Vol. 148, No. 1, 1987 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
October 14, 1987 Pages 279-285

IDENTIFICATION OF A NOVEL IN-FRAME TRANSLATIONAL STOP CODON IN HUMAN INTESTINE ApoB mRNA

Ashok V. Hospattankar, Keiichi Higuchi, Simon W. Law, Nancy Meglin, and H. Bryan Brewer, Jr.

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

Received August 28, 1987

SUMMARY: Human apolipoprotein (apo) B exists in plasma as two isoproteins desiginated apoB-100 and apoB-48. ApoB-100 (512 kDa) and apoB-48 (250 kDa) are synthesized by the liver and intestine respectively. Analysis of apoB cDNA clones isolated from a human intestinal cDNA library revealed that the intestinal apoB mRNA contains a new in-frame translational stop codon. This premature stop codon is generated by a single base substitution of a 'C' to 'T' at nucleotide 6538 which converts the codon 'CAA' coding for the amino acid glutamine residue 2153 to an in-frame stop codon 'TAA'. The generation of a stop codon in the intestinal apoB mRNA appears to be tissue specific since it has not been reported in cDNA clones isolated from human liver cDNA libraries which code for the 4536 amino acid apoB-100. A potential polyadenylation signal sequence 'AATAAA' was also identified 390 bases downstream from the new stop codon. The new stop codon in the human intestinal apoB mRNA provides a potential mechanism for the biosynthesis of intestinal apoB-48.

Apolipoprotein B (apoB), is a major protein constituent of low density lipoproteins, very low density lipoproteins and chylomicrons. ApoB plays an important role in lipid metabolism by serving as the principal ligand for interaction with the low density lipoprotein receptor (1). In human plasma apoB is heterogeneous and exists predominantly as two isoproteins designated apoB-100 and apoB-48 (2). Studies in vitro have established that human liver synthesizes and secretes only apoB-100 (3). In contrast, the human intestine has been proposed to produce both apoB-100 and apoB-48, however the synthesis is developmentally regulated with apoB-100 and apoB-48 synthesized by the fetal and adult intestine respectively (4). Recently we and others have determined the entire sequence of the 14.1 kb long apoB-100 mRNA which encodes for the 4536 amino acid apoB-100 (5-7). The precise structural

0006-291X/87 $1.50

relationship however between apoB-100 and apoB-48 is as yet unknown. Immunological mapping of epitopes of the B-100 and B-48 apolipoproteins by monoclonal apoB antibodies has suggested that apoB-48 is similar in amino acid sequence to the amino terminal half of apoB-100 (8,9). The previous studies conducted in our laboratory have identified a second apoB mRNA of 7.5 kb in the intestine of sufficient size to code for apoB-48 (10-13). In the present study, we have further investigated the apoB mRNA and potential mechanism(s) for the production of apoB-48 in the human intestine.

METHODS

Extraction of Human Liver/Intestine RNA and Northern Blot Analysis: RNA was isolated from adult human liver or intestine as previously reported (10), and fractionated on a 1% agarose gel in the presence of 6% (vol/vol) formaldehyde at 3.5 volts per cm for 6 h and transferred to nitrocellulose filters. The filters were hybridized with a nick translated apoB-100 probe (10^7 cpm) in 10 ml of hybridization solution containing 5 X SSC, 5 X Denhardts solution, 0.1% $NaDodSO_4$, 20 mM Tris HCl (pH 7.4), denatured salmon sperm DNA (100 µg/ml) and 50% (vol/vol) formamide at 42°C for 20 h. After hybridization, filters were washed in 2 X SSC containing 0.1% $NaDodSO_4$ for five minutes at 25°C, for 30 min at 65°C and finally for 1 h at 65°C in 1 X SSC with 0.1% $NaDodSO_4$ followed by autoradiography.

