Apolipoprotein B mRNA sequences 3' of the editing site are necessary and sufficient for editing and editosome assembly

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ABSTRACT

Apolipoprotein B (apoB) mRNA is edited in rat liver and intestine through the direct conversion of cytidine to uridine at nucleotide 6666. Recently, we have proposed the 'Mooring Sequence' model, in which editing complexes (editosomes) assemble on specific apoB mRNA flanking sequences to direct this site-specific editing event. To test this model, apoB mRNA deletion and translocation mutants were constructed and analyzed. Specific sequences 3' of the editing site were absolutely required for editing, while specific sequences and bulk RNA 5' of the editing site were required for efficient editing. Translocation of apoB 3' flanking sequences induced editing of an upstream cytidine, demonstrating that 3' sequences are necessary and sufficient to direct editing *in vitro*. 3' flanking sequences were also shown to be necessary and sufficient for editosome complex assembly. These data provide strong support for a 'Mooring Sequence' model in which 3' apoB flanking sequences direct editosome assembly and subsequent editing *in vitro*, while 5' flanking sequences enhance these functions.

INTRODUCTION

In rats, apolipoprotein B (apoB) exists as high molecular weight (apoB_H) and low molecular weight (apoB_L) isoforms. ApoB_L is synthesized from the same primary transcript as apoB_H through the direct editing of cytidine to uridine at nucleotide 6666, leading to the production of a UAA stop codon at codon 2153 (1,2). This reaction appears to be carried out by a site-specific cytidine deaminase (3), although direct removal and replacement of the cytosine base has not been ruled out. In addition to apoB mRNA editing, diverse forms of RNA editing have also been described in the mitochondria of several organisms, including kinetoplastid protozoa (4–6), slime mold (7), and several plants (8).

Deletion mutagenesis has shown that 55 nucleotides of specific apoB sequence encompassing the editing site are sufficient to support site-specific editing in an *in vitro* assay, utilizing a crude rat liver S100 extract (9). A sequence of 26 nucleotides was not

sufficient to support detectable editing *in vitro*, but could support *in vivo* editing in a rat hepatoma cell line when placed in the context of 900 nucleotides of distal apoB mRNA sequences (10). These results suggest that the requirements for editing *in vitro* are more stringent than *in vivo* and/or that large amounts of distal apoB mRNA sequences can induce editing. Point mutation analysis of a nine nucleotide region encompassing the editing site (6663–6671) suggested a relaxed sequence requirement within this region (11). Mutant RNAs containing additional cytidines adjacent to the editing site (nucleotides 6665–6668) were also edited at these residues. These data suggest that the cytidine deaminase alone may lack the selectivity required for site specific editing and also that nucleotides in the immediate vicinity of the editing site lack the regulatory capacity to account for the specificity of apoB mRNA editing. Recently, point mutagenesis identified an 11 base region (nucleotides 6671–6681) downstream of the editing site which was necessary for apoB mRNA editing (12). The authors concluded that this 11 base region was required for site-specific editing and proposed that this region is a binding site for the apoB editing activity. Close analysis of the data suggests that other flanking sequences, both 5' and 3', may be necessary for site-specific editing.

We have recently identified a 27S protein-containing macromolecular complex (the editosome) which specifically assembles on editing-competent apoB mRNA substrates (13). Assembly of this complex kinetically precedes the accumulation of edited RNA and inhibition of complex formation by vanadyl-ribonucleoside complexes (VRC) is accompanied by inhibition of editing. Utilizing a different RNA gel mobility shift assay, Lau et al. (14) have also described the sequence-specific binding of proteins to apoB mRNA substrates and have identified a 40 kDa protein which specifically UV crosslinks to apoB mRNA near the editing site (nucleotides 6661–6686). These results, along with the mutational analysis detailed above, support the recently proposed 'Mooring Sequence' model for apoB mRNA editing (13) in which sequences flanking the editing site direct site-specific editing through interactions with specific proteins. Cytidine deaminase activity within the editosome complex is positioned to scan for cytidines in the general vicinity of nucleotide 6666. The term

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'mooring' has been proposed for the mechanism which positions cytidine deaminase over the site to be edited without actually docking it to the exact nucleotide position.

