Proc. Natl. Acad. Sci. USA
Vol. 85, pp. 1772–1776, March 1988
Biochemistry

Human apolipoprotein B (apoB) mRNA: Identification of two distinct apoB mRNAs, an mRNA with the apoB-100 sequence and an apoB mRNA containing a premature in-frame translational stop codon, in both liver and intestine

KEIICHI HIGUCHI, ASHOK V. HOSPATTANKAR, SIMON W. LAW, NANCY MEGLIN, JEANNE CORTRIGHT, AND H. BRYAN BREWER, JR.

Molecular Disease Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

Communicated by Donald S. Fredrickson, November 18, 1987

ABSTRACT **Human apolipoprotein B (apoB) is present in plasma as two separate isoproteins, designated apoB-100 (512 kDa) and apoB-48 (250 kDa). ApoB is encoded by a single gene on chromosome 2, and a single nuclear mRNA is edited and processed into two separate apoB mRNAs. A 14.1-kilobase apoB mRNA codes for apoB-100, and the second mRNA, which codes for apoB-48, contains a premature stop codon generated by a single base substitution of cytosine to uracil at nucleotide 6538, which converts the translated CAA codon coding for the amino acid glutamine at residue 2153 in apoB-100 to a premature in-frame stop codon (UAA). Two 30-base synthetic oligonucleotides (nucleotides 6523–6552 of apoB mRNA), designated apoB-Stop and apoB-Gln, were synthesized containing the complementary sequence to the stop codon (UAA) and glutamine codon (CAA), respectively. Analysis of intestinal apoB mRNA by hybridization with apoB-Stop and apoB-Gln probes and sequence analysis of apoB clones in two independent human small intestinal cDNA libraries established that intestinal apoB mRNA contained both the apoB mRNA that codes for apoB-100 and the apoB mRNA containing the premature in-frame stop codon, which codes for apoB-48. Investigation of hepatic apoB mRNA and two hepatic cDNA libraries by hybridization with the apoB-Stop and apoB-Gln synthetic probes as well as by cDNA sequencing revealed that liver apoB mRNA also contains both the apoB-100 mRNA and the apoB-48 mRNA containing the stop codon. The combined results from these studies establish that both human intestine and liver contain the two distinct apoB mRNAs, an mRNA that codes for apoB-100 and an apoB mRNA that contains the premature stop codon, which codes for apoB-48. The premature in-frame stop codon is not tissue specific and is present in both human liver and intestine.**

Human apolipoproteins B (apoB) is a major protein moiety of chylomicrons, very low density lipoproteins, and low density lipoproteins (LDL) (1, 2). ApoB plays a central role in LDL metabolism by serving as the ligand that interacts with the LDL receptor to initiate endocytosis and LDL catabolism (3). Within human plasma, apoB exists predominately as two isoproteins, designated apoB-100 (512 kDa) and apoB-48 (250 kDa) (4, 5). Kane and colleagues have proposed that in humans apoB-100 and apoB-48 are secreted by the liver and intestine, respectively (4, 5). *In vitro* studies with normal human hepatocytes as well as HepG-2 cells have revealed that the major apoB secreted was apoB-100 (6). In contrast, both apoB-100 and apoB-48 equivalent apolipoproteins are secreted from the rat liver, indicating that there are species differences in the apoB isoproteins secreted from the liver (7–9).

No consensus on the specific apoB isoproteins secreted from the human intestine is present in the literature. Glickman *et al.* (10) reported that human fetal intestinal organ cultures initially secreted apoB-100; however, apoB-48 was secreted after the 18th week of gestation. These results were interpreted as indicating that the apoB isoproteins secreted by the human intestine were developmentally regulated. In contrast to these results, analysis of adult human intestine using immunohistochemical techniques with monoclonal antibodies specific for apoB-100 demonstrated the presence of apoB-100 in the adult human intestine (11). Recently our laboratory (12) reported that apoB-100 was secreted from freshly isolated adult intestinal organ cultures *in vitro*. Thus both apoB-48 as well as apoB-100 have been reported to be secreted from the human intestine. In studies in the rat, Lee *et al.* (13) observed that apoB-100 was the primary apolipoprotein secreted by the rat intestine; however, in the absence of protease inhibitors, apoB-100 was cleaved to an apolipoprotein similar in size to apoB-48. In other studies the apoB-48 equivalent isoprotein was identified as the apoB isoprotein secreted by the rat intestine (8, 9, 14).

Recently our laboratory (15), Powell *et al.* (16), and Chen *et al.* (17) have reported that human intestinal apoB mRNA contains a premature in-frame translational stop codon. The premature stop codon analyzed in intestinal cDNA clones was generated by a single base substitution of cytosine to thymine at nucleotide 6538, which converts the codon coding for amino acid glutamine (CAA) at residue 2153 to an in-frame stop codon (TAA). The new stop codon in human intestinal apoB mRNA provides a mechanism for the biosynthesis of intestinal apoB-48. Based on sequence analyses of liver and intestinal apoB cDNA clones and amplified cDNA from hepatic and intestinal apoB mRNA, Powell *et al.* (16) concluded that intestinal apoB mRNA contained only the stop codon resulting in only apoB-48 being secreted by the intestine and that the stop codon was tissue specific and not present in human liver.

