Phylogenetic Analysis of the Apolipoprotein B mRNA-editing Region

EVIDENCE FOR A SECONDARY STRUCTURE BETWEEN THE MOORING SEQUENCE AND THE 3' EFFICIENCY ELEMENT

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Apolipoprotein (apo) B mRNA editing is the deamination of C6666 to uridine, which changes the codon at position 2153 from a genomically encoded glutamine (CAA) to an in-frame stop codon (UAA). The apoB mRNA-editing enzyme complex recognizes the editing region of the apoB pre-mRNA with exquisite precision. Four sequence elements spanning 139 nucleotides (nt) on the apoB mRNA have been identified that specify this precision. In cooperation with the indispensable mooring sequence and spacer element, a 5' efficiency element and a 3' efficiency element enhance editing in vitro. A phylogenetic comparison of 32 species showed minor differences in the apoB mRNA sequence, and the apoB mRNA from 31 species was robustly edited in vitro. However, guinea pig mRNA was poorly edited. Compared with the consensus sequences of these 31 species, guinea pig apoB mRNA has three variations in the 3' efficiency element, and the conversion of these to the consensus sequence increased editing to the levels in the other species. From this information, a model for the secondary structure was formulated in which the mooring sequence and the 3' efficiency element form a double-stranded stem. Thirty-one mammalian apoB mRNA sequences are predicted to form this stem positioning C6666 two nucleotides upstream of the stem. However, the guinea pig apoB mRNA has a mutation in the 3' efficiency element (C6743 to U) that predicts an extension of the stem and hence the lower editing efficiency. A test of this model demonstrated that a single substitution at 6743 (U to C) in the guinea pig apoB mRNA, that should reduce the stem, enhanced editing, and mutations in the 3' efficiency element extended the stem for three base pairs dramatically reduced editing. Furthermore, the addition of a 20-nucleotide 3' efficiency element RNA, to a 58-nucleotide guinea pig apoB mRNA lacking the 3' efficiency element more than doubled the in vitro editing activity. Based on these results, a model is proposed in which the mooring sequence and the 3' efficiency element form a double-stranded stem, thus suggesting a mechanism of how the 3' efficiency element enhances editing.

RNA editing is the alteration of the genetic information present in nascent RNA transcripts. One form of RNA editing, apolipoprotein (apo) B mRNA editing, is the deamination of a specific cytidine (C6666) by an editing complex consisting of the editing enzyme APOBEC-1 (apoB mRNA editing enzyme catalytic polypeptide 1) and one or more as yet unidentified auxiliary proteins (1–7). The resulting U changes codon 2153 from a genomically encoded CAA (glutamine) to an in-frame stop codon (UAA) (8, 9). ApoB mRNA editing, which occurs in the intestine of all mammals, results in the formation of a truncated apoB protein (apoB-48) that is 48% of the size of the full-length genomically encoded apoB (apoB-100) (10, 11).

The apoB mRNA editing occurs with exquisite precision. The apoB mRNA editing complex locates the apoB pre-mRNA among tens of thousands pre-mRNAs (12, 13). No other mRNAs have been identified that are edited by this mechanism, and with the exception of a minor site in the human apoB mRNA, no other cytidines in the apoB mRNA are edited (14). Four sequence elements have been identified in the apoB mRNA that specify this precision. These elements span 139 nucleotides (nt) on the apo-mRNA and are necessary for editing at physiological levels (15, 16). Essential for a basal level of editing at C6666 are the 11-nucleotide (nt) "mooring sequence" (nt 6671–6681) (17–19) and a 4-nt spacer sequence between the edited cytidine and the mooring sequence (17, 18, 20). These two elements are located within a 22-nt editing cassette (21). Together with the editing cassette, a 5' efficiency element (nt 6609–6628) and a 3' efficiency element (nt 6717–6747) are necessary to achieve editing at C6666 at physiological levels in vitro (15, 16), since shorter versions of apoB mRNA are edited less efficiently (15, 16, 21–23).