Hepatic apoB mRNA editing is metabolically, hormonally, and developmentally regulated in rats (15–17), suggesting that editosomal components and/or their interactions may be regulated. Identification of the RNA sequences which direct the editing process and the proteins which bind these sequences is an important step towards understanding apoB mRNA editing and its regulation. To evaluate the flanking sequence requirements for site-specific apoB mRNA editing and editosome assembly *in vitro*, we have constructed and analyzed deletion and translocation mutants. The data presented here support the Mooring Sequence model and provide a direct demonstration that sequences 3' of the editing site are necessary and sufficient to support site-specific editing and editosome assembly, while sequences 5' of the editing site serve to enhance these functions.

MATERIAL AND METHODS

ApoB Plasmid Constructs

Parental ApoB constructs pSX7, pRSA13, pBS55 (17E37), and pBS26 (4E21) contain 2429, 448, 55, and 26 nucleotides, respectively, of specific apoB sequence flanking the editing site and contain cytidine at nucleotide 6666 (9). Deletion mutants of pBS55 were constructed using the Erase-a-Base® deletion mutagenesis kit (Promega) and were screened by double restriction enzyme (Kpn I-Sac I) digestion analysis; clones of interest were subsequently sequenced utilizing the Sequenase® 2.0 kit (United States Biochemical) (Table 1). Deletion of 5' sequences was accompanied by deletion of varying amounts of 5' polylinker, while construction of 3' deletion mutants 17E30 and 17E25 destroyed the wild-type Hind III linearization site and necessitated cleavage at a downstream Bam HI site, resulting in the addition of 11 nucleotides of GC-rich 3' polylinker (5'CCCGGGGGATC3'). 4E21(+)'5'Poly was constructed by sub-cloning 4E21 into pGEM-7Zf(+) by double-digestion of both plasmids with Kpn I and Sac I and subsequent recombination. 3' end 'swap' mutants 17E21-SW and 4E37-SW were constructed by double-digesting 17E37 and 4E21(+)'5'Poly with Bcl I (site at nucleotide 6672 in apoB sequence) and Sac I and subsequent recombination (Table 1). Translocation mutants of pRSA13 and pSX7 (pRSA13Δ3'TL and pSX7Δ3'TL, respectively) were constructed by digestion of both plasmids with Bcl I (sites at nucleotides 6441 and 6672 in apoB sequence) and subsequent vector religation (Figure 2). pRSA13Δφ was constructed by linearization of pRSA13 with EcoR I (site at nucleotide 6511 in apoB sequence). 3' deletion mutants 4E9 and 17E9 were constructed by double-digestion of parental constructs 4E21 and 17E37 with Bcl I and Bam HI (site in 3' polylinker) and subsequent religation of the compatible 5' overhangs in the vector (Table 1). All Bcl I-cut plasmids were grown in DM(–) cells (BRL) prior to Bcl I digestion.

Extract Preparation

Rat liver S100 extracts were prepared as previously described (13). Sequential protein precipitation was performed with 15% and 25% ammonium sulfate by adding a saturated ammonium sulfate solution to the S100 extract. The precipitated protein was dissolved in and dialyzed against 1×Editing Buffer minus glycerol (10 mM Hepes pH 7.9, 50 mM KCl, 50 mM EDTA, and 0.25 mM DTT).

Table 1. Summary of apoB deletion mutant editing efficiencies.

Construct	ApoB Insert (nt)	Nucleotides	5' Polylinker	3' Polylinker	% Editing
17 E 37	55	6649-6703	40	4	100
5' Mutants					
17 E 37 (-)'5' Poly	55	6649-6703	15	4	30
15 E 37	53	6651-6703	5	4	15
13 E 37	51	6653-6703	13	4	7.5
4 E 37	42	6662-6703	15	4	7.5
4 E 37-SW	42	6662-6703	72	4	5
3' Mutants					
17 E 30	48	6649-6696	40	11	10
17 E 25	43	6649-6691	40	11	2.5
17 E 21-SW	39	6649-6687	40	2	5
4 E 21	26	6662-6687	38	2	1.25
17 E 9	27	6649-6675	40	32	N.D.
4 E 9	14	6662-6675	38	32	N.D.

Editing efficiencies were determined by direct scintillation counting of excised first stop (unedited) and second stop (edited) gel bands and were normalized relative to the 6% editing supported by parental construct 17E37. N.D., not detectable.