In this report we present data to establish that human intestine and liver contain two distinct apoB mRNAs, a mRNA with the apoB-100 sequence and an apoB mRNA containing the premature in-frame translational stop codon.* Therefore, the human intestine secretes both apoB-48 and apoB-100, and the premature in-frame translational stop codon is present in both human liver and intestine.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: apoB, apolipoprotein B.

*The sequences reported in this paper are being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J03635).

MATERIALS AND METHODS

Screening of Human Intestinal and Liver cDNA Libraries. Two human intestinal and two hepatic cDNA libraries were utilized in this study. An intestinal cDNA library established in λgt11 was kindly provided by Y. Edwards (Medical Research Council Human Biochemical Genetic Unit, London), and the other intestinal cDNA library established in λgt10 was kindly provided by A. A. Protter (California Biotechnology, San Francisco). A nick-translated cDNA probe (λMDB-4) previously isolated in this laboratory from a human liver cDNA library (18) was used for screening of the intestinal λgt11 library. The liver plasmid and λgt11 cDNA libraries used in this study have been previously described (18, 19).

Two 30-base synthetic oligonucleotides, ApoB-Stop (5′-ATACTGATCAAATTATATCATATATGTCTG-3′) and ApoB-Gln (5′-ATACTGATCAAATTGTATCATATATGTCTG-3′), were synthesized by the modified phosphate triester method (OCS Laboratories, Denton, TX) based on the complementary nucleotide sequences at positions 6523–6552 of the apoB mRNA containing the stop codon (UAA) and apoB-100 glutamine codon (CAA) as previously reported (15). These synthetic oligonucleotides were radiolabeled by end-labeling using standard procedures (20) and were used in hybridization for screening of intestinal and liver cDNA libraries.

DNA Sequence Analysis. Clones were selected from the above libraries, and recombinant phage DNA was isolated by the plate lysate method, followed by polyethylene glycol precipitation and extraction with phenol. DNA inserts were subcloned into M13mp18 or M13mp19 and sequenced by the Sanger dideoxy chain termination method (21). Universal sequence primers (Bethesda Research Laboratories) and synthetic oligonucleotide primers (OCS Laboratories) were used to complete the sequences of the clones.

Extraction and Blot-Hybridization Analysis of Human Liver and Intestinal mRNA. mRNA was isolated from adult human liver and intestine as previously reported (22) and fractionated on a 1.0% agarose gel containing 6.0% (vol/vol) formaldehyde at 50 V for 5 hr, followed by transfer to nitrocellulose filters. The filters were prehybridized followed by hybridization with a nick-translated apoB-100 probe (λMDB-1) as described previously (18). Hybridizations with synthetic oligonucleotide probes were performed for 16 hr at 46°C in a solution containing 5× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate, pH 7), 5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 0.1% $NaDodSO_4$, and 30 mM Tris·HCl (pH 7.4). After hybridization, filters were washed three times in 5× SSC for 5 min at room temperature and finally for 15 min at 50°C in 5× SSC.

Extraction and Southern Blot Analysis of Human DNA from Liver, Intestine, and Leukocytes. Normal human chromosomal DNA was extracted from human liver and intestine as described by Maniatis *et al.* (20). High molecular weight DNA was extracted from human leukocytes as described previously (23). Twenty micrograms of DNA was digested with *Hin*dIII or *Eco*RI, and the fragments were separated by 0.7% agarose gel electrophoresis, followed by transfer to nitrocellulose filters. Hybridization using synthetic oligonucleotides was performed at 50°C for 16 hr, and filters were washed three times in 5× SSC for 5 min at room temperature and for 30 min at 60°C in 5× SSC.

RESULTS

Screening and Identification of an In-Frame Stop Codon (TAA) and an apoB-100 Glutamine Codon (CAA) in Human Intestinal cDNA. In our initial studies on intestinal apoB mRNA, a 1.8-kilobase (kb) cDNA probe (λMDB-4) corresponding to the 6.3- to 8.1-kb region of apoB mRNA (15) was used for screening of 5×10^5 clones of the λgt11 human intestinal cDNA library. A total of 11 positive clones were identified in the λgt11 library, and sequence analysis of 6 clones revealed a single base substitution of cytosine to thymine at nucleotide 6538, which converted the codon coding for glutamine (CAA) to an in-frame stop codon (TAA) (15). Based on these results two probes, designated apoB-Stop and apoB-Gln, were synthesized complementary to the stop codon (TAA) and the apoB-100 glutamine codon (CAA) sequences, respectively, as outlined in *Materials and Methods*. Differential Southern blot analysis of 11 clones using the synthetic probes apoB-Stop and apoB-Gln revealed a total of 9 clones with insert sizes ranging from 0.3 to 1.5 kb that contained the stop codon (Fig. 1*A*), whereas two independent clones (λMDBII-3 and λMDBII-6) with insert sizes of 1.8 kb had the codon for glutamine (CAA) in apoB-100 mRNA (Fig. 1*B*).

