The subcellular compartment in which apolipoprotein (apo) B mRNA is edited is unknown. We studied the site of endogenous apoB mRNA editing and correlated the extent of editing with mRNA maturation in the rat liver. RNA editing activity was demonstrated in both nuclear and cytoplasmic extracts. The specific activity of the editing activity was 5.5-fold higher in the nuclear extract, which was not accounted for by activators, inhibitors, or modulators. However, the total editing activity was 3.1 times higher in the cytoplasmic extract. Highly purified rat liver nuclear apoB mRNA contained 17.3 ± 1.45% edited sequences compared with 56 ± 2.5% and 62.15 ± 6.2% edited sequences in hepatic total and polysomal RNAs, respectively. Because of the significant extent of editing of total nuclear RNA, we fractionated it into a poly(A') and poly(A+) fraction. While the poly(A') fraction contained only 10.4 ± 1.1% edited sequences, which represents a maximum estimate, the poly(A+) nuclear apoB mRNA contained 50 ± 1.8% edited sequences, a value very similar to that for polysomal RNA. By direct sequencing of cDNA and genomic clones, we found that as in the case of the human apoB gene, the rat apoB gene contains an intron 25 immediately upstream of the edited exon 26. Using this information, we developed a method to examine in a highly selective manner apoB mRNA that is present in the nucleus before splicing of intron 25 and after splicing of this intron. The unspliced nuclear pre-mRNA contained 7.4 ± 0.2% edited sequences compared with 51.0 ± 0.9% edited sequences in the spliced nuclear apoB mRNA. Furthermore, in the poly(A') pool of apoB pre-mRNA, unspliced nuclear pre-mRNA contained hardly any (1.56%) edited sequences, and the spliced nuclear pre-mRNA contained 7.8 ± 0.6% edited mRNA. In the poly(A+) fraction, unspliced nuclear pre-mRNA had 25.4 ± 0.05% and spliced nuclear mRNA 53 ± 0.6% of its apoB mRNA in an edited form. We conclude that in the rat liver apoB mRNA editing is not a cotranscriptional event. It occurs posttranscriptionally, but the process is essentially complete in the spliced polyadenylated apoB mRNA before it leaves the nucleus. Little, if any, additional editing occurs in the cytoplasmic compartment.

Apolipoprotein (apo) B is a heterogeneous protein. The two major forms of apoB are apoB-100, a physiological ligand for the low density lipoprotein receptor, and apoB-48, an obligatory component of intestinal chylomicrons (1). In mammals, apoB-100 is produced by the liver, and apoB-48 by the small intestine. The rodents (rat and mouse) are unique among mammals in that apoB-48 is also produced in large amounts in the liver (2, 3). The biogenesis of apoB-48 mRNA is unique; it is produced from apoB-100 mRNA by RNA editing, which involves a C → U conversion of the first base of codon-2153 encoding a glutamine residue, changing it from CAA to UAA, an in-frame stop codon (4, 5). The mechanism of apoB mRNA editing is unclear; most likely it is a sequence-specific cytidine deamination or transamination reaction (6).

Although there are many studies on the tissue-specific (2, 3, 7), developmental (7–9), nutritional (10, 11), and hormonal (2, 12, 13) regulation of apoB mRNA editing, it is not known whether editing occurs cotranscriptionally or posttranscriptionally, in the nucleus or in the cytoplasm of the cell, or at what stage during the production and maturation of apoB mRNA the C → U conversion occurs. In this communication, we present the subcellular topography of apoB mRNA editing in the rat liver, not only in terms of its localization in the nuclear and cytoplasmic compartments, but also with respect to the various stages of apoB mRNA production and maturation. Although the early transcript (nonpolyadenylated unspliced apoB pre-mRNA) is largely unedited, editing is essentially complete by the time the intranuclear apoB mRNA is spliced and polyadenylated. Little additional editing occurs following the export of the mature apoB mRNA into the cytoplasm.