Rat enterocytes were prepared from Sprague-Dawley male rats (300–400 g) as described by Weiser (18) with the following modifications: (1) the initial intestinal wash contained 0.5 μg/ml each leupeptin and aprotinin, 1 mM PMSF, 0.05 mM VRC, and 20 units/ml soybean trypsin inhibitor and (2) enterocyte release was accomplished with a single 30 min incubation at 37°C. An S100 extract was prepared by the method of Dignam *et al.* (19) and was subsequently dialyzed against buffer D {20 mM Hepes pH 7.9, 20% glycerol (v/v), 100 mM KCl, 0.2 mM EDTA, and 0.5 mM DTT}.

In Vitro Editing Reactions

Linearized plasmid DNA (1 μg) was transcribed with T7 or T3 RNA polymerase and capped. Radio-labeled RNAs for editosome analysis were transcribed with [α -³²P]-ATP and PAGE-purified. In the standard editing reaction, 10–20 fmoles of synthetic apoB RNA was incubated at 30°C for 3-hr with extract in a 50 μl reaction containing 10 mM Hepes pH 7.9, 10% glycerol (v/v), 50 mM KCl, 50 mM EDTA, 0.25 mM DTT, and 40 units of RNasin® (Promega). Editing reactions contained 60 μg of ammonium sulfate-precipitated rat liver extract or 60 μg enterocyte S100 extract and were stopped as previously described (9), phenol-chloroform extracted, and ethanol precipitated.

Primer Extension Analysis

Approximately 30 fmoles of the appropriate ³²P-labeled oligonucleotide was mixed with the precipitated RNA, heated to 70°C for 5 min and slow cooled (1.5 hr) to 25°C in 0.5 M KCl. The annealed products were primer extended for 1 hr at 37°C in a reaction containing 50 mM Tris pH 8.3, 100 mM KCl, 10 mM MgCl₂, 10 mM DTT, 0.5 mM spermidine, 50 μM each dATP, dCTP, and dTTP, 250 μM dideoxy-GTP (ddGTP), 10 units of RNasin, and 4 units AMV Reverse Transcriptase (Promega). An excess of ddGTP is added to ensure proper termination of primer extension at nucleotide 6666 (unedited cytidine, first stop) or the next cytidine 5' of nucleotide 6666 (edited cytidine, second stop). The reactions were precipitated and the primer extension products were resolved on a 10% acrylamide / 7M urea gel and autoradiographed. Editing levels were quantitated by scintillation counting of excised first and second stop gel bands.

Editosome Analysis

Editing reactions were carried out with [α -³²P] ATP-labeled RNA substrates; one-fifth of each reaction was resolved on 4%

Figure 1. Primer extension analysis of apoB deletion mutant editing. Deletion mutant apoB RNAs were incubated for 3 h at 30°C with 60 μ g rat enterocyte S100 extract as described in Material and Methods. The products of the editing reactions were analyzed by primer extension in the presence of dideoxy-GTP. The positions of the primer and the extension products generated from unedited (CAA) and edited (TAA) RNAs are indicated. The length of the edited (TAA) primer extension product is dependent on the position of the next cytidine in the RNA and varies between constructs.

Figure 2. Construction of translocation mutant pRSA13 Δ 3'TL. Translocation mutant pRSA13 Δ 3'TL was constructed by digestion of parental vector pRSA13 with Bcl I and subsequent vector religation as described in Material and Methods. pSX7 Δ 3'TL was constructed from parental vector pSX7 in the same fashion.

acrylamide / 0.5% agarose 'Native' gels and autoradiographed as previously described (13). For sedimentation analysis, 10 editing reactions were diluted one-third with 1 $\times$ Editing Buffer minus glycerol, applied to a 36 ml 10–50% glycerol gradient in editing buffer and sedimented at 100,000 $\times$ g for 5 h at 7°C (13). Fractions (1.5 ml) were collected from the top of the gradient and stored at –20°C until used. In specific cases, fractions were brought to 0.1 mg/ml heparin prior to native electrophoresis.

Oligonucleotides

The following primer extension oligonucleotides were purchased PAGE-purified from Genosys:

DD3: AATCATGTAAATCATAACTATCTTTAATATACTGA, 35-mer with 3' end at 6675
 DD5: CGATATCAAGCTTTAATATACTGA, 24-mer with 3' end at 6675 and 5' end in pBS KS(–) polylinker
 JB-1: CTCTGTATTTTCTTACAAATTGATC, 25-mer with 3' end at 6642
 JB-2: GGTGGCGGCCGCTCTAGAACTAGTGG, 26-mer with 3' end at nucleotide 689 in pBS KS (–) vector

All oligonucleotides are complementary to human apoB sequence, except DD5, the 5' end of which is in pBS KS(–) polylinker (underlined sequence which starts complementarity at nucleotide 710 of vector) and JB-2, which is complementary to polylinker sequence. Oligonucleotides were labeled with (γ -³²P)ATP and T4 polynucleotide kinase (United States Biochemical) by the vendor's protocol.

