Characterization of the human *apobec-1* gene: expression in gastrointestinal tissues determined by alternative splicing with production of a novel truncated peptide

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Abstract. In humans, both the expression of apobec-1 and the C to U deamination of apoB mRNA are confined to the small intestine. In order to understand the tissue-restricted pattern of apobec-1 expression, we have isolated the chromosomal gene spanning the human *apobec-1* locus. The human *apobec-1* gene spans 18 kb and contains five exons, all of which are translated. Transcription initiation, determined by RNase protection and primer extension analyses, is localized to a single start site 34 nt upstream of the open-reading frame in exon 1. A common, but functionally silent, gene polymorphism was detected that changes Ile80 to Met. RNase protection and reverse-transcription PCR analysis demonstrated the presence of an exon 2-skipped form of apobec-1 mRNA that arises through use of an alternative splice acceptor. This alternative splicing causes a frame-shift that produces a novel, 36 amino acid peptide. The exon 2-skipped form accounts for ~50% of apobec-1 mRNA in the adult small intestine and up to 90% of apobec-1 mRNA in the developing gut. An antipeptide antibody identified the truncated protein in villus cells of the adult small intestine. These data suggest that exon 2-skipping may represent an important control mechanism regulating *apobec-1* gene expression in humans.–**Hirano, K-I., J. Min, T. Funahashi, D.A. Baunoch, and N.O. Davidson.** Characterization of the human *apobec-1* gene: expression in gastrointestinal tissues determined by alternative splicing with production of a novel truncated peptide. *J. Lipid Res.* 1997. **38:** 847–859.

Supplementary key words apolipoprotein B mRNA • RNA editing • developmental regulation • gene structure • intestinal peptide

Apolipoprotein B (apoB) is an obligate component of triglyceride-rich lipoproteins and circulates in two distinct forms, each encoded by a common gene (reviewed in ref. 1). ApoB-100, the full-length form of the protein, contains 4536 residues and is produced in the human liver (1). ApoB-48 is secreted by the human small intestine as a result of a C → U deamination reaction that introduces a translational stop codon into the nuclear apoB transcript (2, 3). This process, referred to as apoB mRNA editing (reviewed in refs. 4, 5), eliminates from apoB-48 the domains present in apoB-100 that are proposed to be involved in the binding of low density lipoprotein (LDL) to the LDL receptor. In addition, synthesis of apoB-48 also precludes the formation of Lp[a], a process that requires the presence of an unpaired cysteine residue found in the extreme carboxyl terminus of apoB-100 (4–7). The net result of apoB mRNA editing thus fundamentally alters the functional properties of the apoB protein and imparts distinct metabolic characteristics upon lipoproteins associated with the different isoforms.

ApoB mRNA editing is mediated by a multicomponent enzyme complex (8) that includes a catalytic subunit, apobec-1 (9). Apobec-1 is an RNA-specific cytidine deaminase (10, 11) with a distinct pattern of expression in mammalian tissues. Originally isolated from rat small intestinal cDNA (9), apobec-1 was found to be widely expressed in both the rat and mouse (12, 13). This pattern of distribution extended to tissues such as the kidney and spleen which, in both rat and mouse, have virtually no detectable apoB mRNA (12). Expression of the human and rabbit homologs, by contrast, were found to be restricted to the gastrointestinal tract, with maximal levels of apobec-1 mRNA detected in the proximal small intestine (14–16). The tissue-restricted pattern of expression of apobec-1 mRNA in humans is consistent with previous studies demonstrating that human liver

Abbreviations: apobec-1, apolipoprotein B mRNA editing enzyme, catalytic polypeptide #1; RT-PCR, reverse transcription-polymerase chain reaction; apoB, apolipoprotein B; RACE, rapid amplification of cDNA ends.

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contains exclusively unedited apoB mRNA (2, 3). A corollary to this demonstration was the finding that transfection of apobec-1 into human HepG2 cells resulted in the acquired ability of these cells to edit apoB mRNA and to secrete apoB-48 (17). In an extension of this paradigm, the successful introduction of apobec-1 into rabbit liver using adenovirus-mediated gene targeting has also been reported (18, 19). The common theme to emerge from these studies is that delivery of apobec-1 to rabbit and human liver cells provides a crucial ingredient in order that editing of the endogenous apoB RNA template may occur. Conclusive demonstration of the requisite importance of apobec-1 was recently provided through experiments in which gene-targeting of *apobec-1* in mice eliminated apoB mRNA editing in all tissues examined and abolished apoB-48 production (20, 21).

ApoB mRNA editing has been demonstrated to be under developmental, hormonal, nutritional, and metabolic regulation in the liver of rats and mice (22–27). Studies in the developing human small intestine have also demonstrated that apoB mRNA editing increases during the late first and early second trimester, in association with a developmental increase in apobec-1 mRNA abundance (28, 29). These findings were recapitulated in Caco-2 cells, a colon cancer-derived cell line that undergoes spontaneous cytodifferentiation upon reaching confluence (29). While the nature of the cues that promote the developmental increase in intestinal apobec-1 mRNA abundance remain unknown at present, the conservation of this process across species suggests that it represents an important adaptation.

In order to pursue the basis for the tissue-restricted pattern of expression of apobec-1 in humans, we have cloned the chromosomal gene spanning the *apobec-1* locus. The structural gene contains five exons, all of which are translated. A single transcription start site was demonstrated, located in the proximal region flanking the first exon. A major finding of this study is the presence of an alternative splice variant of apobec-1 mRNA in which exon 2 is skipped. This splice variant accounts for up to 50% of the mRNA species in the adult jejunum. Alternative splicing of apobec-1 mRNA produces a frame-shift that results in production of a novel truncated peptide of 36 residues which is expressed in villus cells of the adult small intestine.

MATERIALS AND METHODS

Isolation of genomic clones

A human genomic library, prepared from placenta, was purchased from Stratagene (cat # 946206). Approximately three million recombinants were screened at high stringency using a full-length human apobec-1 cDNA (15). Three overlapping clones were obtained that were characterized by Southern blotting and restriction mapping. In order to characterize the region flanking exon 1, we obtained a P1 clone (P1-1425, Genome Sciences, St. Louis, MO) which was generously provided to us by Dr. Thomas Innerarity of the Gladstone Foundation Laboratories, San Francisco. Analysis of sequence motifs was undertaken using the Genetics Computer Group (GCG) software.

Amplification of the 5′ end of the human apobec-1 cDNA (5′ RACE)

First strand cDNA synthesis was undertaken with oligo dT priming using total RNA from adult human jejunum, colon, and stomach. The following incubation conditions were used, representing minor modifications of the recommendations provided with the Marathon cDNA amplification kit (cat # K1802-1, Clontech): reverse transcription was performed at 42°C for 60 min in 20 μL (final volume) buffer containing 6 mM $MgCl_2$, 1 mM dNTP, 1 mM oligo dT, and 50 units Superscript reverse transcriptase (Life Technologies). The reaction mixture was then transferred to a tube containing 24 units *E. coli* DNA polymerase I, 5 units *E. coli* DNA ligase and 1 unit *E. coli* RNase H, in a final volume of 80 μL second strand buffer (Clontech). PCR amplification was undertaken using the adaptor primer (AP1) and gene-specific primers (below) under the following conditions: Initial denaturation for 3 min at 95°C, followed by 30 cycles of 30 seconds at 95°C, 30 seconds at 55°C, 90 seconds at 72°C. The amplicon was analyzed by agarose electrophoresis and Southern transfer, followed by hybridization with an internal primer. The fragment was purified from the agarose gel and cloned into a TA vector (pGEM T, Promega) for sequencing.

Primer extension analysis

The relevant primer, as indicated in the appropriate figure legend, was gel-purified and end-labeled by T4 polynucleotide kinase. Ten fmol of labeled primer was annealed to 50 μg of total RNA (or 10 μg of tRNA) by heating the mixture to 65°C for 90 min in 15 μL of hybridization buffer (0.15 M KCl, 10 mM Tris-HCl, pH 8.3, and 1 mM EDTA), after which the tubes were allowed to cool slowly to room temperature. After annealing, 0.9 μL of 1 M Tris-HCl, pH 8.3, 0.9 μL of 0.5 M $MgCl_2$, 0.25 μL of 1 M DTT, 6.75 μL of 1 mg/ml actinomycin D, 1.33 μL of 5 mM dNTPs, 20 μL H_2O, and 5 units of Superscript reverse transcriptase (Life Technologies, Inc.) were added to each tube and the mixture was incubated for 1 h at 42°C. After RNase A treatment (500 μg/ml RNase A, 37°C, 30 min), phenol–chloroform ex-

traction, and ethanol precipitation, the pellet was resuspended in 3 μL of loading buffer, denatured at 95°C for 3 min and subjected to denaturing electrophoresis, using either 6 or 8% polyacrylamide (Sequegel, National Diagnostics). Gels were dried and exposed to X-ray film (XAR, Kodak) at −80°C with one intensifying screen.