MATERIALS AND METHODS

Preparation of S-100 and Nuclear Extracts—Male Wistar rats weighing 500–600 g were put to death by carbon dioxide suffocation. The livers were perfused and removed as quickly as possible, then blotted and trimmed of connective tissues before submerging in an ice-cold homogenizing buffer (0.25 M sucrose, 20 mM Tris, pH 7.5, 4 mM MgCl₂, 10 mM KCl, 1 mM 2-mercaptoethanol). The livers were minced with scissors in a cold room and homogenized in a Potter-Elvehjem glass vessel with a motor-driven Teflon pestle for 5–8 strokes. The homogenate was centrifuged for 10 min at 1,500 × g to remove nuclei and cellular debris. The supernatant was further centrifuged for 1 h at 100,000 × g at 4 °C. Nuclear extracts were prepared from isolated rat liver nuclei with a modified procedure of Gorski et al. (14). The S-100 supernatant fractions and nuclear extracts were used immediately for in vitro editing assays as described (15).

Preparation of Total, Polysomal, and Nuclear RNA—Total RNA from rat liver tissue was prepared according to Chirgwin et al. (16). The polysomal fraction was prepared according to Noll (17). The 1,500 × g supernatant was centrifuged for 10 min at 25,000 × g to remove residual cell debris, mitochondria, and lysosomes. The opaque, post-mitochondrial supernatant was treated with sodium deoxycho-
late (final concentration 1.3%) to solubilize the membranes of the endoplasmic reticulum. The supernatant was loaded on a 2-ml portion containing 10 mM Hepes (pH 7.6), 25 mM KCl, 0.15 mM spermine, 50 mM spermidine, 1 mM EDTA, 2 mM sucrose, and 10% glycerol. The homogenate was centrifuged through a 10-ml sucrose cushion at 24,000 rpm for 30 min at 0 °C in a SW 27 rotor. The nuclear pellets were redispersed in an 8:1 (v/v) mixture of buffer A, glycerol, and buffer B (buffer A without sucrose and glycerol) with a loose-fitting Teflon-glass homogenizer. This nuclear suspension was layered over another cushion of buffer A and centrifuged for 1 h at 0 °C. The purified nuclear pellets contained only intact nuclei and were devoid of other subcellular materials as evidenced by electron microscopy. To ensure that the nuclei preparations were not contaminated by any trace amounts of cytoplasmic RNA, the purified nuclei were treated with 50 μg/ml RNase A for 15 min at 25 °C.

RNA from the isolated nuclei and solubilized polysomes were purified by the guanidium isothiocyanate/CsCl method as described by Chirgwin et al. (16). To remove residual nuclear DNA, the purified RNA fractions were treated with 50 μg/ml RQ1 DNase (Promega) for 15 min at 37 °C and were subsequently inactivated by heating at 95 °C for 5 min. The treated RNA was subsequently used for PCR amplification. The RQ1-pretreated RNA fractions contained no intact residual genomic DNA, as there were no PCR products that could be observed when the first cycle of cDNA synthesis with the avian myeloblastosis virus reverse transcriptase was omitted.

Oligo(dt)-Cellulose Column Fractionation of Poly(A)+ and Poly(A−) Nuclear RNA—The guanidium isothiocyanate/CsCl-purified and RQ1-pretreated nuclear RNA fractions were passed through the oligo(dt) spin columns according to the protocol supplied by the manufacturer (Pharmacia LKB Biotechnology Inc.). After the first column, the bound and unbound fractions were each passed through two additional oligo(dt) columns successively to ensure 99% purity in terms of poly(A)+ RNA or poly(A−) RNA.

Assay of apoB-48/ApoB-100 mRNA Ratio—The ratio of apoB-48/apoB-48 + apoB-100 mRNA in individual RNA samples was assayed by the PCR-cloning-colony hybridization assay of Wu et al. (7). A minimum of 2,000 colonies were used for sequence-specific (i.e. B-Gln- or B-Stop-specific (7)) oligonucleotide hybridization in each assay.