Figure 3. Primer extension analysis of apoB translocation mutant editing. Mutant apoB RNAs were incubated for 3 h at 30°C with either 60 μ g rat enterocyte S100 extract (A) or 60 μ g of partially purified rat liver extract (B) as described in Material and Methods. The products of the editing reactions were analyzed by primer extension. The positions of the primer and the extension products generated from unedited (CAA) and edited (TAA) RNAs are indicated. The length of the edited (TAA) primer extension product is dependent on the position of the next cytidine in the RNA and varies between constructs.

RESULTS AND DISCUSSION

We have previously reported that 60 μ g of a rat liver S100 extract edits approximately 2% of input control RNA (pRSA13) in a 3 hr editing reaction (13). The editing efficiency of this extract has been improved by sequential 15% and 25% ammonium sulfate precipitation. 60 μ g of the 25% ammonium sulfate fraction edits approximately 6–8% of input control pRSA13 RNA in a 3 hr reaction, constituting a 3- to 4-fold increase in specific activity over liver S100. In contrast, 60 μ g of rat enterocyte S100 extract edits 20–30% of input control pRSA13 in a 3 hr reaction and was used without further fractionation. Both extracts are stable at –20°C for at least 3 months.

Deletion mutagenesis was carried out on the 5' and 3' end of a 55 nucleotide apoB insert spanning the editing site (17E37) to determine the *in vitro* sequence requirements for editing with rat liver and enterocyte extracts. 3' end 'swap' mutants 17E21-SW and 4E37-SW were constructed through recombination of apoB constructs 17E37 and 4E21(+)'5'Poly (see Material and Methods). RNAs transcribed from these plasmids were incubated with rat liver and enterocyte extract and *in vitro* conversion of cytidine 6666 to uridine was analyzed by primer extension (Figure 1). Editing with rat liver and enterocyte extracts showed identical sequence dependence with all deletion and 'swap' mutants tested (liver data not shown).

5' apoB mRNA sequences are required for efficient editing *in vitro*

Removal of 25 nucleotides of 5' polylinker sequence {17E37-(–)'5'Poly, lane 2} lowered editing efficiency 3-fold relative to

Figure 4. Editosome assembly occurs prior to the onset of editing in enterocyte extracts. A, editing activity of enterocyte extract on pRSA13 RNA was assayed by primer extension as described in Material and Methods for the times indicated at the top of the panel. The positions of the primer and the extension products generated from unedited (CAA) and edited (TAA) RNAs are indicated. B, editosome assembly on radio-labeled pRSA13 RNA was determined by native gel electrophoresis (as described in Material and Methods) at the times indicated at the top of the figure. The migration of unassembled RNA (A) and editosomes (B) are indicated to the left.

parental construct 17E37 (lane 1), suggesting that flanking sequences of non-apoB origin can influence editing efficiency. In contrast, addition of varying amounts of 3' polylinker had no detectable effect on the efficiency of *in vitro* editing (data not shown). Further removal of 2 and 4 nucleotides of specific 5' apoB sequence (15E37 and 13E37, lanes 3 and 4, respectively) lowered editing efficiency 2-fold and 4-fold further, respectively. Removal of an additional 9 bases of specific 5' apoB sequence (4E37, lane 5) had no additional effect on editing efficiency. Addition of 5' polylinker to this construct (4E37-SW, lane 6) could not rescue editing function, suggesting that RNA length alone can not compensate for the loss of specific 5' apoB sequences. These data suggest that specific apoB sequences 14–17 nucleotides upstream of the editing site in addition to non-specific bulk RNA sequences 5' of the editing site are required for efficient editing *in vitro*.