Reverse transcription coupled polymerase chain reaction (RT-PCR)

Aliquots of total RNA (20 μg) from the indicated sources were treated with 0.5 units of DNase RQ1 (Promega, Madison, WI) at 37°C for 45 min in 50 μl of 40 mM Tris-HCl, pH 7.5, 6 mM $MgCl_2$, 10 mM NaCl, 20 units RNase inhibitor (RNasin, Promega). The RNA was then sequentially extracted with phenol–chloroform and chloroform, precipitated with ethanol, washed once in 70% ethanol, and resuspended in water just prior to use. Conditions for RT-PCR used 2 μg total RNA and used downstream priming with primer P5 in the presence of 50 units Superscript reverse transcriptase at 42°C for 60 min. After heating the RT reaction at 75°C for 5 min, one-tenth volume was used for PCR. After heat denaturation at 95°C for 5 min, PCR was conducted in the presence of 0.3 μl of α-^{32}P dCTP (3000 Ci/mmol, 10 mCi/ml, NEN DuPont) using the following parameters: 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 90 seconds, respectively. A final extension of 10 min at 72°C was used for the last cycle. The internal standards (30) were reverse transcribed as described above, using previously described primer-pairs, and amplified in separate tubes (30). Cycle numbers were optimized for each target: 30 cycles for apobec-1, 22 cycles for GAPDH, and 20 cycles for β2-microglobulin (30). Two μL of each reaction was analyzed by 4% nondenaturing PAGE.

RT-PCR amplification of apobec-1 RNA from G292 cells and expression of mutant apobec-1 cDNA

G292 cells were obtained from the American Type Culture Collection (ATCC, Cat# CRL1423) and grown as recommended. Total RNA was used to prime first strand cDNA synthesis as detailed above using primer P10 (see below). RT-PCR conditions were as detailed above, using primers P6 and P10. Identical conditions were used to amplify apobec-1 from genomic DNA with the omission of reverse transcriptase and using primers P7 and P8. The products were cloned into a TA vector (Promega) and sequenced. For expression studies, the cDNAs were amplified with primers P9 and P11, digested with *Bam*HI and *Sal*I, sequenced, and cloned into pGEX4T-3 for expression as GST-fusion proteins. Proteins were expressed in RB79I cells, as previously detailed, and purified over glutathione-agarose beads (31). In vitro apoB RNA editing, RNA binding, and cytidine deaminase activities were determined as previously detailed (31). ApoB RNA editing was conducted on a human apoB RNA template (17) with 10 μg of chicken intestinal S100 extracts as a source of complementation activity.

RNase protection assay

The relevant fragments were generated by restriction digestion of subgenomic clones or by PCR as indicated in the figure legends. All the templates for riboprobe synthesis were fully sequenced and subcloned into pGem 3Zf(+) (Promega), linearized and radiolabeled antisense cRNA synthesized using α-^{32}P-UTP (3000 Ci/mmol, NEN) and either T7 or SP6 RNA polymerase (Promega). These transcripts demonstrated specific activities in the range of $1.7–2.6 \times 10^9$ cpm/μg. Total RNA (50 μg) was coprecipitated with 4×10^4 cpm of the appropriate riboprobe and redissolved in 30 μl of 80% formamide, 40 mM PIPES (pH 6.4), 0.4 M NaCl, and 5 mM EDTA. After denaturation at 90°C for 5 min, the mixture was incubated at 42°C for 16 h. The annealed mixture was incubated at 37°C for 30 min with RNase (A/T1 mixture, Ambion) and the protected fragments were precipitated by Solution Dx (Ambion) and separated on 6 or 8% denaturing PAGE.

Immunocytochemical localization of the truncated peptide produced by alternative splicing of apobec-1 mRNA

Samples of normal human ileum were obtained after surgical resection of colon carcinomas. Four-μM sections were mounted onto Superfrost plus slides (Fisher Scientific, PA), baked at 60°C for 60 min, cleared in xylenes and hydrated through a descending ethanol series. The hydrated sections were placed in a Coplin staining jar containing 0.01 M citrate, pH 7.0, inside a Samsung 1.5 cubic foot, 900 watt microwave. The tissue sections were heated for 2 min on high, followed by 13 min at 20% power. The sections were allowed to cool for 15 min, then were washed in running water. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 20 min. Sections were incubated overnight at 4°C with a 1:300 dilution of rabbit antisera raised against a synthetic peptide (EESNPGSLTSSMTPENFVKRP, prepared by Research Genetics, Huntsville, AL). Control sections were incubated in preimmune serum. Slides were incubated and washed in an automated processor (Ventana Gen II, Ventana Medical Systems, Tucson, AZ) using a biotinylated anti-rabbit antiserum coupled to an indirect (streptavidin-horseradish peroxidase) detection with diaminobenzidine. The slides were counterstained with hematoxylin

and photographed with Fujichrome 100 film using a Zeiss Axiolab microscope.

Oligonucleotides

All oligonucleotides were purchased from GIBCO-BRL and purified, where indicated, by polyacrylamide gel electrophoresis.

AP1: 5′-CCATCCTAATACGACTCACTATAGGGC-3′;

P1: 5′-GGGTCACCGGTTGAAGGACCTTTCAGAAG TCAT-3′
(human apobec-1 translated cDNA +35 to +1);

P3: 5′-CTTTCTCAGAAGTCATGGT-3′
(human apobec-1 translated cDNA nt +16 to −3);

P4: 5′-GTCCTTCAACCGGTGACCCCACT-3′
(human apobec-1 translated cDNA nt +17 to +39);

P5: 5′-CTTCCACGTGATTGGTGGTG-3′
(human apobec-1 translated cDNA nt +190 to +171);

P6: 5′-ACAGAGCACCATGACTTCTGAGAAA-3′
(human apobec-1 translated cDNA nt −10 to +15);

P7: 5′-GACCCCAGAGAACTTCGTAAAGAGGCC-3′
(human apobec-1 translated cDNA nt +82 to +108);

P8: 5′-CCGAGCTACGTAGATCACTAGAGTCAC-3′
(human apobec-1 translated cDNA nt +354 to +328);

P9: 5′-GTAGGATCCATGACTTCTGAGAAAGGTCC TTCA-3′
(human apobec-1 translated cDNA nt +1 to +24 with *Bam* HI linker);

P10: 5′-TCATCTCCAAGCCACAGAAGGATG-3′
(human apobec-1 translated cDNA nt +771 to +688);

P11: 5′-AGTGTCGACTCATCTCCAAGCCACAGAAG GATG-3′
(human apobec-1 translated cDNA nt +771 to +688 with *Sal*I linker).

RESULTS

Structural organization of the human *apobec-1* gene

Characterization of a human P1 clone, illustrated in **Fig. 1,** revealed that the *apobec-1* gene spans approximately 18 kb and contains five exons. The longest of three lambda clones isolated from a human genomic library contained only the last four exons, presumably due to the large size of intron 1 (over 13 kb, see **Table 1**). The exons correspond to those reported for the mouse and, with the exception that exon 1 contains 34 untranslated nucleotides, there were no untranslated exons identified in the human *apobec-1* gene (13). Sequencing of all the exons and the exon-intron boundaries, revealed that all splice-sites conform to the gt-ag rule (Table 1). The exon sizes varied from 28 bp (exon 2) to 398 bp (exon 3) (Table 1) and revealed a similar pattern of codon interruption as noted for the mouse *apobec-1* gene (13).

Delineation of a single transcription start site in exon 1

Two complementary approaches were taken, as indicated schematically in **Fig. 2A.** RNase protection revealed a protected fragment of 50 nt which was detectable in both adult stomach and jejunum (Fig. 2B). Primer extension analysis using adult jejunum revealed a dominant extension product (Fig. 2C), corresponding to the position predicted by RNase protection, and indicating a start site located 34 nt upstream of the initiator ATG codon. In addition, sequence analysis of 2 clones obtained after 5′ RACE (data not shown) confirmed the presence of a single transcription start site at nucleotide −34. The transcription initiation site for *apobec-1* thus creates an mRNA with a short 5′ untranslated region.