To definitively establish that the codon for glutamine (CAA) in apoB-100 mRNA was present in the human intestine, we determined the 5′ nucleotide sequence of the insert of one clone, designated λMDBII-3, which hybridized with apoB-Gln, and compared it to the complete sequence of a second clone, λMDBII-1 (1.5 kb), which was positive on hybridization with apoB-Stop and contained the stop codon (Fig. 2). There was no difference in sequence between these two clones except for the single base substitution of cytosine to thymine at nucleotide 6538. The regions of the nucleotide sequences of clones λMDBII-1 and λMDBII-3 containing the

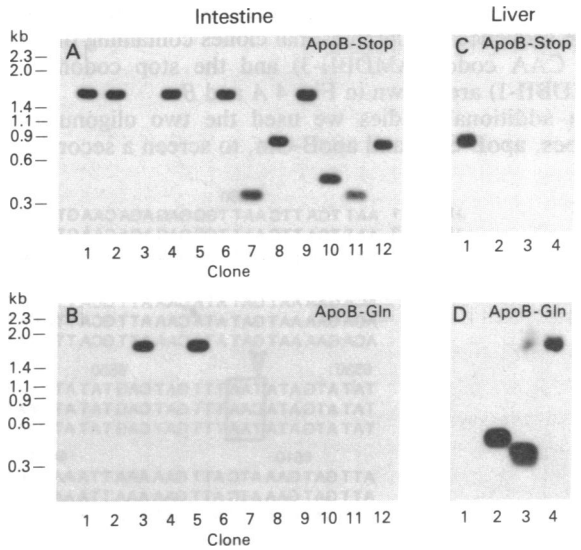

FIG. 1. Differential Southern blots of human intestinal and liver apoB cDNA clones hybridized with 30-base synthetic oligonucleotide probes. *Eco*RI-digested clones were hybridized at 50°C for 16 hr with apoB-Stop (*A* and *C*) or apoB-Gln (*B* and *D*) probes, which contain sequences complementary to the in-frame stop codon and the glutamine codon (CAA) in apoB-100 mRNA, respectively. Filters were washed in 5× SSC at 60°C for 30 min. (*A* and *B*) Intestinal cDNA clones. Lanes: 1, λMDBII-1; 2, λMDBII-2; 3, λMDBII-3; 4, λMDBII-5; 5, λMDBII-6; 6, λMDBII-7; 7, λMDBII-8; 8, λMDBII-10; 9, λMDBII-11; 10, λMDBIII-1; 11, λMDBIII-2; 12, λMDBIII-3. Two additional clones, λMDBII-4 and λMDBII-9, had the same size insert as λMDBII-1 and λMDBII-8, respectively. (*C* and *D*) Liver cDNA clones. Lanes; 1, λMDBI-1; 2, λMDBI-2; 3, λMDBI-3; 4, λMDBI-4. cDNA clones present in lanes 1, 2, 4, and 6–12 in *A* and lane 1 in *C* contain the stop codon (TAA), whereas the codon for glutamine (CAA) in the apoB-100 mRNA sequence is present in clones in lanes 3 and 5 in *B* and lanes 2–4 in *D*. Molecular size markers (in kb) are indicated at left.

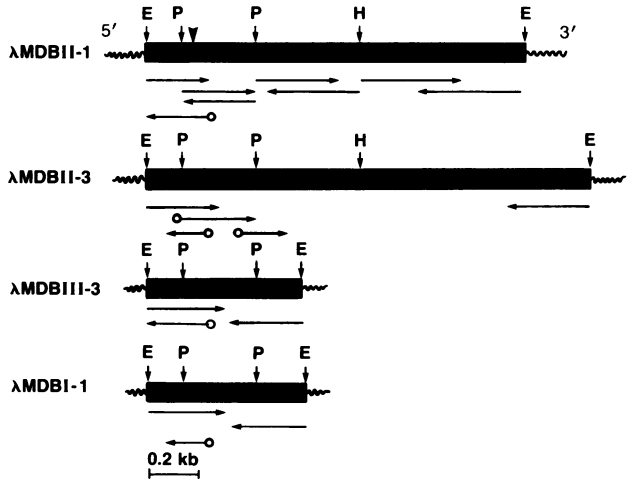

FIG. 2. Restriction map and sequence strategy of the human intestinal and liver apoB cDNA clones. The strategy for the sequence analysis of intestinal (λMDBII-1, λMDBII-3, and λMDBIII-3) and liver (λMDBI-1) clones is indicated with arrows, and ←○ denotes sequences determined by using synthetic oligonucleotide primers. The arms of the cloning vector are indicated by wavy lines. Restriction endonuclease sites are represented as follows: E, *Eco*RI; H, *Hin*dIII; P, *Pst* I. The position of the new in-frame stop codon is indicated by an arrowhead.