3' apoB mRNA sequences are absolutely required for editing *in vitro*

Removal of 7 and 12 nucleotides of 3' specific apoB sequence (17E30 and 17E25, lanes 8 and 9, respectively) reduced editing efficiency 10-fold and 40-fold relative to parental construct 17E37; however, deletion of 16 nucleotides of 3' sequence (17E21-SW, lane 10) reduced editing efficiency only 13-fold. The enhanced editing efficiency of 17E21-SW relative to 17E30 and 17E25 may be due to the addition of 11 nucleotides of GC-rich polylinker to the 3' end of these clones. Parental clone 4E21 (lane 11) edited at an efficiency approximately 80-fold less than 17E37. In contrast, 3' deletion mutants 17E9 and 4E9 (lanes 13 and 15, respectively) did not edit at detectable levels in liver or enterocyte extracts. These data corroborate the data of Shah et al. (12) and suggest an absolute requirement of specific apoB mRNA sequences 10–21 nucleotides 3' of the editing site for editing and a requirement for additional 3' apoB flanking

Figure 5. Glycerol gradient sedimentation of editosomes formed by enterocyte extracts. Enterocyte editing reactions containing radio-labeled pRSA13 RNA were sedimented through 10–50% glycerol gradients as described in Materials and Methods; 10 μ l of each fraction was resolved by native gel electrophoresis and autoradiographed. The position of unassembled RNA (A) and the editosome (B) are indicated to the left. The upper two thirds of the gradient from top to bottom are shown as lanes 1–10. The trailing edge of fraction 2 and the midpoint of fraction 2 correspond to 11S and 27S, respectively.

Figure 6. Electrophoretic comparison of the complexes formed by enterocyte and liver extracts. Enterocyte and liver editing reactions were sedimented through glycerol gradients as described in Figure 5 and 10 μ l from fractions 2 and 3 (11S and 27S respectively) were resolved by native gel electrophoresis and autoradiographed. The position of unassembled RNA (A) and the editosome (B) are shown to the right. Lanes 1 and 4 correspond to enterocyte 11S and 27S fractions treated with heparin prior to electrophoresis; lanes 2 and 5 correspond to enterocyte 11S and 27S fractions untreated; lanes 3 and 6 correspond to liver 11S and 27S fractions untreated.

sequences for efficient editing. Given that 17E9 contains all 5' sequence present in the parental clone (17E37) yet does not edit at a detectable level, we conclude that specific 5' apoB mRNA flanking sequences enhance editing efficiency *in vitro* but are unable to direct site-specific editing without a minimum amount of 3' flanking sequences.

Translocation of 3' apoB mRNA flanking sequences induces editing at an upstream cytidine

Given that deletion and 3' end 'swap' constructs with only 4 nucleotides of specific 5' apoB sequence can support *in vitro* editing at a detectable level, specific 3' apoB sequences alone may be sufficient to direct editing of an upstream cytidine and would therefore be translocatable. To test this hypothesis, deletion mutants of pRSA13 and pSX7 lacking specific 5' flanking sequences (pRSA13 Δ 3'TL and pSX7 Δ 3'TL, respectively) were constructed (see Figure 2) in which 3' flanking sequences were translocated just downstream of another CAA (C at nucleotide 6435). In these translocation mutants, sequences 3' of cytidine 6435 differed from wild-type sequences only by insertion of an A residue four nucleotides downstream of the cytidine. pRSA13 Δ 3'TL and pSX7 Δ 3'TL contain 217 and 2159

Figure 7. Editosomes assemble on apoB translocation mutant RNA substrates. Editing reaction containing radio-labeled RNA substrates were resolved by native gel electrophoresis and autoradiographed with Kodak XRP-5 (lanes 1 and 2) or XAR-5 (lanes 3–6) film. Unassembled RNA (A) and editosomes (B) are indicated to the left. The RNA substrates used for assembly are shown at the bottom of the figure and are described in Materials and Methods. Complexes formed by liver and enterocyte extracts are shown in panels A and B, respectively. Lanes 1, 3, and 5 show the absence of complex assembly at time zero and lanes 2, 4, and 6 show the maximum level of B complex formation following 2 h (liver) or 30 min (enterocyte) reactions.

nucleotides of apoB sequence, respectively. Translocation of 3' apoB flanking sequences induced editing of cytidine 6435 (Figure 3, lanes 3 and 5 of panels A and B) which was not edited in the parental constructs (lanes 7 and 9). The level of editing supported by pRSA13 Δ 3'TL (lane 3) was approximately 4% of that supported by its parental clone pRSA13 (lane 1) for both enterocyte and liver extracts (panels A and B, respectively). In contrast, pSX7 Δ 3'TL (lane 5) supported editing at 4% and 18% of its parental construct pSX7 (lane 2) in enterocyte and liver extracts (Panels A and B, respectively). Liver extract also showed enhanced editing efficiency with pSX7 (120% the level seen with pRSA13), while enterocyte extract edited the two constructs at approximately equivalent efficiencies.