A common, but functionally silent, polymorphism detected in the coding region of exon 3

ApoB mRNA editing has been previously demonstrated in G292 cells, an osteosarcoma cell line, after transfection of a chimeric apoB-E expression plasmid containing sufficient sequence flanking the edited site (32). G292 cells do not express endogenous apoB mRNA and thus it was of interest to determine whether apobec-1 mRNA was present in these cells, as extraintestinal expression of apobec-1 in human tissues and cell lines has not been documented to date (15, 16). Accordingly, apobec-1 mRNA was amplified by RT-PCR and the products were sequenced. Six of 6 clones revealed a sequence identical to the previously published human apobec-1 cDNA (15, 16) with a single nucleotide difference noted in all, namely a C → T transition at position 240 of the cDNA that alters an AT*C* codon to an AT*G*, thus changing an isoleucine to a methionine residue (**Fig. 3A**). Genomic DNA was isolated from this cell line and subjected to PCR amplification and sequence analysis, revealing the identical substitution (data not shown). Because the C → T transition de-

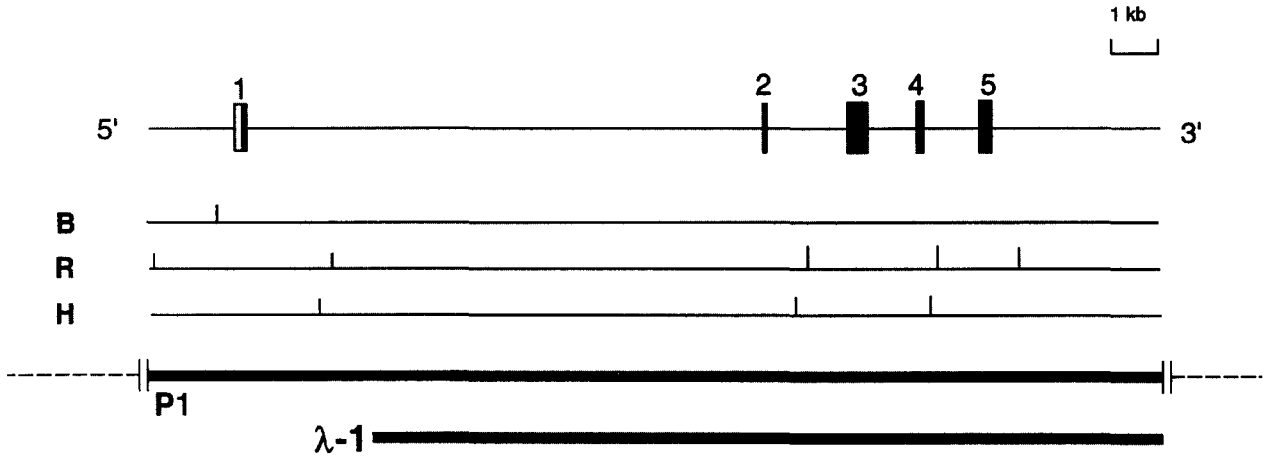


Fig. 1. Structural organization and partial restriction map of the human *apobec-1* gene. Filled and open boxes indicate translated and untranslated exons, respectively. B, *Bam*HI; R, *Eco*RI.

stroys a *Pvu*II restriction site (Fig. 3A), screening was undertaken, as shown in Fig. 3B, to determine the allele frequency of this polymorphism. Analysis of 50 unrelated individuals from three different ethnic groups revealed that the mutant (A2) allele is more prevalent in African-American and Chinese populations than in those of Caucasian descent (Fig. 3C).

In order to determine the functional importance of this mutation, both the mutant and wild-type alleles were expressed as GST-fusion proteins (31). Expression of the two proteins (Fig. 3D), allowed an analysis of their relative ability to catalyze in vitro editing of a synthetic human apoB RNA template. This assay revealed no difference in the ability of either protein to mediate C to U RNA editing, over a 5-fold range of apobec-1 input (Fig. 3E). These data suggest that this common polymorphism is functionally silent.

Regulation of intestinal apobec-1 gene expression through alternative splicing: exon 2-skipping

Two complementary approaches demonstrate the presence of alternative splicing which produces exon 2-skipping in apobec-1 RNA. As shown in **Fig. 4A,** RNase protection using a riboprobe spanning exons 2 and 3 produces a 174 nt fragment with the full length mRNA; exon 2-skipping produces a fragment of 146 nt. Similarly, RT-PCR amplification using primers in exon 1 and exon 3 generates an amplicon of 200 bp in the presence of exon 2 while exon 2-skipping results in a product that is 28 bp smaller (172 bp). The results of these approaches are demonstrated in Figs. 4B and 4C, which indicate that approximately 50–60% (by densitometric scanning) of apobec-1 mRNA in the adult stomach and small intestine contains the exon 2-skipped form.

Studies of the developmental regulation of human small intestinal apobec-1 gene expression have previously demonstrated an increase in apobec-1 mRNA and protein abundance during the late first trimester (29). In view of the demonstration of exon 2-skipping in the adult small intestine, it was of interest to determine whether a similar phenomenon occurs in the fetal gut. Accordingly, RT-PCR was performed using primers P5 and P6, as illustrated in Fig. 4A, to distinguish the splice variants. Several samples were examined from different gestational age fetal small intestine in addition to Caco-2 RNA samples isolated at different times post-plating (29, 30). The results indicate a progressive decrease in the proportion of the exon 2-skipped apobec-1 mRNA

TABLE 1. Organization of human *Apobec 1* gene

Exon Number	Exon Size	Sequence at Exon-Intron Junction		Codon Interrupted	Intron Size
		5′ Splice Donor	3′ Splice Acceptor		
	bp	↓	↓		*kb*
1	50	GAG AAA G gtaaaat	ggtcaacag GT CCT TCA	Gly^{6}	13.0
2	28	ACT CTG AG gtaagaa	tatttcag G AGA AGA	Arg^{15}	1.8
3	398	GCA TCA G gtaaaa	ccttag AG TAT TAT	Gly^{148}	1.0
4	119	TA ATT CTA gtaagt	attccag AGT CTT CCA	Leu^{187}/Ser^{188}	1.1
5	280				

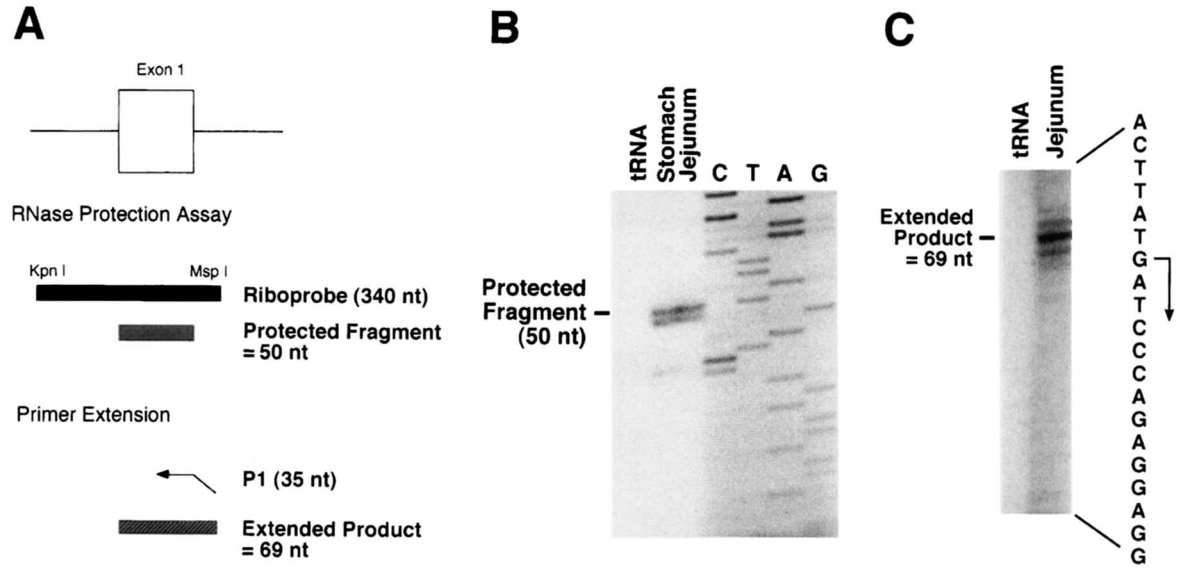


Fig. 2. Determination of the transcription start site in the human *apobec-1* gene. A. Schematic overview of transcription start site mapping. For RNase protection assay the riboprobe template was generated by subcloning a *Kpn* I/*Msp* I genomic fragment into a pGem 3Z vector. Primer extension was performed with antisense primer P1, corresponding to nucleotide +1 to +35 of human apobec-1 translated cDNA. B. RNase protection mapping of major transcription start site in human stomach and jejunum. Fifty μg of total RNA from adult human stomach and jejunum were hybridized with a ^{32}P-labeled riboprobe and the protected fragment was analyzed by 8% PAGE. Lanes G, A, T, C represent a dideoxynucleotide chain-termination sequencing reaction performed with primer P3. C. Primer extension results using 50 μg of total RNA from human jejunum, hybridized to the end-labeled primer P1 and extended with reverse transcriptase. Primer extension products were separated by 8% PAGE. The corresponding nucleotide sequence is shown and the arrow indicates the single, major transcription start site.

from a maximum of ~90% at 10 weeks gestational age to ~50% in the adult sample (**Fig. 5A**). Caco-2 RNA demonstrated over 90% of the exon 2-skipped form at all time points examined (Fig. 5B).