Although the mechanism for this precise editing is unknown, we and others have hypothesized that the RNA secondary structure is critical (24). In this study, we used a phylogenetic comparison approach (25) to infer the secondary structure of a 139-nt apoB mRNA containing both the 5' and 3' efficiency elements. Here we report that the mooring sequence and the 3' efficiency element form a double-stranded stem that facilitates the editing of the apoB mRNA by the editing complex.

EXPERIMENTAL PROCEDURES

Amplification of the ApoB cDNA and DNA Sequence—Total RNA was extracted by Trizol (Life Technologies, Inc., Gaithersburg, MD) from...
mammalian livers that were quick-frozen in liquid nitrogen. The reverse transcriptase (RT) reaction was performed at 42 °C for 1 h with one of the human apoB-specific lower primers (see Table I) and avian myeloblastosis virus reverse transcriptase (Life Sciences, Inc., St. Petersburg, FL). cDNA was extracted from tissue with the DynaZyme Tissue Kit (Qiagen, Valencia, CA). Polymerase chain reaction (PCR) was performed on liver cDNA or genomic DNA with a set of human apoB upper (HB) and lower primer using the Expand High Fidelity PCR System (Roche Molecular Biochemicals, Indianapolis, IN) and the following cycling parameters: 94 °C for 2 min, four cycles of 94 °C for 30 s, 45 °C for 30 s, 72 °C for 1 min followed by 32 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. All PCR products were analyzed on agarose gels, and the PCR product of the expected size was detected. A second nested PCR amplification was performed on 1 μL of the PCR product. The single-banded PCR products (337–397 nt) were either purified by agarose gel electrophoresis and eluted by Qiagen II gel extraction (Qiagen, Valencia, CA) or purified over S-300 HR columns (Amerham Pharmacia Biotech, Piscataway, NJ). Sequence analysis was performed on both strands of the PCR products using the Big Dye d-Rhodamine dye terminator mixture on a 373 DNA Sequencing System (Applied Biosystems/Perkin-Elmer Corp., Norwalk, CT). The sequences were aligned with the DNASTAR software package (DNASTAR Inc., Madison, WI).

**Generation of ApoB mRNA**—ApoB RNAs were produced by in vitro transcription from PCR constructs that included a T7 promoter (16). Primers T7HBU6605 and T7HBU6533 were used to amplify DNA templates for transcription. The small RNAs, designated EFFG and EFFramboad, were transcribed from oligonucleotides T7EFFG (Table I) and T7EFFS after annealing to the upper oligo T7U, necessary for T7 transcription.

**Generation of Mutant ApoB mRNAs**—Two-step PCR was used to incorporate mutations into the guinea pig apoB PCR products (26). The PCR products were purified, and the RNAs were produced as described for the apoB RNAs. The sequence of each mutated guinea pig apoB PCR template was verified by sequence analysis.

**In Vitro ApoB mRNA Editing Assay**—For the recombinant in vitro apoB mRNA editing assay (27), 100 μg of synthetic RNA (prepared as described above), 100 μg of rabbit liver S100 extract, 5 μg of recombinant rabbit APOBEC-1 (MBP-APOBEC-1) (28), 1 μg of Escherichia coli tRNA, and 40 units of RNasin (Promega, Madison, WI) in buffer D containing 1 mM diethiothreitol in a reaction volume of 100 μl were incubated at 30 °C for the indicated times and then extracted as described (27). In control experiments, the RNAs were incubated in the assay mixture lacking rabbit liver S100 extract. For the in vitro apoB mRNA editing assay with guinea pig enterocyte S100 extract (29), 100 μg of synthetic RNA was incubated with 150 μg of guinea pig enterocyte (S100) extract, 1 μg of E. coli tRNA, and 40 units of RNasin (Promega, Madison, WI) in buffer D containing 1 mM diethiothreitol in a reaction volume of 100 μl. All RNAs were amplified by RT-PCR, and the resulting single-band PCR products were purified over a microspin S-300 HR column (Amerham Pharmacia Biotech). For the in vitro apoB mRNA editing assay where two RNAs were incubated, GP6641 and EFFG or EFFramboad, the two RNAs were preincubated at 65 °C for 5 min and cooled to room temperature before they were added to the in vitro apoB mRNA editing assay.