Screening of Human Intestinal cDNA Library With apoB cDNA Probes: A human intestinal cDNA library established in λgt-11 was kindly provided by Dr. Yvonne Edwards, MRC Human Biochemical Genetics Unit, London, UK. The screening procedure employed was essentially the same as previously described (10,14). In brief, approximately 5 X 10^5 clones were screened by hybridization to a radiolabeled cDNA clone (λMDB-4) previously isolated in this laboratory from a human liver cDNA library (5). λgt-11 apoB clones which were positive to the probe were plaque purified to homogeneity. Plate lysate stocks were prepared by transfection of E. Coli 1088 (14). Recombinant phage DNA was isolated by polyethylene glycol precipitation followed by phenol/chloroform/isoamyl alcohol (24:24:1) extraction.

DNA Sequence Analysis: The human intestinal λgt-11 apoB cDNA clones were analyzed by the Sanger's dideoxynucleotide chain termination procedure (15). Single stranded DNA templates were generated by subcloning into M13mp19 or M13mp18. Universal sequencing primers (BRL) and synthetic oligonucleotide primers (OCS Labs. Denton, Texas) were used to complete the sequence of the apoB clones.

RESULTS AND DISCUSSION

ApoB mRNA in human liver and intestine were evaluated by RNA blot hybridization. A nick translated apoB-100 cDNA probe designate λ MDB-1

Figure 1. Northern blot analysis of poly(A)$^+$RNA isolated from human liver (L) and intestine (I). The blot was hybridized with a nick translated apoB cDNA probe λMDB-1 (5).

(10) which is 1.9 kb in length and 1.5 kb from the 5' region of the apoB-100 mRNA was utilized as a probe. As illustrated in Fig. 1, this probe hybridized to a single 14.1 kb RNA species in the human liver, and two RNA species (14.1 kb and 7.5 kb) in the human intestine (10-13). The presence of two RNA species in the human intestine raises the possibility of two functional mRNA's in the intestine, encoding for the B-100 and B-48 apolipoproteins. Previous studies conducted in our laboratory utilizing cDNA probes derived from different regions of the apoB-100 mRNA indicated that the 7.5 kb mRNA observed in human intestine hybridized only to the cDNA probes derived from the first 7.5 kb 5' segment of the apoB-100 mRNA (10-13). Structural and immunological analysis of the B-48 apolipoprotein (8,9,16) have also suggested that apoB-48 is the amino terminal half of apoB-100.

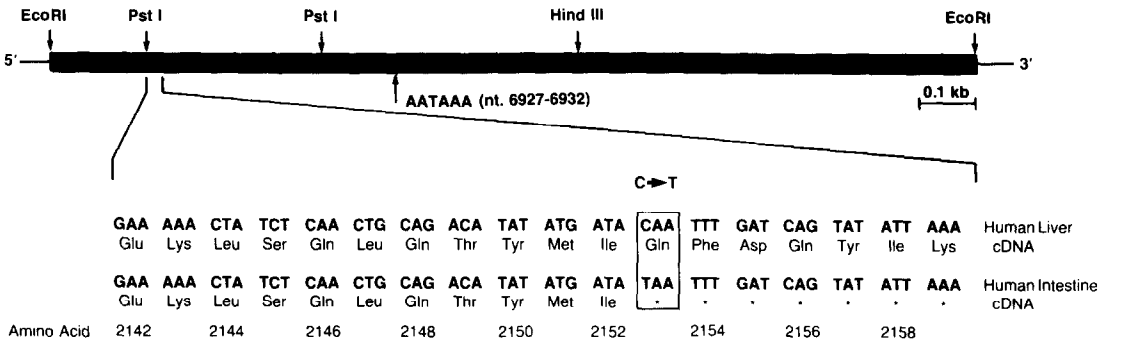

Figure 2. Restriction enzyme map of the 1.5 kb cDNA insert (λMDB-II) isolated from a human intestinal cDNA library. The complete sequence of the insert was established by the dideoxy method. Directly below are the nucleotide sequence and derived amino acid sequence that span the region corresponding to residues 2142-2159 present in the 14.1 kb long apoB mRNA. In the human intestine, a single base substitution of a 'C' to 'T' (at residue 2153) introduces a new in-frame stop codon in the apoB mRNA. Also shown is the location (nt 6927-6932) of a potential polyadenylation signal 390 base downstream from the new stop codon; nt 1 is the 'A' of the 'ATG' codon that encodes the initiator methionine.