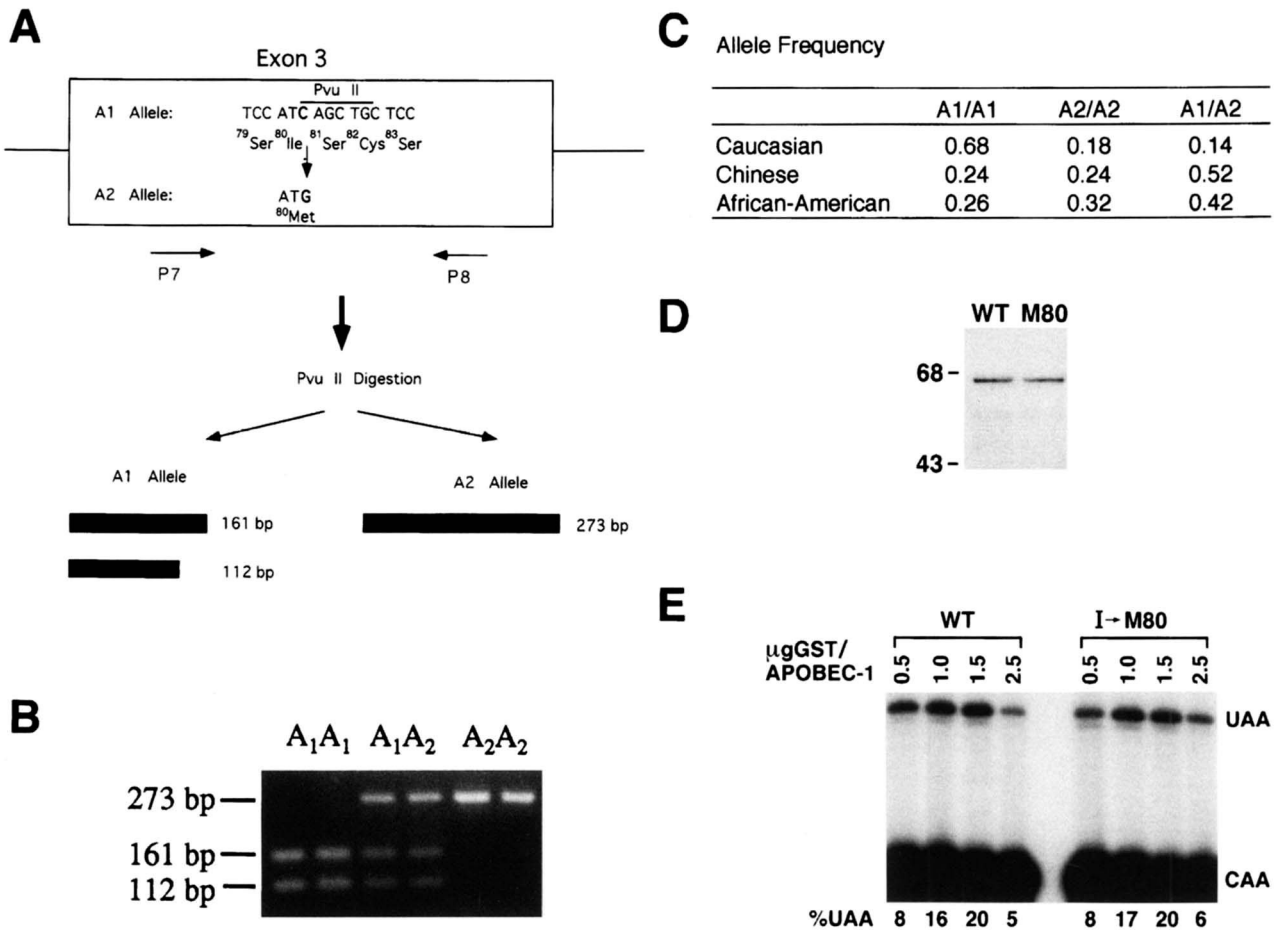
Exon 2-skipping produces a frame-shift and creates a novel truncated peptide

As a consequence of the alternative splicing described above, there is a frame-shift that alters the reading frame of the exon 2-skipped mRNA, as detailed in **Fig. 6A.** Translation of the splice-variant produces a novel, 36 amino acid peptide in which only the first 6 residues are shared with apobec-1 (Fig. 6A). As summarized schematically in Fig. 6B, the data suggest that exon 2-skipping is a quantitatively important mechanism regulating the expression of *apobec-1* in the human gastrointestinal tract.

In order to determine whether a corresponding protein product of this alternatively spliced form of apobec-1 mRNA is present in the human small intestine, an antipeptide antiserum was raised against a portion of the truncated peptide and used in immunocytochemical analysis. A reactive product was demonstrated in adult small intestinal villi (**Fig. 7A, B**) suggesting that this truncated peptide is detectable in an appropriate cellular distribution. No reaction product was detectable using preimmune antiserum (Fig. 7C).

Sequence analysis of the 5′ flanking region of human *apobec-1*

The proximal ~1.5 kb of 5′ flanking sequence upstream of the translation initiation site was sequenced as shown in **Fig. 8.** The most proximal ~130 nucleotides (heavy dashed line in Fig. 8) demonstrates ~75% homology to the corresponding region of the mouse promoter (13). Further upstream of this region, however, the sequence diverges with less than 40% homology over the next ~1400 nucleotides. No canonical TATA or CAAT box were demonstrated in the proximal promoter (Fig. 8), a finding similar to that in the mouse *apobec-1* gene which does not contain a canonical TATA box in the region flanking exon 1 (13). Two *Alu* sequences, of the Alu-S subfamily (33), were identified in the region flanking the transcription start site (underlined in Fig. 8), in addition to a series of 27 GT repeats in the proximal region of intron 1 (Fig. 8). Binding sites for PEA-3 (34, 35) were identified in addition to sites for several other known transcription factors (36–42). The functional analysis of this region is discussed below.



	A1/A1	A2/A2	A1/A2
Caucasian	0.68	0.18	0.14
Chinese	0.24	0.24	0.52
African-American	0.26	0.32	0.42

Fig. 3. A common polymorphism of apobec-1 discovered in G292 cells and its distribution in different populations: functional implications as determined using in vitro apoB RNA editing assays with recombinant mutant and wild-type proteins. A. Sequence of wild-type (A1) and mutant (A2) allele. The polymorphism converts an ATC to an ATG codon and disrupts a *Pvu* II site in the PCR product. B. Screening for the A1 and A2 alleles using PCR and *Pvu* II digestion. C. Allele frequency among 50 unrelated individuals from three different populations. D. Expression of the wild-type (WT) and mutant (M80) forms of human apobec-1, each as GST-fusion proteins. The proteins were purified using glutathione-agarose and analyzed by denaturing SDS-PAGE followed by Western blotting with anti-apobec-1 antisera. E. In vitro apoB RNA editing assays performed on a human apoB RNA template using the indicated amounts of recombinant apobec-1 and 10 µg chicken intestinal S100 extract as a source of complementation activity. After incubation with the indicated amounts of apobec-1, the RNA was extracted and analyzed by primer extension. The numbers at the bottom of each lane indicate the percent editing, determined by laser scanning densitometry of the autoradiograms.

DISCUSSION

RNA editing is an important molecular mechanism as it allows the production of functionally distinct protein products from a common gene, thereby increasing the genetic diversity of the organism at little metabolic cost. ApoB mRNA editing in the mammalian small intestine has been proposed to represent a potential adaptation whereby intestinal triglyceride-rich lipoproteins, principally chylomicron remnants containing apoB-48 and apoE, are preferentially directed to receptors such as the low density lipoprotein-related receptor (4, 5), thereby allowing the rapid delivery of intestinal triglyceride to the liver. The absence of intestinal apoB mRNA editing in chickens and frogs, coupled with the lack of expression of a homolog of apoE in these species, lends support to the hypothesis that the emergence of a dual-receptor mechanism for clearance of lipoproteins coincides with the mammalian divergence (43–45). In attempting to understand the emergence of apoB mRNA editing, the current studies provide insight into the molecular mechanisms that may regulate *apobec-1* gene expression in the human gastrointestinal tract.