**Primer Extension Analysis**—Primer extension was performed essentially as described (4). Primer Cons51 (Table I) was used for primer extension of the 29 apoB mRNAs, except for the primer extension of the guinea pig RNA where primer GP51 was used. PE6666 (Table I) was used to reanalyze editing in the guinea pig apoB mRNA and to analyze editing in GP6641. In these experiments, the coding strand instead of the noncoding strand was analyzed for editing. For these experiments, 1 μM each of dATP, dGTP, and dTTP and 5 μM ddCTP were used for the poisonous primer extension. The extent of editing was determined with a radioanalytic imaging system (AMBIS, San Diego, CA). The percent editing is given as the mean of the number (n) of experiments.

**RESULTS**

**Phylogenetic Comparative Analysis**—Phylogenic comparisons are the most incisive a priori approach to inferring RNA secondary structure (25). Double-stranded helices in RNA are tested by inspecting the equivalent base paring in homologous RNAs in which the sequence varies. The method is based on the assumption that RNAs with similar function will have similar secondary structure even though there are variations in the sequence. We expected to find co-variation of paired residues in helical regions so that the pairing potential is maintained. We used a phylogenetic comparative approach to attempt to infer the secondary structure of the 139-nt sequence encompassing the apoB mRNA editing site at C<sup>6666</sup>. This sequence includes the 5′ efficiency element, the editing site, the mooring sequence, and the 3′ efficiency element. The apoB mRNA editing region from 30 eutherian mammals was amplified either by PCR from genomic DNA or by RT-PCR from liver RNA, and the sequences were aligned (Fig. 1). The apoB sequences were very similar (at least 81%) and did not have sufficient co-variations necessary to predict conserved base pairing or to formulate a unique consensus secondary structure model by using genebee or m-fold software (30, 31). However, the analysis showed two highly conserved sequences present within the editing region. Nine nucleotides are conserved within the 5′ efficiency element (nt 6616–6624), and 22 nt are conserved within the editing cassette, which includes C<sup>6666</sup>, the spacer region, and the mooring sequence (nt 6662–6863). Only the guinea pig (nt 6616 and 6677) and the cow apoB (nt 6619 and 6681) sequences have mutations within these regions.

**In Vitro Editing of the ApoB mRNAs**—We next determined if all 30 apoB RNA sequences are good substrates for APOBEC-1. To test the 30 amplified apoB sequences for editing, we transcribed the PCR products in vitro and analyzed the apoB mRNAs for editing in a recombinant in vitro editing assay (Fig. 2). The apoB mRNAs were 337–397 nt in length. Interestingly, the apoB RNAs from 29 of the 30 species were good substrates for APOBEC-1 and were edited between 33 and 59% by the rabbit APOBEC-1. Previously, it has been shown that rat and mouse apoB mRNA are good substrates for APOBEC-1, in vitro and in vivo, respectively (2, 28, 32–34). However, the apoB mRNA from one of those species, the guinea pig, was edited poorly (3%). To exclude a possible artifact caused by the guinea pig-specific primer for primer extension (GP51, Table I), we repeated the primer extension experiments on the sense strand with primer PE6666. Again, editing of the guinea pig apoB mRNA was only 3% (data not shown). To rule out species incompatibility (the previous experiment used recombinant rabbit APOBEC-1 plus auxiliary factors from rabbit liver), we repeated the experiment with guinea pig S100 extract (Fig. 3). These experiments showed that the guinea pig apoB mRNA is an intrinsically poorer substrate than the apoB mRNA from other species tested.