PCR Amplification of Unspliced and Spliced Nuclear RNA—The genomic sequence of the rat apoB-100 gene spanning the exons 25/26 (exon 25/exon 26 was determined by Sanger dideoxynucleotide sequencing of the cloned rat liver cDNA in Agt10 and cloned DNAs from PCR products of rat genomic DNA. Multiple clones were sequenced to ensure that PCR errors were excluded. For examination of rat liver nuclear RNA, an upstream primer (CCCCCGGCTCTGGTACACATGAAGGT) located in exon 25 and a downstream primer (GGGGCGGCTCTGGTACATGATGATCTTCG) positioned in exon 26 were used to amplify the spliced and unspliced apoB nuclear RNA. Either total nuclear RNA or nuclear poly(A)+ or poly(A−) fractions were used for amplifications. In each case, the 3.0- and 2.5-kb PCR-amplified DNA fragments corresponding to the unspliced apoB pre-mRNA and the spliced, mature nuclear mRNA, respectively. The two PCR bands were gel-purified and assayed for the ratio of apoB-48/apoB-48 + apoB-100 sequences by the method of Wu et al. (7).

RESULTS

ApoB mRNA Editing Activities in the Nucleus and Cytosol Extracts—As an initial check on the possible location of the RNA editing activity, we have tested nuclear and cytosolic extract from rat liver for apoB mRNA editing activity in vitro (15). Editing activity is easily detected in both types of extracts. The specific activity of the editing activity in the nuclear extract (0.55 × 10−15 mol/μg/h) is approximately 5.5-fold higher than the cytosol fraction (0.1 × 10−15 mol/μg/h). Mixing experiments indicate that the difference in specific activity is not due to the presence of inhibitors or modulators. However, when the total activities extracted from each compartment are compared, there is 3.1 times more activity isolated from the cytosol (14.720 × 10−15 mol/h/g tissue) compared with nuclear extract (4.774 × 10−15 mol/h/g tissue).

Quantitation of Endogenous ApoB-48 mRNA in Highly Purified Nuclear RNA and in Total and Polysomal RNAs—To compare the endogenous level of edited (apoB-48) mRNA in the various subcellular compartments, we prepared highly purified nuclei from rat liver. The nuclei were treated with ribonuclease before they were used for RNA extraction to eliminate any trace of cytoplasmic mRNA contamination. Electron microscopic examination of the purified nuclei (Fig. 1) indicates that they were of high quality and not contaminated by cytoplasmic tags. An occasional nuclear ghost was seen, indicating that small amounts of intranuclear content could have been released and lost during the preparation. The proportion of apoB-48 mRNA compared with total apoB (i.e. apoB-48 + apoB-100) mRNA was determined by a sensitive assay developed by Wu et al. (7) (Fig. 2a). By this assay, the nuclear apoB mRNA contained 17.3 ± 1.45% edited mRNA, the total cellular and polysomal apoB mRNA contained 56 ± 2.5% and 62.5 ± 6.2% edited mRNA, respectively. Although these values suggest that RNA editing could occur predominantly in the cytoplasmic compartment, the presence of 17.3 ± 1.45% edited mRNA in the nuclear compartment is well above background and is highly significant.

To characterize further the extent of RNA editing in subpopulations of the apoB nuclear mRNA, we fractionated the highly purified nuclear RNA by oligo(dt)-cellulose chromatography. The bound (poly(A+)') and unbound (poly(A−)') RNA fractions were analyzed with respect to the proportion of edited apoB mRNA (Fig. 2b). By DNA excess hybridization, 83% of the nuclear apoB mRNA was in the poly(A+) fraction and 17% in the poly(A−) fraction. We found that the poly(A−) fraction contained 10.4 ± 1.1% edited apoB mRNA whereas the poly(A+) fraction contained 50 ± 1.8% edited apoB mRNA, a value approaching that in the polysomal mRNA.