Certain structural features of the human *apobec-1* gene detailed in this report confirm earlier predictions (13) concerning the tissue-specific pattern of apoB

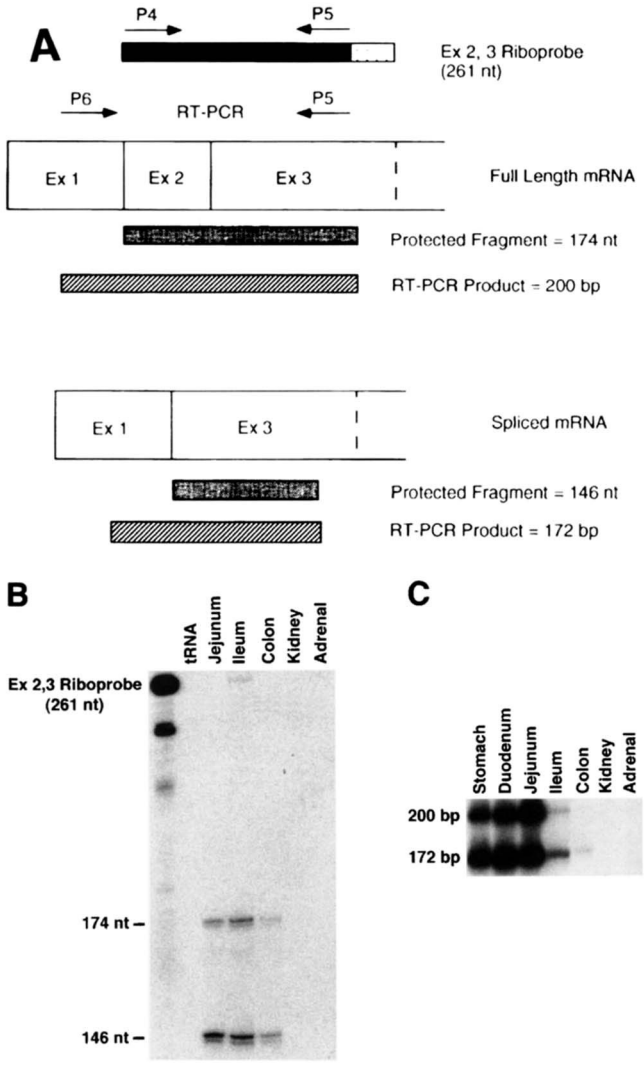


Fig. 4. Demonstration of exon 2-skipped apobec-1 mRNA in the human gastrointestinal tract. A. Schematic overview of the strategies for detecting alternatively spliced mRNA by both RNase protection and RT-PCR analysis. The exon 2, 3 riboprobe was generated by PCR using primers P4 and P5, and the product was cloned into pGEM3Z and sequenced. RT-PCR was conducted using primers P5 and P6. The lengths of the protected fragments and RT-PCR products are shown. B. RNase protection assay. Fifty μg of total RNA from adult human jejunum, ileum, colon, kidney, and adrenal were hybridized with the ^{32}P-labeled riboprobe and the protected fragments were analyzed by 6% PAGE. C. Two μg of total RNA was used for initial priming with primer P5 in a 20 μl reaction volume. Two μl of this reaction was used for PCR in the presence of α-^{32}P-dCTP. PCR-products were analyzed by 4% nondenaturing PAGE.

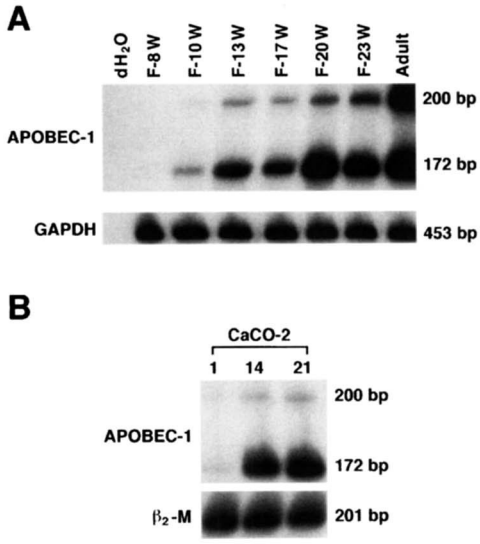


Fig. 5. Developmental regulation of alternative splicing of apobec-1 mRNA in human fetal small intestine and in Caco-2 cells. A. Fetal human small intestinal apobec-1 mRNA splicing. Two-hundred ng of total RNA from fetal small intestinal samples from 8, 10, 13, 17, 20, and 23 weeks gestation and adult human jejunum was used for RT-PCR amplification in the presence of α-^{32}P-dCTP. RT-PCR amplification of apobec-1 (28 cycles) and GAPDH (22 cycles) was conducted in separate tubes. Aliquots of the reaction mix were analyzed by 4% nondenaturing PAGE and autoradiography. B. Regulation of exon 2-skipping in pre-and post-confluent Caco-2 cells. Two-hundred ng of total RNA from Caco-2 cells grown on plastic for 1, 14, and 21 days (post-plating) was used for RT-PCR amplification in the presence of α-^{32}P-dCTP. RT-PCR amplification of apobec-1 (30 cycles) and β2 microglobulin (20 cycles) was conducted in separate tubes. Aliquots of the reaction mix were analyzed by 4% nondenaturing PAGE and autoradiography.

mRNA editing in humans (2–5) and the restriction of apobec-1 mRNA to the small intestine (15, 16). This includes the observation that the human gene contains only the last five exons, in contrast to the presence of three untranslated exons demonstrated in the mouse *apobec-1* gene (13). The significance of this observation is illustrated by the high levels of expression previously obtained in a variety of cells (including HeLa cells) of a heterologous reporter using a mouse apobec-1 promoter fragment containing ~1.7 kb of flanking sequence immediately 5′ to the first untranslated exon (13). The same study revealed that intestinal (Caco-2 cell) reporter gene expression was increased with a construct containing ~1.7 kb of flanking sequence upstream of exon 4, which represents the first translated exon (13). Additionally, expression of the exon 4-reporter construct in non-intestinal cells was extremely low (13). Taken together, these data suggest that intestine-specific expression of apobec-1 may be regulated through sequences immediately upstream of the coding region of the gene. We undertook similar studies using luciferase reporter constructs linked to genomic fragments containing from 2 to 6kb flanking the translation start site of human *apobec-1*, either with or without regions containing up to 3 kb of intron 1. These constructs produced no detectable activity in HeLa or HepG2 cells (K. Hirano, J. Min, and N.O. Davidson, unpublished observations). Surprisingly, there was also no detectable activity from these constructs when