**Guinea Pig ApoB mRNA Editing**—This difference in editing efficiency had to be due to sequence differences between the guinea pig mRNA and the consensus sequences of the other 31 apoB RNAs. There were two regions we thought were the most important for editing efficiency. One was the 5′ efficiency element, and the other was the mooring sequence. Since the guinea pig apoB sequence has a mutation both in the 5′ efficiency element and in the mooring sequence, we surmised that changing these nucleotides to the consensus sequences would result in more efficient editing. Analysis showed that the substitution of the U for a consensus C (6616) within the 5′ efficiency element of the guinea pig apoB mRNA had no influence on editing efficiency (data not shown). However, when the guinea pig mooring sequence was changed to the consensus mooring sequence, we obtained unexpected results. Instead of increasing editing activity as expected, restoring the consensus mooring sequence drastically reduced editing (GPM-MOOR, Fig. 4). Because of the importance of the exact mooring sequence, these results were difficult to understand (19). The possible explanation that the guinea pig enterocyte S100 extract is species specific for the guinea pig RNA and favors a U at position 6677 was eliminated from consideration since the rabbit apoB mRNA, which has a G at this position, is edited...
more efficiently than guinea pig apoB mRNA by the guinea pig enterocyte extract (Fig. 3).

Compensatory Mutations in the Guinea Pig ApoB mRNA—To explain this apparent paradox, we hypothesized that, at some point in evolution, a mutation in guinea pig RNA reduced editing activity and a corresponding compensatory mutation restored editing activity, albeit at a lower level. Thus, we set out to find the other mutations that would compensate for the mooring sequence mutation. Again, assuming that the secondary structure of the apoB RNA is important and that it is maintained in the guinea pig apoB RNA, we reasoned that these experiments should help us with the secondary structure analysis.

To investigate whether compensatory mutations within the guinea pig apoB mRNA could possibly restore editing efficiency, we searched for unique mutations in the guinea pig apoB mRNA. We found seven sites where the guinea pig sequence differs from the consensus sequence of the other 31 species (Fig. 5). We then prepared RNA constructs with six sets of two co-variations. In each of these, the U was changed to a consensus sequence G in the mooring sequence (GPM-MOOR), and one other base was altered. The purpose was to determine if any of these changes would rescue the decreased editing caused by the mooring sequence mutation. Three of the co-mutations increased editing efficiency (Fig. 5), GPM-MOOR-6665, GPM-MOOR-6743, and GPM-MOOR-6745. One was located directly upstream of C6666 (GPM-MOOR-6665), and the other two were located within the 3' efficiency element (GPM-MOOR-6743, GPM-MOOR-6745). The three co-mutations that did not alter editing were located in the 5' efficiency element or in regions that were previously shown not to be necessary for efficient editing (16). Since the co-mutations that rescued ed-

### TABLE I

| Oligonucleotide sequence |
|--------------------------|
| HBU6507                  |
| HBU6526                  |
| HBU6533                  |
| T7HBU6507                |
| T7HBU6526                |
| HBL6845                  |
| HBL6865                  |
| HBL6876                  |
| Cons51                   |
| GP51                     |
| PE6666                   |
| T7U                      |
| T7EFFG                   |
| T7EFFS                   |

The oligonucleotide orientation is 5' to 3'.

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**FIG. 1. Sequence alignment of the apoB-editing site from 32 mammals.** The apoB-editing region was amplified from genomic DNA or liver cDNA using a set of human apoB upper (HBU) and human apoB lower (HBL) primers (Table I) with the exception of the rat and the mouse sequences, which were retrieved from GenBank. Accession numbers: rat (X59598) and mouse (AA980571). Conserved nucleotides are shown in normal font and variations to the consensus are shown in **bold**. The locations of part of the 5’ efficiency element, C^6^6^6^6, the mooring sequence, and the 3’ efficiency element are marked. The residue number according to the human apoB cDNA is shown at the bottom.