cytosine to thymine substitution are shown in Fig. 3. The additional 1.8-kb clone, designated λMDBII-6, which was positive on hybridization with apoB-Gln, was identical in sequence to λMDBII-3 and contained the CAA codon. Autoradiograms of the DNA sequencing gels illustrating the nucleotide sequences of the intestinal clones containing the apoB-100 CAA codon (λMDBII-3) and the stop codon (TAA) (λMDBII-1) are shown in Fig. 4 *A* and *B*.

In additional studies we used the two oligonucleotide probes, apoB-Stop and apoB-Gln, to screen a second intestinal cDNA library established in λgt10. After screening 2.5 × 10^5 plaques, three clones were identified that hybridized to apoB-Stop (λMDBIII-1,-2,-3). The insert sizes of these clones were 0.3, 0.4, and 0.6 kb, respectively. Sequence analysis of all three clones revealed the stop codon sequence at the same position as λMDBII-1; however, no clones contained the apoB-100 glutamine codon (CAA). In the present studies, no clones that were sequenced in the λgt11 and λgt10 libraries contained a poly(A) tail.

Screening and Identification of an In-Frame Stop Codon (TAA) in Human Liver cDNA. The identification of the in-frame stop codon (UAA) in human intestinal apoB mRNA prompted a reanalysis of the liver cDNA libraries that were utilized during the sequence analysis of apoB-100 mRNA (18). The two synthetic probes, apoB-Stop and apoB-Gln, were used to screen 1 × 10^6 clones of a λgt11 liver cDNA library and 5 × 10^5 clones of a plasmid human liver cDNA library. Differential Southern blot analysis of the apoB positive clones in the λgt11 cDNA identified 4 clones with inserts ranging in size from 0.3 kb to 0.7 kb that contained the stop codon, whereas 16 other clones hybridized to the apoB-Gln probe of apoB-100 mRNA (Fig. 1 *C* and *D*). The nucleotide sequence of one of the clones, designated λMDBI-1, that hybridized to the apoB-Stop probe (Fig. 1*C*) is illustrated in Fig. 3. This sequence contained a single substitution of cytosine to thymine at nucleotide 6538, which confirmed the presence of the stop codon (TAA) in the human liver cDNA clone. An autoradiogram of the DNA sequencing gel illustrating the nucleotide sequence of the λMDBI-1 clone and the stop codon (TAA) is shown in Fig. 4*C*.

In the plasmid liver cDNA library, four clones hybridized to the apoB-Gln probe; however, no clones hybridized to the apoB-Stop probe.