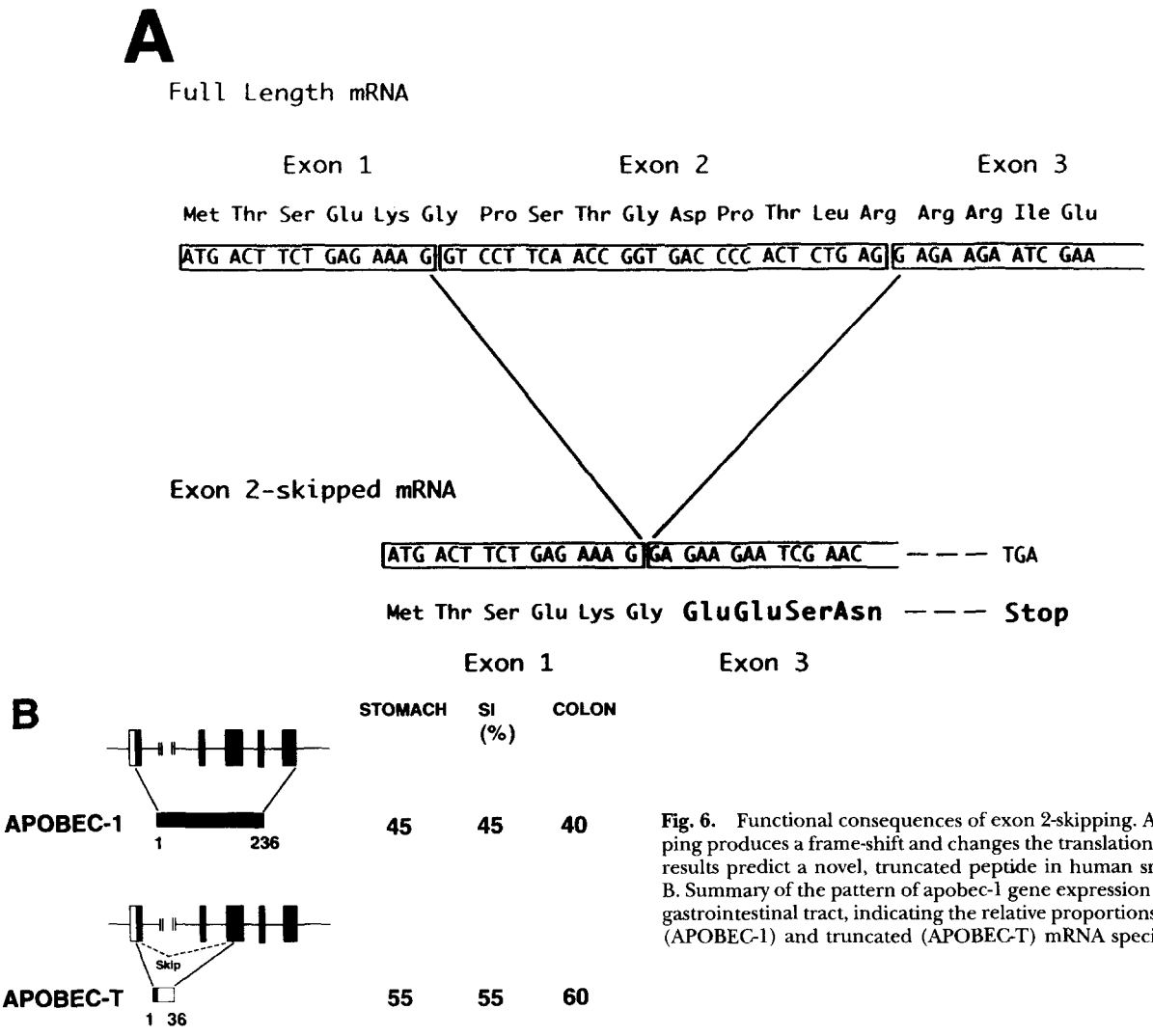


Fig. 6. Functional consequences of exon 2-skipping. A. Exon 2-skipping produces a frame-shift and changes the translation product. The results predict a novel, truncated peptide in human small intestine. B. Summary of the pattern of apobec-1 gene expression in the human gastrointestinal tract, indicating the relative proportions of full length (APOBEC-1) and truncated (APOBEC-T) mRNA species.

transfected into postconfluent Caco-2 cells (K. Hirano, J. Min, and N.O. Davidson, unpublished observations). In this regard, one noteworthy feature of the human apobec-1 gene is the presence, at position $-53 \rightarrow -48$, of the central core (AACAAT, Fig. 8) of a 20 nucleotide motif recently identified by Simon, Roberts, and Gordon (42) as a colon-specific repressor element in the human *Fabpi* gene. The entire 20 nucleotide region of apobec-1 flanking the core motif contains only 12 of 20 matches with the corresponding region of the human *Fabpi* gene (42), but certain implications for this *cis*-acting element may extend to the current studies. Specifically, as the expression studies were carried out in Caco-2 cells, a colon cancer-derived cell line, it is possible that the colon-specific silencer element may repress activity of the constructs that we examined in this cell line. It bears emphasis that findings to be reported elsewhere demonstrate that reporter constructs prepared from rat genomic DNA fragments, containing from 200 bp to ~2 kb, flanking the first translated exon are active in Caco-2 cells (K. Hirano, J. Min, and N.O. Davidson, unpublished observations). These observations diminish the possibility that a technical limitation was responsible for the apparent absence of activity of human promoter fragments in Caco-2 cells. Nevertheless, identification of a functional intestinal promoter for the human *apobec-1* gene will require further study.

Two other findings emerged from the current studies, both of which were unanticipated. The first was the demonstration that *apobec-1* is polymorphic. This observation was initially made through an analysis of apobec-1 mRNA in G292 cells, an osteosarcoma cell line previously demonstrated to support RNA editing of a chimeric apoB-E RNA (32). Expression of apobec-1 mRNA was examined in these cells in view of the fact that a functional transcript has not previously been demon-

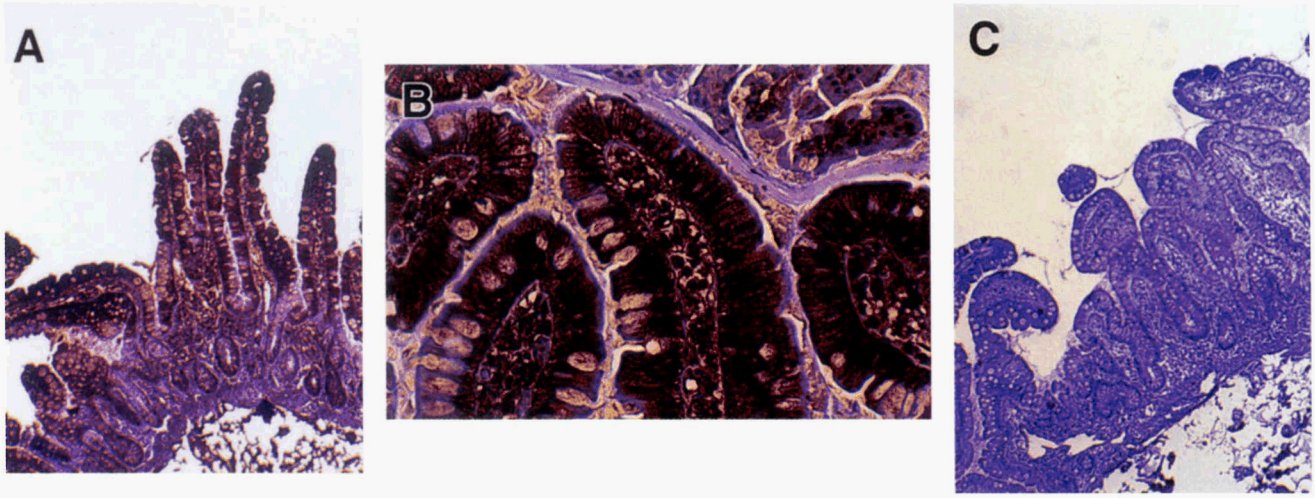
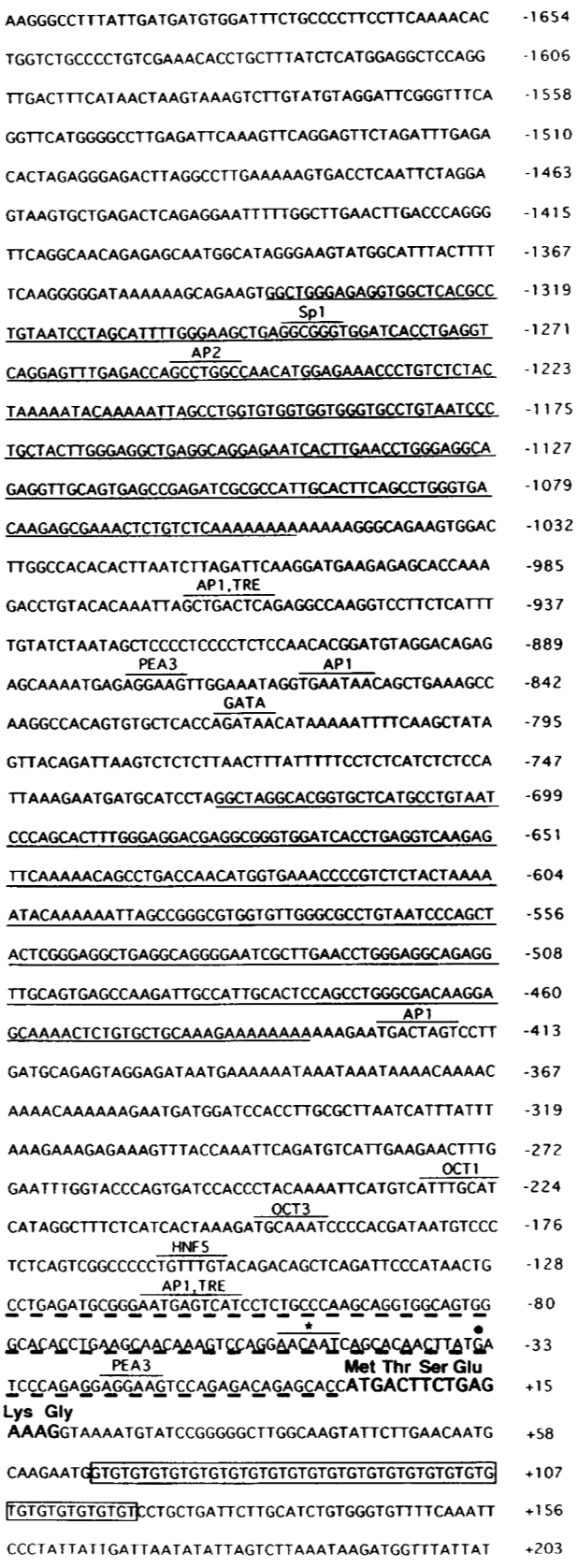


Fig. 7. Immunocytochemical demonstration of a protein product corresponding to the alternatively spliced apobec-1 mRNA in human small intestine. Diffuse staining is evident along the crypt-villus axis of the adult small intestine (panel A) and appears to be within enterocytes as the large, vacuolated goblet cells shown at higher power (panel B) do not stain. No reaction product was detectable with preimmune serum (panel C).

strated outside of the human gastrointestinal tract (15, 16). RT-PCR amplification and sequencing of multiple PCR products from both RNA and genomic DNA demonstrated a single nucleotide change from the previously published human cDNA sequence (15, 16). Further analysis demonstrated that this mutant is functionally competent as regards in vitro apoB RNA editing. Additional studies also demonstrated essentially wild-type levels of cytidine deaminase and RNA-binding activity using the recombinant protein (K. Hirano, S. Anant, and N.O. Davidson, unpublished observations). These observations, coupled with the apparent frequency of the polymorphism, lend support to the prediction that the mutant form of the protein is likely to be fully functional in vivo. It should be emphasized, however, that a subtle phenotype cannot be excluded.