Development of a Method to Examine Specifically Unspliced and Spliced ApoB mRNA—Since the results from the nuclear RNA experiments suggest that RNA editing is almost complete in the polyadenylated nuclear apoB mRNA, we developed a method to compare the extent of RNA editing in unspliced versus spliced apoB mRNA.

It is difficult to analyze apoB pre-mRNAs because the apoB gene is highly complex containing 28 introns and 29 exons (18). If the rat apoB gene organization is similar to that in humans, highly complex pre-mRNAs of different sizes are expected. We developed a method that enabled us to correlate

![FIG. 1. Electron micrographs of isolated rat nuclei from livers.](image-url)
of the editing site (Fig. 3), we amplified the apoB mRNA sequences between these primers. As shown in Fig. 4a, when we used highly purified rat liver nuclear RNA as a template, we observed two discrete bands of PCR products with sizes expected for unspliced and spliced apoB mRNA. In contrast, only single bands corresponding to the spliced mRNA were detected in total RNA (which is essentially total cytoplasmic RNA because its preparation involved the removal of nuclear debris and therefore had little nuclear contamination) and polysomal RNA. The upper (3 kb) band of the nuclear RNA PCR product was not caused by DNA contamination because (i) the nuclear RNA had been treated with DNase to remove any DNA during its preparation, (ii) the upper band was resistant to DNase treatment prior to PCR (compare the control genomic DNA amplification product, which was completely eliminated), and (iii) it required reverse transcriptase for amplification (Fig. 4a). Southern blot hybridization (Fig. 4b) using intron- and exon-specific oligonucleotides (Fig. 3) and direct sequencing (data not shown) confirmed that the lower band was a cDNA copy of spliced apoB mRNA and the upper band a cDNA copy of the unspliced apoB pre-mRNA containing intron 25. PCRs using a limited number of cycles (5, 10, and 15) indicate that the unspliced apoB mRNA is about 5–10-fold more abundant than the spliced species.

Correlation of Extent of RNA Editing with RNA Splicing and Polyadenylation—The proportion of edited apoB mRNA was determined in the PCR products from both the unspliced pre-mRNA (Fig. 4b, upper band) and the nuclear spliced mRNA (Fig. 4b, lower band, and Fig. 5, left panel). The unspliced mRNA contained 7.4 ± 0.2% edited sequences,

FIG. 2. Proportion of edited apoB mRNA in rat liver RNA (a) and nuclear RNA (b) subfractions. The percent of apoB-48/ (apoB-48 + apoB-100) mRNA in each RNA fraction was assayed by the method of Wu et al. (7). Values are means ± S.E. from triplicate determinations from five independent experiments.

FIG. 3. Intron-exon junctional structure of rat apoB exons 25 and 26 and intron 25. This region of cloned rat apoB genomic DNA and cDNA was completely sequenced. Only the crucial segments are shown. The sequences used for PCR primers are marked by arrows above them and the exon- and intron-specific oligonucleotide probe sequences are underlined. The 5′ PCR primer sequence is that shown, whereas the 3′ PCR primer sequence is the complement of the sequence shown. Artificial XmaI and NotI restriction sites have been added to the 5′ end of the two primers. The edited C is marked by an asterisk. Intron 25 contains 506 nucleotides. Intron 25/exon 26 junction is located 2,521 nucleotides upstream of the edited C.

the editing event with the splicing of the intron immediately upstream of the exon containing the edited C.