Blot-Hybridization Analysis of Liver and Intestinal mRNA. Blot-hybridization analysis of human liver and intestinal mRNA was performed using λMDB-1, a cDNA probe that covers the 5′ region of apoB-100 mRNA, as well as the synthetic apoB-Stop and mRNA-Gln probes (Fig. 5). Hy-

```
                    6390                6410                6430                6450
λMDBII-1 AAT TCA TTC AAT TGG GAG AGA CAA GTT TCA CAT GCC AAG GAG AAA CTG ACT GCT CTC ACA AAA AAG TAT AGA ATT
λMDBII-3 AAT TCA TTC AAT TGG GAG AGA CAA GTT TCA CAT GCC AAG GAG AAA CTG ACT GCT CTC ACA AAA AAG TAT AGA ATT
λMDBI-1  AAT TCA TTC AAT TGG GAG AGA CAA GTT TCA CAT GCC AAG GAG AAA CTG ACT GCT CTC ACA AAA AAG TAT AGA ATT

                    6470                6490                6510
ACA GAA AAT GAT ATA CAA ATT GCA TTA GAT GAT GCC AAA ATC AAC TTT AAT GAA AAA CTA TCT CAA CTG CAG ACA
ACA GAA AAT GAT ATA CAA ATT GCA TTA GAT GAT GCC AAA ATC AAC TTT AAT GAA AAA CTA TCT CAA CTG CAG ACA
ACA GAA AAT GAT ATA CAA ATT GCA TTA GAT GAT GCC AAA ATC AAC TTT AAT GAA AAA CTA TCT CAA CTG CAG ACA

6530                6550                6570                6590
TAT ATG ATA TAA TTT GAT CAG TAT ATT AAA GAT AGT TAT GAT TTA CAT GAT TTG AAA ATA GCT ATT GCT AAT ATT
TAT ATG ATA CAA TTT GAT CAG TAT ATT AAA GAT AGT TAT GAT TTA CAT GAT TTG AAA ATA GCT ATT GCT AAT ATT
TAT ATG ATA TAA TTT GAT CAG TAT ATT AAA GAT AGT TAT GAT TTA CAT GAT TTG AAA ATA GCT ATT GCT AAT ATT

    6610                6630                6650                6670
ATT GAT GAA ATC ATT GAA AAA TTA AAA AGT CTT GAT GAG CAC TAT CAT ATC CGT GTA AAT TTA GTA AAA ACA ATC
ATT GAT GAA ATC ATT GAA AAA TTA AAA AGT CTT GAT GAG CAC TAT CAT ATC CGT GTA AAT TTA GTA AAA ACA ATC
ATT GAT GAA ATC ATT GAA AAA TTA AAA AGT CTT GAT GAG CAC TAT CAT ATC CGT GTA AAT TTA GTA AAA ACA ATC

                    6690                6710                6730                6750
CAT GAT CTA CAT TTG TTT ATT GAA AAT ATT GAT TTT AAC AAA AGT GGA AGT AGT ACT GCA TCC TGG ATT CAA AAT
CAT GAT CTA CAT TTG TTT ATT GAA AAT ATT GAT TTT AAC AAA AGT GGA AGT AGT ACT GCA TCC TGG ATT CAA AAT
CAT GAT CTA CAT TTG TTT ATT GAA AAT ATT GAT TTT AAC AAA AGT GGA AGT AGT ACT GCA TCC TGG ATT CAA AAT

                6770                6790                6810
GTG GAT ACT AAG TAC CAA ATC AGA ATC CAG ATA CAA GAA AAA CTG CAG CAG CTT AAG AGA CAC ATA CAG AAT ATA
GTG GAT ACT AAG TAC CAA ATC AGA ATC CAG ATA CAA GAA AAA CTG CAG CAG CTT AAG AGA CAC ATA CAG AAT ATA
GTG GAT ACT AAG TAC CAA ATC AGA ATC CAG ATA CAA GAA AAA CTG CAG CAG CTT AAG AGA CAC ATA CAG AAT ATA

6830                6850                6870                6890
GAC ATC CAG CAC CTA GCT GGA AAG TTA AAA CAA CAC ATT GAG GCT ATT GAT GTT AGA GTG CTT TTA GAT CAA TTG
GAC ATC CAG CAC CTA GCT GGA AAG TTA AAA CAA CAC ATT GAG GCT ATT GAT GTT AGA GTG CTT TTA GAT CAA TTG
GAC ATC CAG CAC CTA GCT GGA AAG TTA AAA CAA CAC ATT GAG GCT ATT GAT GTT AGA GTG CTT TTA GAT CAA TTG

    6910                6930                6950                6970
GGA ACT ACA ATT TCA TTT GAA AGA ATA AAT GAT GTT CTT GAG CAT GTC AAA CAC TTT GTT ATA AAT CTT ATT GGG
GGA ACT ACA ATT TCA TTT GAA AGA ATA AAT GAT GTT CTT GAG CAT GTC AAA CAC TTT GTT ATA AAT CTT ATT GGG
GGA ACT ACA ATT TCA TTT GAA AGA ATA AAT GAT GTT CTT GAG CAT GTC AAA CAC TTT GTT ATA AAT CTT ATT GGG
```

FIG. 3. Nucleotide sequence of apoB cDNA clones. The sequences of clones from intestine (λMDBII-1 and λMDBII-3) and liver (λMDBI-1) were identical except for the single substitution of cytosine to thymine at nucleotide 6538 (box), which results in a premature in-frame stop codon. The potential polyadenylylation signal 390 base pairs downstream from the new stop codon is boxed. Nucleotide 1 is the adenosine of the ATG codon that codes for the initiator methionine.

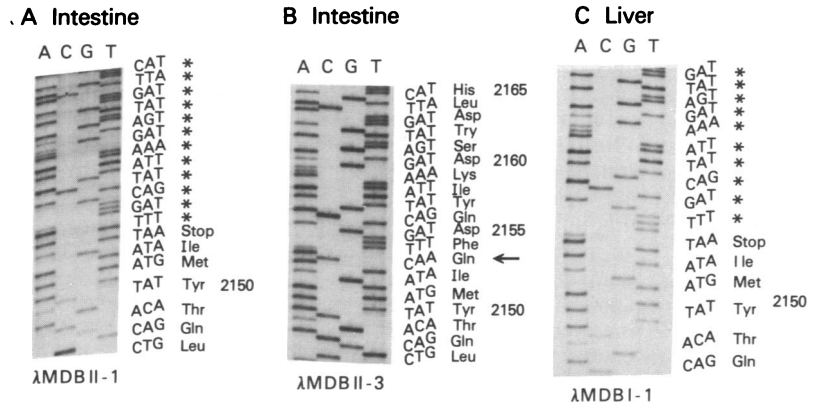

FIG. 4. Autoradiograms of DNA sequencing gels. (*A*) Portion of the coding sequences of intestinal clone λMDBII-1 that contains the in-frame stop codon, which terminates translation. (*B*) Portion of the coding sequence of intestinal clone λMDBII-3 that has a CAA triplet (indicated by arrow) coding for glutamine at residue 2153 in apoB-100. (*C*) Portion of the coding sequences of the liver cDNA clone λMDBI-1 containing the stop codon sequence.