A second and equally unexpected observation was the presence of an alternatively spliced form of apobec-1 mRNA which accounts for ~50% of the transcript in the adult human small intestine. This truncated peptide is predicted to have no apoB mRNA editing or cytidine deaminase activity as it does not contain the catalytic motif previously demonstrated to be required for these functions (10, 11, 31). A detailed examination of apobec-1 mRNA from numerous tissues of both the rat and mouse, to be reported elsewhere, demonstrates that generally less than 10% of the transcripts contain an alternatively spliced form corresponding the exon 2-skipped species identified in human intestine (K. Hirano, J. Min, and N.O. Davidson, unpublished observations). However, alternative splicing of the mouse apobec-1 transcript was reported earlier and involves an alternate splice acceptor site in the noncoding region of the first translated exon (13). Thus there is a precedent for the regulation of apobec-1 gene expression through mechanisms involving alternative processing of the transcript. The current studies, demonstrating an alternatively spliced mRNA in which exon 2 is skipped, raise the possibility that an alternative pathway might exist for the regulation of apobec-1 gene expression in the human intestine. Why might such a mechanism have evolved? Studies in transgenic mice and rabbits have indicated that the unregulated, high-level production of apobec-1 in the liver is associated with the development of hepatocellular carcinoma in a large percentage of animals (46). Based upon this association, it is tempting to speculate that alternative splicing of apobec-1 mRNA in the human small intestine may provide a mechanism to limit the expression of apobec-1. Further analysis of the structural evolution of the *apobec-1* gene, however, will be required to permit more solid conclusions to be made concerning this point.

Numerous studies have demonstrated that apoB mRNA editing is regulated in mouse and rat liver via mechanisms involving hormonal, metabolic, and nutritional cues (22–27). Although less is known concerning the regulation of apoB mRNA editing in the human small intestine, earlier work demonstrated a developmental increase in apoB-48 secretion in association with an increasing proportion of edited apoB mRNA (28). This increase occurs in temporal association with an increase in small intestinal apobec-1 mRNA abundance (29), suggesting that *apobec-1* gene expression is subject to modulation in a physiologically relevant context. These features notwithstanding, addressing the tran-



scriptional regulation of intestinal gene expression has been difficult to address in cell culture models, the most significant issue being the lack of an entirely representative model of a small intestinal enterocyte. Indeed, other workers have pointed out the limitations of Caco-2 cells in studies of intestinal apolipoprotein gene expression (47). Further work involving transgenic mice will be required to assist in the dissection of *cis*-acting elements important in the cell-specific expression of *apobec-1* in the human small intestine, in particular the relevance of the sequence divergence in the promoter region of the human versus the rat and mouse *apobec-1* genes. These and other issues concerning the tissue-specific expression of this gene will be the focus of future reports.

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REFERENCES

1. Young, S. G. 1990. Recent progress in understanding apolipoprotein B. *Circulation.* **82:** 1574–1594.
2. Powell, L. M., S. C. Wallis, R. J. Pease, Y. H. Edwards, T. J. Knott, and J. Scott. 1987. A novel form of tissue-specific RNA processing produces apolipoprotein-B48 in intestine. *Cell.* **50:** 831–840.
3. Chen, S. H., G. Habib, C. Y. Yang, Z. W. Gu, B. R. Lee, S. A. Weng, S. R. Silberman, S. J. Cai, J. P. Deslypere, M. Rosseneu, A. M. Gotto, W. H. Li, and L. Chan. 1987. Apolipoprotein B-48 is the product of a messenger RNA with an organ-specific in-frame stop codon. *Science.* **238:** 363–366.
4. Scott, J., N. Navaratnam, S. Bhattacharya, and J. R. Morrison. 1994. The apolipoprotein B messenger RNA editing enzyme. *Curr. Opin. Lipidol.* **5:** 87–93.
5. Davidson, N. O., S. Anant, and A. J. MacGinnitie. 1995. Apolipoprotein B messenger RNA editing: insights into

Fig. 8. 5′ Flanking sequence of exon 1 in human apobec-1 gene. The locations of potential transcription factor binding sites are indicated by a solid line. Two *Alu* sequences are indicated with continuous underlining. The asterisk indicates the putative colon-specific-repressor site identified in the human *Fabpi* gene (42). The solid circle indicates the major transcription start site; 27 repeats of GT are boxed. The heavy dashed line indicates the portion of flanking sequence with 75% homology to that in the mouse *apobec-1* gene (13). Translated nucleotides are indicated in large, bold characters.

