. Inset. Molecular mass standards used were identical to previously published human apoB mRNA data from two different mouse lines are shown. The different lanes are as shown. Molecular mass standards are: 91.4, ovalbumin; 69 kDa, serum bovine albumin; 46 kDa, ovalbumin; 30 kDa, carbonic anhydrase; 21.5 kDa, trypsin inhibitor; 14.3 kDa, lysozyme. The molecular mass standards used were identical to previously published human apoB mRNA data from two different mouse lines are shown. The different lanes are as shown. Molecular mass standards are: 91.4, ovalbumin; 69 kDa, serum bovine albumin; 46 kDa, ovalbumin; 30 kDa, carbonic anhydrase; 21.5 kDa, trypsin inhibitor; 14.3 kDa, lysozyme.
has been shown to contain tissue-specific enhancer sequences for apoB expression in vitro (Brooks et al., 1991). (ii) The construct contains a total of seven introns (natural introns 1–3 and 25–28). The reason for including a large number of introns is because apoB is a large gene and introns have been shown to facilitate expression of foreign genes in transgenic mice (Brinster et al., 1988; Palmiter et al., 1991). (iii) The apoB minigene construct spans the complete functional transcription unit of apoB as defined by DNase I sensitivity and chromosomal anchorage sites (Levy-Wilson and Fortier, 1989), which extends well beyond the cap site in the 5′- and the polyadenylation site in the 3′-flanking DNA. It has been shown that the microinjection of similar complete functional units of other genes predictably leads to high level position-independent expression in transgenic animals (Bonifer et al., 1990). (iv) The construct was assembled from five cloned cassettes (Fig. 1). If mutagenesis of a particular region of apoB is desired in the future, it can be easily performed on one of the component cassettes. The transgenic mouse expresses human apoB mRNA at high levels in liver and small intestine. We have previously shown the 5′-flanking DNA and first three introns are sufficient for transcription of a very short partial human apoB mRNA present in liver and intestine (Xiong et al., 1991). In the mouse, human apoB mRNA in liver is expressed at a much lower level compared to that in intestine. Whether this difference in expression pattern is related to the presence or absence of introns in the apoB gene is unclear. Compared to the endogenous mouse apoB mRNA, the transgenic apoB mRNA is more similar in human apoR mRNA. (i) During the assembly of the gene construct, the sequence of all exons and ligation sites was defined by DNase I sensitivity and polyadenylation site in the 3′flanking DNA. (ii) It has been shown that the transgenic mRNA is authentic and recombined (unRt). (iii) The sequence of all exons and ligation sites was defined by DNase I sensitivity and polyadenylation site in the 3′-flanking DNA. (iv) The construct was assembled from five cloned cassettes (Fig. 1). (v) The construct was assembled from five cloned cassettes (Fig. 1).
Why Is Human ApoB mRNA Edited in Vitro and in Cultured Cells but Not in Transgenic Mice?—We showed that the human apoB mRNA in transgenic animals consists of >99% of the unedited apoB-100 sequence by (i) primer-extension assay (Fig. 5a), (ii) direct sequencing of PCR amplification products (Fig. 5b), and (iii) PCR cloning and colony hybridization using allele-specific oligonucleotides (Table II). Three independent transgenic lines were investigated; in each, >99% of the human apoB mRNA was in the unedited (apoB-100) form in both liver and small intestine. The almost complete absence of edited human apoB mRNA in these transgenic animals is surprising. Previous observations on apoB mRNA editing either by tissue extracts in vitro (Driscoll et al., 1988; Chen et al., 1990) or in cultured cells by transfection (Boström et al., 1989; Yao et al., 1992) uniformly demonstrated that human apoB mRNAs are good substrates for editing enzymes from other mammalian species. Although the reason for the discrepancy is unclear, there are important differences in experimental design that might explain the nonsusceptibility of human apoB mRNA to editing by the murine enzyme in vivo.

Most of the previous observations were made on very short human apoB mRNA transcripts that vary from 16–400 bp. There may be sequences further upstream or downstream to these transcripts that confer the sequence specificity. Most of the in vitro editing experiments also utilized purified synthetic human apoB mRNA segments as substrates. Chen et al. (1990) found that such substrates occasionally produced results that were different from those derived from apoB mRNA produced in a coupled transcription-editing system using rat liver nuclear extracts. In those instances that showed a difference, the substrates with the “natural” secondary structure transcribed by the extract, were not edited in vitro with a potentially unnatural secondary structure. RNA substrates that had been added to the editing extract in vitro suggest that when these substrates are not necessarily followed by the natural editing event in vivo, second exons that are not edited in vivo, secondary to C-6666, may be important.

The fact that transfection of murine apoB mRNA into cultured cells in transfection produced predominantly unedited mouse apoB mRNA, whereas the same type of murine apoB mRNA was efficiently edited. In fact, attempts by tissue extracts to inhibit editing were generally unsuccessful (Yao et al., 1992). There are, however, important differences in design between the construct used in transfection experiments and that used in the transgenic mouse experiments. The previous study used the cytomegalovirus promoter-enhancer and human growth hormone polyadenylation signals instead of the authentic human promoter and polyadenylation signals used in this study. Furthermore, the previously reported DNA construct contained only the last three apoB introns (introns 26–28), and the editing site was within an artificial giant first exon, which spans 11.9 kb (Blackhart et al., 1990). In contrast, the editing site in the construct used for transgenic mouse production is inside a natural exon 26, bounded on both sides by natural introns (nos. 25 and 26). Lau et al. (1991) recently showed that apoB mRNA editing in vivo is an intranuclear event that occurs posttranscriptionally coincident with splicing and polyadenylation. Therefore, the splicing of the introns flanking the edited exon may be important in determining both the specificity and efficiency of apoB mRNA editing. An ex oron the size of exon 26 (7.5 kb) is normally very inefficiently spliced, if at all (Robberson et al., 1990). The occurrence of the editing site within this unusually large exon suggests that there may be specific interactions between the editing enzyme and the RNA-splicing machinery. The majority of edited (apoB 48) mRNA in human intestine is prematurely polyadenylated, i.e. poly(A) addition occurs within 1 kb of nucleotide 6666 (the edited nucleotide) instead of another 7 kb downstream as in apoB 100 mRNA (Chen et al., 1987). Therefore, interactions between the editing enzyme and the polyadenylation machinery is also possible. Perhaps the different polyadenylation sequence in the apoB minigene construct used in this study and that used in the transfection experiments also contributed to the difference in susceptibility to editing between the two types of constructs.