In order to gain further insight to the mechanisms involved in the biosynthesis of apoB-48, studies were undertaken to screen a human intestinal cDNA library and to preferentially select the clones which are likely to contain the 3' end of the apoB-48 mRNA. We used a cDNA probe designated λ MDB-4 which has been previously isolated from a human liver cDNA library (5). This probe contained a 1.8 kb insert corresponding to the 6.3 - 8.1 kb segment of the apoB-100 mRNA. A total of 5×10^5 cDNA clones from the human intestinal library were screened with this probe and seven positive cDNA clones were identified. Restriction enzyme mapping of these seven clones indicated that each clone contained overlapping inserts, and their size range from 0.9 - 1.5 kb. The inserts from these clones were subcloned into the M13mp19 vector and single stranded templates were generated for sequence analysis.

The restriction enzyme map of one of these clones, designated MDB-II (1.5 kb), is illustrated in Fig. 2. The sequence analysis of MDB-II indicated that there was a single base substitution of a 'C' to 'T' at nucleotide 6538 of the 14.1 kb apoB mRNA (nucleotide 1

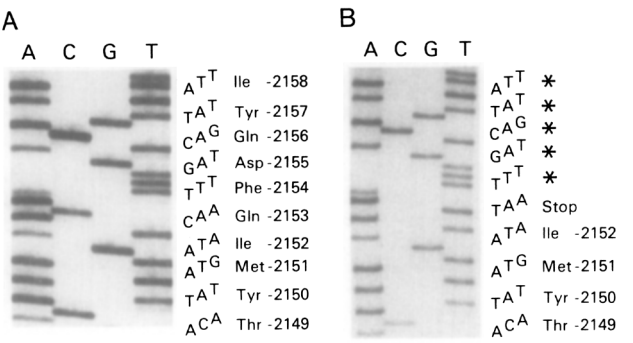

Figure 3. Autoradiograms of DNA sequencing gels. (A) illustrates a portion of the coding sequence (residues 2149-2158) of human liver apoB mRNA; (B) contains a portion of the coding sequence (residues 2149-2158) of human intestinal apoB mRNA. A single base substitution of a 'C' to 'T' converts residue 2153 into an in-frame 'stop' codon.

corresponds to 'A' of the codon 'ATG' encoding the initiator methionine) which was in-frame with the coding sequence. As a result, the nucleotide sequence 'CAA' which codes for residue 2153 'Gln' in the 14.1 kb apoB mRNA is changed to 'TAA' which generates a 'stop' codon (Fig. 2).

Autoradiograms of the DNA sequencing gels illustrating the nucleotide sequence and deduced amino acids in two cDNA clones isolated from cDNA libraries prepared from the human liver and intestine are shown in Fig 3. The human liver mRNA contained the sequence 'CAA' coding for glutamine as amino acid residue 2153 (Fig. 3A) whereas the intestinal mRNA contained a stop codon (TAA) at this location (Fig. 3B). Sequence analysis of 6 additional clones also confirmed the single base substitution, and the introduction of the new stop codon in the human intestinal apoB mRNA.

The 'C' to 'T' substitution which introduces a premature termination codon appears to be tissue specific, since it has not been identified in any liver mRNAs thus far examined. The base substitution is in exon 26 of the apoB gene and the resulting stop codon is in-frame with the coding sequence. It is interesting to note that a potential polyadenylation signal sequence (AATAAA) was identified 390 base

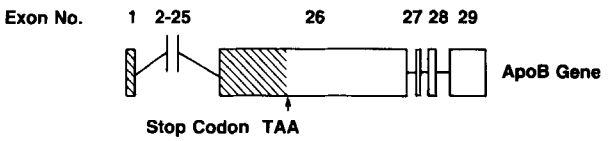

Figure 4. Schematic diagram of the site of the stop codon in the apoB gene. The exon organization of the apoB gene is illustrated, and the stop codon (TAA) identified in exon 26 of the human intestinal apoB mRNA is indicated. The striped segments (exons 1-26) represent the proposed region present in apoB-48.

downstream from the newly introduced stop codon (Fig. 2). This polyadenylation sequence is identical to nucleotides 6927 to 6932 of the 14.1 kb apoB mRNA (Fig. 2).