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the molecular regulation of post-transcriptional cytidine deamination. *Curr. Opin. Lipidol.* **6:** 70–74.
6. McCormick, S. P., J. K. Ng, S. Taylor, L. M. Flynn, R. E. Hammer, and S. G. Young. 1995. Mutagenesis of the human apolipoprotein B gene in a yeast artificial chromosome reveals the site of attachment for apolipoprotein [a]. *Proc. Natl. Acad. Sci. USA.* **92:** 10147–10151.
7. Callow, M. J., and E. M. Rubin. 1995. Site-specific mutagenesis demonstrates that cysteine 4326 of apolipoprotein B is required for covalent linkage with apolipoprotein [a] in vivo. *J. Biol. Chem.* **270:** 23914–23917.
8. Harris, S. G., I. Sabio, E. Mayer, M. F. Steinberg, J. W. Backus, J. D. Sparks, C. E. Sparks, and H. C. Smith. 1993. Extract-specific heterogeneity in high-order complexes containing apolipoprotein B mRNA editing activity and RNA-binding proteins. *J. Biol. Chem.* **268:** 7382–7392.
9. Teng, B., C. F. Burant, and N. O. Davidson. 1993. Molecular cloning of an apolipoprotein B messenger RNA editing protein. *Science.* **260:** 1816–1819.
10. Navaratnam, N., S. Bhattacharya, T. Fujino, D. Patel, A. L. Jarmuz, and J. Scott. 1995. Evolutionary origins of apoB mRNA editing: catalysis by a cytidine deaminase that has acquired a novel RNA-binding motif at its active site. *Cell.* **81:** 187–195.
11. Anant, S., A. J. MacGinnitie, and N. O. Davidson. 1995. apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, is a novel RNA-binding protein. *J. Biol. Chem.* **270:** 14762–14767.
12. Funahashi, T., F. Giannoni, A. M. DePaoli, S. F. Skarosi, and N. O. Davidson. 1995. Tissue-specific, developmental and nutritional regulation of the gene encoding the catalytic subunit of the rat apolipoprotein B mRNA editing enzyme: functional role in the modulation of apoB mRNA editing. *J. Lipid Res.* **36:** 414–428.
13. Nakamuta, M., K. Oka, J. Krushkal, K. Kobayashi, M. Yamamoto, W. H. Li, and L. Chan. 1995. Alternative mRNA splicing and differential promoter utilization determine tissue-specific expression of the apolipoprotein B mRNA-editing protein (Apobec1) gene in mice. Structure and evolution of Apobec1 and related nucleoside/nucleotide deaminases. *J. Biol. Chem.* **270:** 13042–13056.
14. Yamanaka, S., K. S. Poksay, M. E. Balestra, G. Q. Zeng, and T. L. Innerarity. 1994. Cloning and mutagenesis of the rabbit apoB mRNA editing protein. A zinc motif is essential for catalytic activity, and noncatalytic auxiliary factor(s) of the editing complex are widely distributed. *J. Biol. Chem.* **269:** 21725–21734.
15. Hadjiagapiou, C., F. Giannoni, T. Funahashi, S. F. Skarosi, and N. O. Davidson. 1994. Molecular cloning of a human small intestinal apolipoprotein B mRNA editing protein. *Nucleic Acids Res.* **22:** 1874–1879.
16. Lau, P. P., H. J. Zhu, A. Baldini, C. Charnsangavej, and L. Chan. 1994. Dimeric structure of a human apolipoprotein B mRNA editing protein and cloning and chromosomal localization of its gene. *Proc. Natl. Acad. Sci. USA.* **91:** 8522–8526.
17. Giannoni, F., D. K. Bonen, T. Funahashi, C. Hadjiagapiou, C. F. Burant, and N. O. Davidson. 1994. Complementation of apolipoprotein B mRNA editing by human liver accompanied by secretion of apolipoprotein B48. *J. Biol. Chem.* **269:** 5932–5936.
18. Hughes, S. D., D. Rouy, N. Navaratnam, J. Scott, and E. M. Rubin. 1996. Gene transfer of cytidine deaminase apoBEC-1 lowers lipoprotein[a] in transgenic mice and induces apolipoprotein B editing in rabbits. *Hum. Gene Ther.* **7:** 39–49.
19. Kozarsky, K. F., D. K. Bonen, F. Giannoni, T. Funahashi, J. M. Wilson, and N. O. Davidson. 1996. Hepatic expression of the catalytic subunit of the apolipoprotein B mRNA editing enzyme (apobec-1) ameliorates hypercholesterolemia in LDL receptor-deficient rabbits. *Hum. Gene Ther.* **7:** 943–957.
20. Hirano, K., S. G. Young, R. V. Farese, Jr., J. Ng, E. Sande, C. Warburton, L. M. Powell-Braxton, and N. O. Davidson. 1996. Targeted disruption of the mouse apobec-1 gene abolishes apolipoprotein B mRNA editing and eliminates apolipoprotein B48. *J. Biol. Chem.* **271:** 9887–9890.
21. Morrison, J. R., C. Paszty, M. E. Stevens, S. D. Hughes, T. Forte, J. Scott, and E. M. Rubin. 1996. Apolipoprotein B RNA editing enzyme-deficient mice are viable despite alterations in lipoprotein metabolism. *Proc. Natl. Acad. Sci. USA.* **93:** 7154–7159.
22. Wu, J. H., C. F. Semenkovich, S. H. Chen, W. H. Li, and L. Chan. 1990. Apolipoprotein B mRNA editing. Validation of a sensitive assay and developmental biology of RNA editing in the rat. *J. Biol. Chem.* **265:** 12312–12316.
23. Higuchi, K., K. Kitagawa, K. Kogishi, and T. Takeda. 1992. Developmental and age-related changes in apolipoprotein B mRNA editing in mice. *J. Lipid Res.* **33:** 1753–1764.
24. Davidson, N. O., L. M. Powell, S. C. Wallis, and J. Scott. 1988. Thyroid hormone modulates the introduction of a stop codon in rat liver apolipoprotein B messenger RNA. *J. Biol. Chem.* **263:** 13482–13485.
25. Davidson, N. O., R. C. Carlos, and A. M. Lukaszewicz. 1990. Apolipoprotein B mRNA editing is modulated by thyroid hormone analogs but not growth hormone administration in the rat. *Mol. Endocrinol.* **4:** 779–785.
26. Baum, C. L., B. B. Teng, and N. O. Davidson. 1990. Apolipoprotein B messenger RNA editing in the rat liver. Modulation by fasting and refeeding a high carbohydrate diet. *J. Biol. Chem.* **265:** 19263–19270.
27. Lau, P. P., D. J. Cahill, H. J. Zhu, and L. Chan. 1995. Ethanol modulates apolipoprotein B mRNA editing in the rat. *J. Lipid Res.* **36:** 2069–2078.
28. Teng, B., M. Verp, J. Salomon, and N. O. Davidson. 1990. Apolipoprotein B messenger RNA editing is developmentally regulated and widely expressed in human tissues. *J. Biol. Chem.* **265:** 20616–20620.
29. Giannoni, F., S. C. Chou, S. F. Skarosi, M. S. Verp, F. J. Field, R. A. Coleman and N. O. Davidson. 1995. Developmental regulation of the catalytic subunit of the apolipoprotein B mRNA editing enzyme (APOBEC-1) in human small intestine. *J. Lipid Res.* **36:** 1664–1675.
30. Giannoni, F., F. J. Field, and N. O. Davidson. 1994. An improved reverse transcription-polymerase chain reaction method to study apolipoprotein gene expression in Caco-2 cells. *J. Lipid Res.* **35:** 340–350.
31. MacGinnitie, A. J., S. Anant, and N. O. Davidson. 1995. Mutagenesis of apobec-1, the catalytic subunit of the mammalian apolipoprotein B mRNA editing enzyme, reveals distinct domains that mediate cytosine nucleoside deaminase, RNA binding, and RNA editing activity. *J. Biol. Chem.* **270:** 14768–14775.
32. Bostrom, K., Z. Garcia, K. S. Poksay, D. F. Johnson, A. J. Lusis, and T. L. Innerarity. 1990. Apolipoprotein B mRNA editing. Direct determination of the edited base and occurrence in non-apolipoprotein B-producing cell lines. *J. Biol. Chem.* **265:** 22446–22452.

33. Jurka, J., and A. Milosavljevic. 1991. Reconstruction and analysis of human Alu genes. *J. Mol. Evol.* **32:** 105–121.
34. Martin, M. E., J. Piette, M. Yaniv, W. J. Tang, and W. R. Folk. 1988. Activation of the polyomavirus enhancer by a murine activator protein 1 (AP1) homolog and two contiguous proteins. *Proc. Natl. Acad. Sci. USA.* **85:** 5839–5843.
35. Wasylyk, B., C. Wasylyk, P. Flores, A. Begue, D. Leprince, and D. Stehelin. 1990. The c-ets proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature.* **346:** 191–193.
36. Lee, W., P. Mitchell, and R. Tjian. 1987. Purified transcription factor AP-1 interacts with TPA-inducible enhancer elements. *Cell.* **49:** 741–752.
37. Grange, T., J. Roux, G. Rigaud, and R. Pictet. 1991. Cell-type specific activity of two glucocorticoid responsive units of rat tyrosine aminotransferase gene is associated with multiple binding sites for C/EBP and a novel liver-specific nuclear factor. *Nucleic Acids Res.* **19:** 131–139.
38. Schmidt, M. C., Q. Zhou, and A. J. Berk. 1989. Sp1 activates transcription without enhancing DNA-binding activity of the TATA box factor. *Mol. Cell Biol.* **9:** 3299–3307.
39. Barnhart, K. M., C. G. Kim, and M. Sheffery. 1989. Purification and characterization of an erythroid cell-specific factor that binds the murine alpha- and beta-globin genes. *Mol. Cell Biol.* **9:** 2606–2614.
40. Ho, I. C., N. K. Bhat, L. R. Gottschalk, T. Lindsten, C. B. Thompson, T. S. Papas, and J. M. Leiden. 1990. Sequence-specific binding of human Ets-1 to the T-cell receptor alpha gene enhancer. *Science.* **250:** 814–818.
41. Okamoto, K., H. Okazawa, A. Okuda, M. Sakai, M. Muramatsu, and H. Hamada. 1990. A novel octamer binding transcription factor is differentially expressed in mouse embryonic cells. *Cell.* **60:** 461–472.
42. Simon, T. C., L. J. Roberts, and J. I. Gordon. 1995. A 20-nucleotide element in the intestinal fatty acid binding protein gene modulates its cell lineage-specific, differentiation-dependent, and cephalocaudal patterns of expression in transgenic mice. *Proc. Natl. Acad. Sci. USA.* 92: 8685–8689.
43. Mehta, K. D., W. J. Chen, J. L. Goldstein, and M. S. Brown. 1991. The low density lipoprotein receptor in *Xenopus laevis.* I. Five domains that resemble the human receptor. *J. Biol. Chem.* **266:** 10406–10414.
44. Mehta, K. D., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1991. The low density lipoprotein receptor in *Xenopus laevis.* II. Feedback repression mediated by conserved sterol regulatory element. *J. Biol. Chem.* **266:** 10415–10419.
45. Teng, B., and N. O. Davidson. 1992. Evolution of intestinal apolipoprotein B mRNA editing. Chicken apolipoprotein B mRNA is not edited, but chicken enterocytes contain in vitro editing enhancement factor(s). *J. Biol. Chem.* **267:** 21265–21272.
46. Yamanaka, S., M. E. Balestra, L. D. Ferrell, J. Fan, K. S. Arnold, S. Taylor, J. M. Taylor, and T. L. Innerarity. 1995. Apolipoprotein B mRNA-editing protein induces hepatocellular carcinoma and dysplasia in transgenic animals. *Proc. Natl. Acad. Sci. USA.* **92:** 8483–8487.
47. Bisaha, J. G., T. C. Simon, J. I. Gordon, and J. L. Breslow. 1995. Characterization of an enhancer element in the human apolipoprotein C-III gene that regulates human apolipoprotein A-I gene expression in the intestinal epithelium. *J. Biol. Chem.* **270:** 19979–19988.