bridization with the λMDB-1 probe demonstrated a single 14.1-kb apoB mRNA in the liver and two separate apoB mRNAs of 14.1 and 7.5 kb in the intestine, as we have previously reported (24, 25). Hybridizations were performed with the apoB-Stop synthetic probe to determine which intestinal mRNA species contained the stop codon. Both the 14.1- and 7.5-kb apoB mRNA hybridized to the apoB-Stop probe, indicating that the stop codon was present in both apoB mRNAs (Fig. 5). In addition, the apoB-Gln probe hybridized to the 14.1-kb apoB mRNA band in the intestine, which confirmed the presence of the apoB-100 codon for glutamine (CAA) in intestinal apoB mRNA (Fig. 5).

Hybridizations were also performed with the apoB-Stop probe on human liver mRNA. The hepatic 14.1-kb apoB mRNA hybridized with the apoB-Stop probe confirming that the stop codon (UAA) was present in the apoB mRNA in human liver (Fig. 5*C*).

Southern Blot Analysis of Chromosomal DNA. Chromosomal DNAs were isolated from liver, intestine, and leukocytes and were digested with *Eco*RI and *Hin*dIII. Southern blot hybridization analyses utilizing the synthetic probes apoB-Stop and apoB-Gln were performed to determine if the single base substitution of cytosine to thymine could be detected in the genomic DNA sequences. As illustrated in Fig. 6, liver, intestine, and leukocyte DNAs digested with *Eco*RI or *Hin*dIII contained 1.8-kb and 5.5-kb bands, respectively, that hybridized with apoB-Gln. However, no hybridization of any DNA band occurred with apoB-Stop. These results were interpreted as indicating that only the CAA codon of apoB-100 mRNA was present at the DNA level in both the liver and intestine.

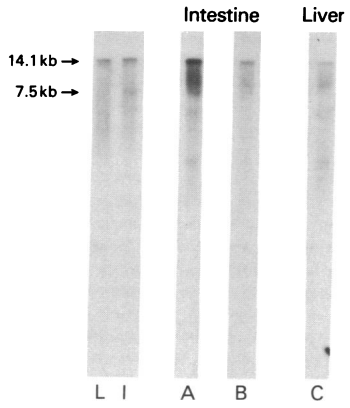

FIG. 5. Blot-hybridization analysis of poly(A)$^+$ RNA isolated from human intestine and liver. Two micrograms of poly(A)$^+$ RNA isolated from human liver (L) and intestine (I) were hybridized with a nick-translated apoB cDNA probe (λMDB-1), which contains the 5′ region of the apoB-100 mRNA. Five micrograms of poly(A)$^+$ RNA isolated from human intestine was electrophoresed and hybridized with one of the end-labeled oligonucleotide probes, apoB-Stop (lane A) or apoB-Gln (lane B), which are specific for the in-frame stop codon (TAA) and the glutamine codon (CAA) in the apoB-100 sequence, respectively. Five micrograms of poly(A)$^+$ RNA isolated from human liver was hybridized with the apoB-Stop probe (lane C).

DISCUSSION

The combined results from our studies have established that human intestinal apoB mRNA contains the CAA codon, which codes for the biosynthesis of apoB-100, in addition to the apoB mRNA containing the in-frame stop codon, which codes for apoB-48 as previously reported (15–17). Using two independent approaches, direct sequencing of intestinal apoB cDNA clones as well as blot-hybridization analysis of intestinal mRNA with the apoB-Gln synthetic probe, which is complementary to the codon for glutamine (CAA) in apoB-100 mRNA, have established that apoB-100 mRNA is present in the adult human intestine. The presence of apoB-100 mRNA, which codes for the biosynthesis of apoB-100 in the human intestine, is consistent with our results on the secretion of apoB-100 from adult human organ cultures (12) and the identification of apoB-100 in enterocytes by immunohistochemical techniques with apoB-100 specific monoclonal antibodies (11).