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The oligonucleotide orientation is 5' to 3'.
iting efficiency in the GPM-MOOR were located directly upstream of C\(^{6666}\) or within two nucleotides in the 3' efficiency element, we analyzed secondary structures of the guinea pig apoB mRNA that would bring these residues into close association, paying special attention to possible interactions between the two RNA sequence elements.

Secondary Structure Prediction—The m-fold minimal energy software (30, 31) predicted a hairpin structure for the 139-nt guinea pig apoB mRNA in which the mooring sequence and the 3' efficiency element form a double-stranded helix (Fig. 6). The predicted secondary structures for the human apoB mRNA and the 30 other apoB mRNAs are similar. However, in the human secondary structure, C\(^{6666}\) is positioned two nucleotides upstream of the double-stranded stem, whereas in the guinea pig secondary structure, C\(^{6666}\) is located one base upstream of the stem. This difference in the secondary structures is due to the unique mutations in the 3' efficiency element of the guinea pig apoB mRNA compared with the other 31-apoB mRNAs (Fig. 1). The co-mutation that increased editing efficiency of GPM-MOOR over the editing efficiency of the guinea pig apoB mRNA (GPM-MOOR-6743) is predicted to open the double-stranded region, mimicking the human secondary structure (C-6743) (Fig. 6).

Test of the Predicted Guinea Pig Secondary Structure—We next sought to test the secondary structure model of the guinea pig apoB mRNA by assaying the editing of RNAs that had all three mutations that enhanced the editing of the RNA (GPM-MOOR) (Fig. 5). One RNA had mutations at the two unique nucleotides in the 3' efficiency element plus one additional unique nucleotide difference directly upstream (nt 6743, 6745, and 6747) and the one directly upstream of C\(^{6666}\) (nt 6665) (GPM-A/CUG) (Fig. 7). Another RNA had the three changes in the 3’ efficiency sequence but omitted the G to A change at nucleotide 6665 (GPM-CUG) (Fig. 7). The editing efficiency of
radioanalytic imaging system.

pig apoB mRNA editing. The extent of editing was determined with a (UGAUCAGUAUA).

mRNA with the consensus mooring sequence (GPM-MOOR) in an analysis (MOOR.

editing activity. Editing of C6666 was determined by primer extension assay using guinea pig enterocyte S100 extracts as source for editing activity, and the editing of C6666 was determined by primer extension analysis (n = 3). A, quantitation of rabbit and guinea pig apoB mRNA editing. The extent of editing was determined with a radioanalytic imaging system. B, primer extension analysis of C6666 as shown. The upper band represents the deamination of C6666 to a uridine (U at 6666). The middle band represents the unmodified cytidine (C at 6666).

Both sets of experiments (Figs. 8 and 9) indicate a direct interaction between A6667 and the U6743, which is located 76 nt further downstream in the guinea pig apoB mRNA. These data support the predicted secondary structure of the guinea pig apoB mRNA.

Testing for the Presence of the Bulge in the Secondary Structure Model—Our data show that the guinea pig apoB mRNA has decreased editing because C6666 is located directly upstream of the extended stem structure and that opening of the extended stem by one nucleotide significantly increased editing. Therefore, if the model is correct the further extension of the stem until C6666 is part of the putative double helix should dramatically decrease editing of the RNA. To accomplish this, we changed two nucleotides in the 3’ efficiency element to base pairs with U6661 and C6666 (GPM-NEG) (Fig. 10). Elongation of the stem drastically decreased editing in GPM-NEG, emphasizing the positioning of C6666 in a single-stranded bulge. The two substitutions introduced into GPM-NEG are located between the three substitutions in GPM-CUG (Fig. 7) that increased editing of the guinea pig apoB mRNA to levels comparable to that of rabbit apoB mRNA.