In humans, the RNA editing site (codon 2153) is located within a 7.5 kb exon (exon 26). It is approximately 2.5 kb downstream of intron 25. We wished to correlate the splicing of this intron with the extent of RNA editing. First, we needed to know if the apoB gene has a similar structural organization around the homologous exon in the rat. We isolated rat apoB cDNA clones from a rat liver cDNA library and isolated genomic clones obtained by PCR of rat genomic DNA spanning the editing site. Approximately 3 kb of the sequences extending at the 5′ end from exon 25 through intron 25 into exon 26 just past the editing site were completely sequenced. The crucial parts of the sequence are shown in Fig. 3. Sequence alignment with the human sequences (19) indicates that intron 25 is conserved in the rat apoB gene where it occurs at the identical location at the nucleotide level as the human apoB gene.

Knowledge of the genomic structure of the intron 25/exon 26 region enabled us to devise a technique to compare specifically unspliced and spliced apoB mRNA isolated from highly purified rat liver nuclei. Using a 5′ PCR primer in exon 25 upstream to intron 25 and a 3′ primer in exon 26 downstream of the editing site (Fig. 3), we amplified the apoB mRNA sequences between these primers. As shown in Fig. 4a, when we used highly purified rat liver nuclear RNA as a template, we observed two discrete bands of PCR products with sizes expected for unspliced and spliced apoB mRNA. In contrast, only single bands corresponding to the spliced mRNA were detected in total RNA (which is essentially total cytoplasmic RNA because its preparation involved the removal of nuclear debris and therefore had little nuclear contamination) and polysomal RNA. The upper (3 kb) band of the nuclear RNA PCR product was not caused by DNA contamination because (i) the nuclear RNA had been treated with DNase to remove any DNA during its preparation, (ii) the upper band was resistant to DNase treatment prior to PCR (compare the control genomic DNA amplification product, which was completely eliminated), and (iii) it required reverse transcriptase for amplification (Fig. 4a). Southern blot hybridization (Fig. 4b) using intron- and exon-specific oligonucleotides (Fig. 3) and direct sequencing (data not shown) confirmed that the lower band was a cDNA copy of spliced apoB mRNA and the upper band a cDNA copy of the unspliced apoB pre-mRNA containing intron 25. PCRs using a limited number of cycles (5, 10, and 15) indicate that the unspliced apoB mRNA is about 5–10-fold more abundant than the spliced species.

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the two compartments in extracts from both the nuclear and cytoplasmic compartments. Activities may be in a cryptic form and may not be accessible quality nuclei from rat enterocytes. We have therefore combined two subcellular compartments from the rat small intestine. The specific activity in the nuclear extract is higher than that in the cytosol extract. However, the total activity is slightly higher in the latter because the volume of the latter is much larger. Whereas the spliced mRNA contained 51.0 ± 0.9% edited sequences, a value approaching that in polysomal apoB mRNA.

To characterize further the stage at which nuclear apoB mRNA is edited during its processing and maturation, we fractionated the poly(A-) and poly(A+) nuclear RNAs into unspliced and spliced fractions using PCR amplification (Fig. 5). In the poly(A-) fraction, the unspliced portion contained 1.56% edited apoB mRNA, which is close to PCR background, whereas the spliced poly(A-) RNA contained 7.8 ± 0.6% edited RNA. In the poly(A+) pool, 25.4 ± 0.05% of the unspliced apoB mRNA was in an edited form compared with 53 ± 0.6% in the spliced apoB mRNA, which is very similar to the value for polysomal apoB mRNA.

![Graph showing the proportion of edited apoB mRNA in unspliced and spliced poly(A-) and poly(A+) rat liver nuclear RNA](image)

**Fig. 5.** Proportion of edited apoB mRNA in unspliced and spliced, poly(A-) and poly(A+) rat liver nuclear RNA. The unspliced and spliced PCR products correspond to the upper and lower bands in Fig. 4. The poly(A-) and poly(A+) subfractions were obtained by oligo(dT)-cellulose spin columns and were subsequently separated into unspliced and spliced fractions by PCR and agarose gel electrophoresis. Proportion of edited apoB mRNA in each fraction was determined by the method of Wu et al. (7). A total of 15 animals were used in three separate experiments. Values were means ± S.E. Where the S.E. bars are so small that they fall within the line of the figure, they have been omitted.

whereas the spliced mRNA contained 51.0 ± 0.9% edited sequences, a value approaching that in polysomal apoB mRNA.