The apparent differences in bulk apoB sequence requirements seen with liver and enterocyte extracts on long RNA substrates supports previous observations in other systems. Innerarity *et al.* (personnel communication) have observed that an apoB-apoE chimeric construct with a 63 nucleotide apoB insert (nucleotides 6636–6698) can be edited *in vivo* by rat liver McArdle 7777 cells, but not by human intestinal CaCo-2 cells, suggesting that the *in vivo* sequence requirements for editing in these two cell lines is substantially different. The enhancing effect in rat liver extract of additional apoB flanking sequences in translocation mutant pSX7 Δ 3'TL may explain the high level of editing seen *in vivo* with apoB construct 4E21 when placed in the context of

900 nucleotides of distal apoB flanking sequences (10). The difference in bulk sequence requirements between liver and enterocyte extracts *in vitro* is potentially important, given that editing in rat liver is highly regulated, while editing in rat enterocytes appears to be constitutive (15–17). Metabolic regulation of apoB mRNA editing may occur through the regulation of protein factors which specifically bind to bulk 5' and/or 3' apoB mRNA flanking sequences. Alternatively, protein factors which recognize and bind the 'mooring' and 'enhancer-like' sequences identified above may be targets for metabolic regulation.

27S editosome assembly in enterocyte extracts

Enterocyte editosomes have not been described previously and have even been proposed not to exist (20). In our *in vitro* assay system, enterocyte extracts clearly assembled editosomes (Figure 4, panel B) with more rapid kinetics than those seen previously with liver extract (13). These data show that although enterocyte extracts have more editing activity than liver extracts, editosome assembly in enterocyte extracts precedes the onset of detectable apoB RNA editing. Enterocyte editosomes were not as stable as liver editosomes due in part to the presence of an RNasin®-resistant RNase activity. Enterocyte (Figure 5) and liver (ref. 13, and Figure 6) editosomes sedimented at approximately 27S. Enterocyte editosome complexes resolved best when 0.1 mg/ml heparin was included in the sample loading buffer (Figure 6, lane 4) which reduced high background levels of protein binding to RNA otherwise visible even at time zero (data not shown). Liver editosomes did not have a heparin requirement. Enterocyte and liver native gel complexes from the 11S and 27S regions of glycerol gradients demonstrated similar electrophoretic properties.

Editosome assembly requires 3' apoB mRNA flanking sequences

An important premise of the mooring sequence model is that site-specific editing arises from selective interaction(s) between flanking sequences and components of the editing apparatus (the editosome) (13). Editing and editosome assembly should therefore have similar, if not identical, flanking sequence requirements. This possibility was evaluated in both enterocyte and liver extracts utilizing 3' flanking sequence translocation and deletion mutants (Figure 7). Both liver and enterocyte extracts assembled editosomes (B complexes) of virtually identical electrophoretic mobility on parental pRSA13 RNA (lane 2, panels A and B, respectively). Editosomes assembled on the translocation mutant pRSA13Δ3'TL in extracts from both tissue sources (lane 4) but were not assembled on pRSA13 Δφ (lane 6), an RNA substrate which does not contain 3' flanking sequences. These data demonstrate that translocation of editing function is accompanied by translocation of editosome assembly and provide strong support for the role of 3' flanking sequences in editosome complex assembly.

CONCLUSIONS

We have presented direct evidence for the role of flanking sequences in apoB mRNA site-specific editing and editosome assembly and have shown that sequences 3' of the editing site are both necessary and sufficient for these processes in liver and enterocyte extracts. Our data also suggest that specific 5' apoB sequences and bulk RNA enhance the efficiency of editing (and

editosome assembly). The editing activities from rat liver and intestinal enterocytes were shown to have identical sequence requirements for editing within 55 nucleotides encompassing the editing site. We conclude that the 'Mooring Sequence' model originally proposed for the liver editing activity is also appropriate for the enterocyte editing activity. Experiments are in progress to evaluate the composition of liver and enterocyte editosomes and to determine how protein-RNA interactions are involved in the regulation of apoB mRNA editing.

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