Editing of a Small apoB mRNA Is Increased by the Coincubation of an RNA Containing the 3’ Efficiency Element—To test further the validity of our RNA secondary model, we used a small guinea pig apoB mRNA, GP6641 (58 nt, 6641–6698), that lacks the 3’ efficiency element. This RNA was incubated in an in vitro editing assay with or without a small RNA encoding the 3’ efficiency element. Co-incubation of GP6641 and the 20-nt 3’ efficiency element (EFFG, 6728–6747) increased editing of GP6641 in a time-dependent manner (Fig. 11). This increased editing efficiency was sequence specific for the 3’ efficiency element since a scrambled 3’ efficiency element did not increase editing of GP6641. Again, this specific enhancement by the consensus 3’ efficiency element supports the helix formation between the mooring sequence and the 3’ efficiency element.

**DISCUSSION**

The apoB mRNA editing complex exactly edits a single cytidine (C6666) of the 43-kilobase apoB pre-mRNA. Several sequence elements have been identified on the apoB mRNA that specify this precision. The mooring sequence and two efficiency elements have been shown to cooperatively increase editing in the apoB mRNA. This in vitro study shows that the mooring sequence and the 3’ efficiency element form a double-stranded stem that facilitates the recognition of the apoB mRNA by the editing complex (Fig. 6).

We have constructed a model of the secondary structure of apoB mRNA in which C6666 is located in a single-stranded region upstream of the double-stranded helix. Thirty-one mammalian apoB mRNA sequences (Fig. 1) are predicted to position both of these altered guinea pig apoB RNAs was markedly increased (GPM-A/CUG, GPM-CUG) (Fig. 7), approaching that of rabbit apoB mRNA (Fig. 9). The predicted secondary structures of GPM-CUG and GPM-ACUG RNAs have reduced stems similar to the secondary structure model of human apoB mRNA. Thus, these results support our model of different editing efficiencies based on secondary structure.

Testing the Opening and Closing Base Pair of the Stem—To test more directly the negative influence of the longer stem in the guinea pig apoB mRNA secondary structure model on editing, we assayed a mutation that would reduce the stem structure (Fig. 8). The U to C mutation at nucleotide 6743 in the 3’ efficiency element increased editing of the guinea pig apoB mRNA (GPM-C) to levels comparable to those of rabbit apoB mRNA. Moreover, introducing a co-mutation downstream of C6666 to restore the longer stem of the guinea pig apoB mRNA drastically decreased editing efficiency (GPM-G/C). This opening and re-closing of the stem by single nucleotide substitutions was repeated with a U to A mutation in the 3’ efficiency element (Fig. 9). Again, editing efficiency increased (GPM-A) with the U to A mutation at 6743 and decreased upon a complementary A to U co-mutation of the nucleotide downstream of C6666 (GPM-U/A). The A to U substitution downstream of C6666 (GPM-A) alone did not increase editing efficiency. Previously, substitution of U for A at nt 6667 decreased editing, and became the nearest neighbor preference for editing (20). Therefore, the unchanged editing efficiency of GPM-A may reflect the sum of increased editing by the opening of the stem and decreased editing by an unfavorable base substitution of U6667 next to C6666. Both sets of experiments (Figs. 8 and 9) indicate a direct interaction between A6667 and the U6743, which is located 76 nt further downstream in the guinea pig apoB mRNA. These data support the predicted secondary structure of the guinea pig apoB mRNA.