Blot-hybridization analysis of the 14.1- and 7.5-kb apoB mRNAs present in the intestine with the apoB-Stop synthetic probe established that the stop codon (UAA) was present in both the 14.1- and 7.5-kb apoB mRNA (Fig. 5). A polyadenylylation consensus signal sequence (AATAAA) at

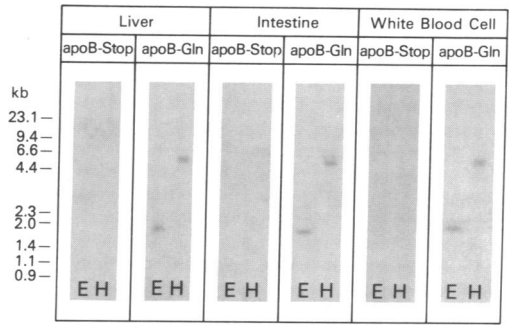

FIG. 6. Southern blot analysis of human genomic DNA isolated from liver, intestine, and leukocytes. Twenty micrograms of genomic DNA was digested with *Eco*RI (E) or *Hin*dIII (H) and was separated by 0.7% agarose gel electrophoresis. Hybridization with end-labeled synthetic oligonucleotide probes apoB-Stop and apoB-Gln was performed at 50°C. The filters were washed at 60°C for 30 min with 5× SSC and then were exposed to x-ray film for 7 days. Molecular size markers (in kb) are shown at left.

nucleotides 6927–6932 is present 390 nucleotides downstream from the stop codon (Fig. 3).

Of particular interest in the studies reported here was the identification of the in-frame stop codon in apoB mRNA in human liver. Use of the apoB-Stop synthetic probe markedly facilitated the identification of the clones in the liver cDNA libraries that contained the stop codon. Both direct sequencing of the liver cDNA clones that hybridized with the apoB-Stop probe and blot-hybridization analysis of liver mRNA with the specific apoB-Stop probe definitively established that the human liver contains apoB mRNA with the stop codon. These results indicate that the premature stop codon in apoB mRNA is not tissue specific as reported (16, 17). In previous *in vitro* studies (6) with normal hepatocytes and HepG-2 cells, apoB-100 was virtually the only apoB isoprotein secreted. The question remains if apoB-48 is secreted from the human liver. In previous *in vitro* studies (6), the culture conditions may not have been correct for the secretion of apoB-48; only minimal secretion of apoB-48 may occur from the human liver due to the low level of apoB mRNA containing the stop codon, or the majority of the apoB isoprotein translated from the stop codon mRNA may not be secreted from the cell. Borchardt and Davis (26) have reported that a significant fraction of the apoB protein translated in the rat liver is not secreted from hepatocytes, and this could also be the case for apoB-48 in human liver. However, both apoB-100 and apoB-48 equivalent isoproteins are secreted from the rat liver (7–9). In a separate series of studies, the stop codon (TAA) was identified by DNA sequencing in three cDNA clones from a rat liver cDNA library, thereby establishing that the rat as well as the human liver contains mRNA containing the stop codon, which may code for apoB-48.

Southern blot analysis of genomic DNA from both the liver and intestine with specific stop codon and CAA codon probes established that only the CAA codon of apoB-100 is present at the DNA level, indicating that the stop codon was not the result of a tissue specific DNA mutation. These results are in agreement with those of Powell *et al.* (16) and indicate that the premature in-frame stop codon is introduced at the RNA level by an RNA editing mechanism.

A schematic overview of the processing of the apoB gene and mRNA is depicted in Fig. 7. Previous studies have shown that apoB is encoded by a single gene on chromosome 2 (25, 27, 28). In this model a single nuclear RNA would be synthesized from the apoB gene. With RNA processing and editing, two separate apoB mRNAs are produced in both the liver and intestine. One apoB mRNA contains the CAA codon and codes for apoB-100. The second mRNA contains an in-frame stop codon that provides a mechanism for the biosynthesis of apoB-48 (Fig. 7). It should be stressed that the apoB isoprotein(s) in plasma that migrates as apoB-48 on

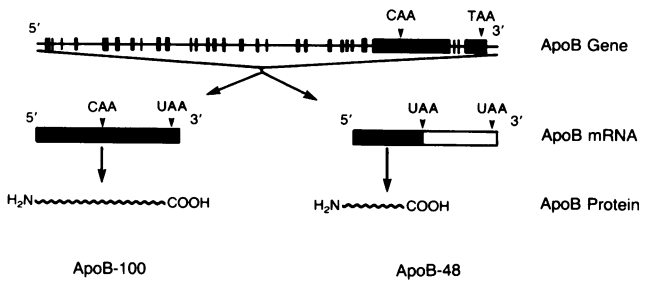

FIG. 7. Schematic diagram of the apoB gene and the processing of intestinal and liver mRNA and B apolipoproteins. The 29 exons of the apoB gene are illustrated by solid boxes (see text for details).

$NaDodSO_4$ gel electrophoresis may be the direct translational product of the apoB mRNA containing the stop codon, may contain the end product of either intracellular or extracellular proteolytic cleavage of apoB-100, or may be a combination of all three.