Figure 4 summarizes the location of the new stop codon in the exon 26 of the apoB mRNA. The addition of an in-frame stop codon in the middle of an exon is unique, and represents a new mechanism for modulating translation of a eukaryotic mRNA. At present we do not know the mechanism by which the stop codon is introduced into the human intestinal apoB mRNA. Alternative splicing, RNA editing and DNA rearrangements (17-19) have been identified as mechanism involved in the production of functional mRNA. The introduction of an in-frame stop codon is novel, and additional studies will be required to further delineate this new mechanism involved in apolipoprotein biosynthesis.

REFERENCES

1. Brown, M.S. and Goldstein, J.L. (1986) Science 232, 34-47.
2. Kane, J.P. (1983) Ann. Rev. Physiol. 45, 637-650.
3. Edge, S.B., Hoeg, J.M., Schneider, P.D, and Brewer, H.B., Jr. (1985) Metabolism 34, 726-730.
4. Glickman, R.M., Rogers, M. and Glickman, J.N. (1986) Proc. Natl. Acad. Sci., USA 83, 5296-5300.
5. Law, S.W., Grant, S.M., Higuchi, K., Hospattankar, A., Lackner, K., Lee, N. and Brewer, H.B., Jr. (1986) Proc. Natl. Acad. Sci. USA 83, 8142-8146.
6. Knott, T.J., Pearse, R.J., Powell, L.M., Wallis, S.C., Rall, S.C., Innerarity T.L. Blackhart, B., Taylor, W.H., Marcel, Y., Milne, R., Johnson, D., Fuller, M., Lusis, A.J., McCarthy, B.J., Mahley, R.W., Levy-Wilson, B. and Scott, J. (1986) Nature, 323, 734-738.
7. Chen, S.H., Yang, C.Y., Chen, R.F., Scbzer, D., Tanimura, M., Li, W.M., Gotto, A.M. and Chan, L. (1986) J. Biol. Chem. 261, 12918-12921.
8. Marcel, Y.L., Hogue, M., Theolis, R. and Milne, R.W. (1982) J. Biol. Chem. 257, 13165-13168.
9. Curtiss, L.K. and Edgington, T.S. (1982) J. Biol. Chem. 257, 15213-15221.

10. Law, S.W., Lackner, K.J., Hospattankar, A.V., Anchors, J.M., Sakaguchi, A.Y., Naylor, S.L. and Brewer, H.B., Jr. (1985) Proc. Natl. Acad. Sci, USA 82, 8340-8344.
11. Law, S.W., Lackner, K.J., Monge, J.C., Hospattankar, A.V., Lee, N., Hoeg, J.M. and Brewer, H.B., Jr. (1986) DNA 5, 81 (Abst.).
12. Law, S.W., Monge, J.C., Lackner, K.J., Hospattankar, A.V., Grant, S.M., Higuchi. L., Gregg, R.E., Lee, N. and Brewer, H.B., Jr. (1986) In Cardiovascular Disease 86. Molecular and cellular mechanisms and preventive treatment. Sixth International Washington Spring Symposium (Gallo, L.L. ed.) (In press).
13. Higuchi, K., Monge, J.C., Lee, N., Law, S.W. and Brewer, H.B. Jr. (1987) Biochem. Biophys. Res. Commun. 144, 1332-1339.
14. Young, R.A. and Davis R. (1983) Proc. Natl. Acad. Sci. USA 80, 1194-1198.
15. Sanger, F., Nicklen, S. and Coulson, A.R. (1977) Proc. Natl. Acad. Sci. USA 74, 5463-5467.
16. Hardman, D.A., Kane, J.P. (1986) Methods Enzymology, 128, Part A, 262-272.
17. Sharp, P.A. The Harvey Lectures (1987). The Harvey Lectures. Series 81, 1-31.
18. Boardman, M., Basi, G.S. and Storti, R.V. (1985) Nucleic Acids Res. 13, 1763-1776.
19. Craigen, W.J. and Caskey, C.T. (1987) Cell, 50, 1-2.