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two nucleotides upstream of the stem (Fig. 6). These apoB mRNAs are conserved immediately 5' of the editing site and within the 3' half of the 3' efficiency element (Fig. 1). All 31 apoB mRNAs that are predicted to position C6666 two nucleotides upstream of the stem were efficiently edited in vitro (Fig. 2), whereas the guinea pig apoB mRNA was edited with low efficiency (Fig. 2). Guinea pig apoB mRNA has a mutation within the 3' efficiency element that results in a 1-base pair extension of the double-stranded stem, positioning C6666 only one nucleotide upstream of the stem (Fig. 6). The extension of the double-stranded stem in the guinea pig apoB mRNA apparently decreases editing efficiency. Restoring the 2-nucleotide distance in the guinea pig apoB mRNA between C6666 and the stem resulted in editing efficiency comparable to that of the other apoB mRNAs (Figs. 3 and 7–9). Single mutations in the 3' efficiency element (U6743) that abolished base pairing with A6667 (GPM-C and GPM-A) were sufficient to enhance editing efficiency (Figs. 8 and 9). Furthermore, this increased editing efficiency was reversible by complementary substitutions at A6667 (GPM-G/C, GPM-U/A). These co-mutations restored base pairing between A6667 and U6743 and drastically decreased editing efficiency (Figs. 8 and 9), emphasizing the negative influence of the extended stem on the editing efficiency of the guinea pig apoB mRNA.

The current model for the apoB mRNA editing complex proposes that APOBEC-1 is a “head to toe” dimer (35, 36). Furthermore, one active site of the APOBEC-1 dimer binds to C6666 and the second site binds to a downstream U in the 3' end of the mooring sequence (19, 36–40). In our model of the secondary structure of apoB mRNA, C6666 is located in a single-stranded
region of the apoB mRNA, suggesting that this is the preferential structure for the efficient access and deamination of C6666 by the apoB mRNA-editing complex (Fig. 10). We can speculate that the interaction of the second active site of the APOBEC-1 with the U in the 3′ end of the mooring sequence may also prefer a single-stranded or bulged U. In our model, the secondary structures of human and guinea pig apoB mRNA have a bulged U in the stem that could be used to position the APOBEC-1 dimer (Fig. 6). When the mooring sequence of the guinea pig apoB mRNA was mutated so that the bulged U was base paired, the editing efficiency decreased (Fig. 4).

Recently, another model of the secondary structure of apoB mRNA was reported. Based on a much smaller sequence of apoB mRNA lacking the efficiency elements (24), this secondary structure model predicts a double-stranded stem between the mooring sequence and sequences directly upstream of C6666. Interestingly, this model locates C6666 two nucleotides upstream of a double-helix stem in a hairpin loop, similar to the positioning of C6666 in our model. Because shorter apoB RNAs are edited less efficiently than apoB RNAs containing the 3′ efficiency element (15, 16, 21–23), this hairpin loop in the short apoB mRNAs may not be as efficient for editing as the double-stranded stem between the mooring sequence and the 3′ efficiency element. Therefore, the hairpin loop structure (24)
may reflect a structure unique to small apoB mRNAs.

The guinea pig editing system poses a paradox. Greeve et al. (41) reported that the apoB mRNA in guinea pig intestine is edited about 95%; however, in vitro guinea pig apoB mRNA is edited with lower efficiency than apoB mRNAs from the 29 other mammals (Fig. 2). Our limited studies indicated that the guinea pig enterocyte S100 extract has a higher editing activity than the rabbit S100 extract (data not shown). Furthermore, the guinea pig extract also edited rabbit apoB mRNA more efficiently than rabbit S100 extract, suggesting that there is not a species-specific auxiliary protein and that the guinea pig efficiently than rabbit extract, suggesting that there is not a species-specific auxiliary protein and that the guinea pig enterocyte S100 extract has a higher editing activity, which compensates for the lower editing efficiency of the guinea pig apoB mRNA.

In summary, our results indicate that the mooring sequence and the 3’-efficiency element form a double-stranded helical stem that facilitates the editing of the apoB mRNA. Thirty-one mammalian apoB mRNA sequences are predicted to form this stem positioning C6666 two nucleotides upstream of the double-stranded stem. However, the guinea pig apoB mRNA has lower editing efficiency than apoB mRNAs from the 29 other mammals, suggesting that there is a structure unique to small apoB mRNAs.

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Phylogenetic Analysis of the Apolipoprotein B mRNA-editing Region: EVIDENCE FOR A SECONDARY STRUCTURE BETWEEN THE MOORING SEQUENCE AND THE 3′ EFFICIENCY ELEMENT

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