In humans the intestine secretes both apoB-48 and apoB-100. The secretion of apoB-100 by the adult human intestine may lead to the formation of intestinal apoB-100 remnants that under certain situations may be atherogenic and lead to an increased risk of premature cardiovascular disease. The human liver, however, appears to secrete almost exclusively apoB-100 under the conditions analyzed (6), suggesting that the predominant apoB mRNA in the liver contains the CAA codon, which codes for apoB-100. Research directed toward an analysis of the factors including diet, drugs, and hormones as well as other effectors that determine the percentage of intestinal and liver apoB mRNA containing the CAA codon, which encodes for apoB-100, and the apoB mRNA, which contains the premature stop codon and codes for apoB-48, will be important.

1. Jackson, R. L. Morrisett, J. D. & Gotto, A. M., Jr. (1976) *Physiol. Rev.* **56**, 259–316.
2. Osborne, J. C., Jr. & Brewer, H. B., Jr. (1977) *Adv. Protein Chem.* **31**, 253–337.
3. Brown, M. S. & Goldstein, J. L. (1986) *Science* **232**, 34–47.
4. Kane, J. P., Hardman, D. A. & Paulus, H. E. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 2465–2469.
5. Kane, J. P. (1983) *Annu. Rev. Physiol.* **45**, 637–650.
6. Edge, S. B., Hoeg, J. M., Schneider, P. D. & Brewer, H. B., Jr. (1985) *Metabolism* **34**, 726–730.
7. Elovson, J., Huang, Y. O. Baker, N. & Kannan, R. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 157–161.
8. Wu, A.-L. & Windmueler, H. G. (1981) *J. Biol. Chem.* **256**, 3615–3618.
9. Sparks, C. E., Hnatiuk, O. & Marsh, J. B. (1981) *Can. J. Biochem.* **49**, 693–699.
10. Glickman, R. M., Rogers, M. & Glickman, J. N. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 5296–5300.
11. Dullaart, R. P. F., Speelberg, B., Schuuman, H.-J., Milne, R. W., Havekes, L. M., Marcel, Y. L., Geuze, H. J., Hulshof, M. M. & Erkelens, D. W. (1986) *J. Clin. Invest.* **78**, 1397–1404.
12. Hoeg, J. M., Svvudov, D. D., Law, S. W., Demosky, J. F., Jr., Meng, M. S., Repin, V. S., Smimov, V. N. & Brewer, H. B., Jr. (1987) *Atherosclerosis* **7**, 491 (abstr.).
13. Lee, D. M., Koren, E., Singh, S. & Mok, T. (1984) *Biochem. Biophys. Res. Commun.* **123**, 1149–1156.
14. Krishnaiah, K. V., Walker, L. F., Borensztajn, J., Schonfeld, G. & Getz, G. S. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3806–3810.
15. Hospattankar, A. V., Higuchi, K., Law, S. W., Meglin N. & Brewer, H. B., Jr. (1987) *Biochem. Biophys. Res. Commun.* **148**, 279–285.
16. Powell, L. W., Wallis, S. C., Pease, R. J., Edwards, Y. H., Knott, T. J. & Scott, J. (1987) *Cell* **50**, 831–840.
17. Chen, S.-H., Habib, G., Yang, C.-Y., Gu, Z.-W., Lee, B. R., Weng, S.-A., Silberman, S. R., Cai, S.-J., Deslypere, J. P., Rosseneu, M., Gotto, A. M., Jr., Li, W.-H. & Chan, L. (1987) *Science* **238**, 363–366.
18. Law, S. W., Grant, S. M., Higuchi, K., Hospattankar, A., Lackner, K., Lee, N. & Brewer, H. B., Jr. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8142–8146.
19. Law, S. W., Lackner, K. J., Hospattankar, A. V., Anchors, J. M., Sakaguchi, A. Y., Naylor, S. L. & Brewer, H. B., Jr. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8340–8344.
20. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
21. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
22. Law, S. W., Gray, G. & Brewer, H. B., Jr. (1983) *Biochem. Biophys. Res. Commun.* **112**, 257–264.
23. Lackner, K. J., Law, S. W. & Brewer, H. B., Jr. (1985) *Nucleic Acids Res.* **13**, 4597–4608.
24. Law, S. W., Lackner, K. J., Monge, J. C., Hospattankar, A. V., Lee, N., Hoeg, J. M. & Brewer, H. B., Jr. (1986) *DNA* **5**, 81 (abstr.).
25. Higuchi, K., Monge, J. C., Lee, N., Law, S. W. & Brewer, H. B., Jr. (1987) *Biochem. Biophys. Res. Commun.* **144**, 1332–1339.
26. Borchardt, R. A. & Davis, R. A. (1987) *J. Biol. Chem.* **262**, 16394–16402.
27. Law, S. W., Lee, N., Monge, J. C., Brewer, H. B., Jr., Sakaguchi, A. Y. & Naylor, S. L. (1985) *Biochem. Biophys. Res. Commun.* **131**, 1003–1012.
28. Young, S. G., Bertics, S. J., Scott, T. M., Dubois, B. W., Curtiss, L. K. & Witzum, J. L. (1986) *J. Biol. Chem.* **261**, 2995–2998.