Implications for Mechanism of RNA Editing—We speculate that apoB mRNA editing is species-specific in vivo because of species-specific differences in apoB mRNA structure involving sequences further upstream or downstream that were not included in the constructs used in previously published in vitro editing assays (Chen et al., 1990; Shah et al., 1991). Apparently, in the mouse apoB, the full-length human apoB mRNA assumes a form that is poorly recognized for editing. The coevolution of apoB mRNA and editing machinery between humans and rodents may have resulted in the apoB editing enzyme (ApoB) mRNA poorly when viewed from a whole-cell perspective. Furthermore, the difference in the editing machinery may account for the species-specific editing in vivo. Our observations in interpreting editability of different constructs (transfection vs. in vivo) suggest that sequences further upstream or downstream to C-6666 may be important. In designing experiments to identify the sequence-specificity of human apoB mRNA, cut-and-paste experiments using murine apoB mRNA segments, allogeneic mouse apoB mRNA segments, or one specifically engineered construct may be needed to obtain reliable data. Based on the available evidence, the catalytic domain of the editing enzyme is distinct from the recognition domain (Chen et al., 1990; Shah et al., 1991; Backus and Smith, 1991; Chan, 1992). The sequence specificity of the catalytic domain is relatively lax; C-6666 and any C nucleotides introduced into its vicinity by site-directed mutagenesis can be edited to a U (Chen et al., 1990). In contrast, mutations involving an 11-bp sequence 5′-GACAGTCAGTGCA-3′ downstream to C-6666 markedly impair the editability of C-6666 (Shah et al., 1991). Therefore, this short sequence which is not species-specific may be part of the recognition domain. The presence of additional recognition domain sequences in apoB mRNA is quite likely because the previously reported mutagenesis experiments covered only 9 bases 5′ and 19 bases 3′ to C-6666, an extremely small proportion of apoB mRNA, which contains more than 14,000 bases.

The relative non-editability of full-length human apoB mRNA strongly suggests the presence of species-specific sequences elsewhere that is an integral part of the recognition domain. The fact that the production of full-length human apoB mRNA did not affect the efficiency of editing of endogenous mouse apoB mRNA (Table II) indicates that there is little competition between the human and the murine mRNA sequences for binding to the editing enzyme. This suggests
that the species-specific recognition sequences (and not the species-non-specific 11-base sequence common to both human and mouse apoB mRNAs) are either the major binding domain needed for editing or they initiate binding of apoB mRNA to the editing enzyme, bringing in the canonical C-6666 to the catalytic domain of the enzyme. The definition of the specific interactions between apoB mRNA sequences and the editing enzyme must await the purification, cloning, and structural analysis of the latter. In the meantime, we should exercise caution in our interpretation of editing experiments utilizing tissue extracts and synthetic substrates in vitro.

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REFERENCES

Backus, J. W., and Smith, H. C. (1990) J. Biol. Chem. 265, 3858-3860.

Baezconde, E., Xiong, W., Foureast, E., and Chan, L. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 212-216.

Bonifer, C., Vidal, M., Grosveld, F., Sippel, A. E. (1990) EMBO J. 9, 2943-2948.

Boström, K., Lauer, S. J., Piskay, K. S., Garcia, Z., Taylor, J. M., and Inseristi, T. L. (1989) J. Biol. Chem. 264, 15701-15708.

Boström, K., Garcia, Z., Piskay, K. S., Johnson, D. F., Luís, A. J., and Inseristi, T. L. (1990) J. Biol. Chem. 265, 22446-22452.

Brinster, R. L., Allen, J. A., Behringer, R. R., Gelinas, R. E., and Palmiter, R. D. (1988) Proc. Natl. Acad. Sci. U. S. A. 85, 8358-8360.

Blackhart, B. D., Yao, Z., and McCarthy, B. J. (1990) J. Biol. Chem. 265, 7844-7850.

Brown, G., Allers, J. J., Fisher, L. D., Schaefer, S. M., Lin, J.-T., Zhao, X.-Q., Biscou, B. D., Fitzpatrick, V. F., and Dodge, Engl. J. Med. 323, 1299-1306.

Brunzell, H. D., Sniderman, A. D., Albers, J. J., and Levy-Wilson, B. (1989) Nature 340, 79-83.

Arteriosclerosis 4, 79-83.

Chan, L. (1992) BioEssays, in press.

Chan, S. H., Yang, C. Y., Chen, P. F., S., Gotto, A. M. Jr., and Chan, L. (1992) J. Biol. Chem. 267, 5955-5959.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Chen, S.-H., Li, X., Liao, W.-H., and Chan, L. (1991) J. Biol. Chem. 266, 5811-5816.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.

Cherwine, J. M., Przybyla, A. E., and Palmiter, R. D. (1989) J. Biol. Chem. 265, 5221-5226.