**DISCUSSION**

The subcellular site of apoB mRNA editing has never received any in-depth analysis. It has been variously postulated by different laboratories to be a cotranscriptional (2, 4, 15) or posttranscriptional (5, 6) process that occurs predominantly in the nucleus (5, 6, 15, 20) or in the cytoplasm (21). Editing has been demonstrated following transfection of hybrid apoB/E gene constructs in various cell lines, but the site of the C → U conversion was not determined (20). Editing has also been demonstrated in vitro using rat liver nuclear (15) and cytoplasmic extracts (15, 21) as well as rat and baboon small intestinal cytoplasmic extracts (22). In this study, we directly compared the editing activities isolated from the nuclear and cytoplasmic fractions of the rat liver. The specific activity in the nuclear extract is higher than that in the cytosol extract. However, the total activity is slightly higher in the latter because the volume of the latter is much larger. We also attempted to compare the activities in these two subcellular compartments from the rat small intestine. Unfortunately, it was technically not feasible to prepare high quality nuclei from rat enterocytes. We have therefore confined all our other experiments to the rat liver.

Although efficient RNA editing activity is easily detected in extracts from both the nuclear and cytoplasmic compartments, we do not understand the significance of this finding in terms of the functional roles of the editing activity from the two compartments in vivo. Potentially part or all of the activities may be in a cryptic form and may not be accessible to the endogenously produced apoB mRNA. Furthermore, at least part of the cytosolic activity could have resulted from leakage from the nuclear compartment during the preparation of the editing extract. Well known nuclear activities, such as RNA splicing activities, can be easily detected in cytoplasmic extracts (23-25). Thus, in order to obtain information on the functional significance of the RNA editing activities detected in tissue extracts in vitro, we directly measured the extent of editing of apoB mRNAs isolated from the nuclear and cytoplasmic compartments, which would reflect the degree of RNA editing in vivo.

In examining the proportion of edited apoB mRNA among the RNA samples isolated from the different compartments, we used the PCR-cloning-colony hybridization assay of Wu et al. (7) because, compared with four other less labor-intensive assays, this one is the most sensitive and reproducible; it enabled us to detect subtle differences among the different RNA samples. The first comparison we made was between rat liver polysomal and total nuclear apoB mRNAs. The difference of 62 versus 17.3% in the proportion of edited apoB mRNA in polysomal and nuclear RNA (Fig. 2a) is impressive and highly significant. A simple interpretation would be that these values reflect the higher total apoB mRNA editing activity isolated from the cytoplasmic compartment and that RNA editing occurs mainly in the latter. However, the 17.3% editing in the RNA isolated from the highly purified nuclei is substantial. It cannot be explained by cytoplasmic contamination because not only were the nuclear preparations of high quality (Fig. 1), they were also treated with ribonuclease to remove any cytoplasmic contamination prior to RNA extraction. Furthermore, since nuclear RNA is highly heterogeneous, we wished to examine the extent of editing in apoB mRNA during the various stages of its maturation.

The first maturation event we studied was polyadenylation. We found that the polyadenylated nuclear apoB mRNA contained approximately 50% edited sequences, whereas the nonpolyadenylated fraction had only 10.4% of its mRNA in an edited form (Fig. 2b). It should be pointed out that the value obtained for the nonpolyadenylated form is probably an overestimate; for an mRNA as large as apoB mRNA, any partially degraded nuclear apoB mRNA that has lost its poly(A) tail would be included in this fraction. It is likely that, in vivo, total nonpolyadenylated nuclear mRNA is edited only to a very small extent.

By completely sequencing rat apoB cDNA and genomic clones that span exons 25 and 26 and intron 25 (Fig. 3), we documented that like the human apoB genomic sequence, in the rat there is a homologous intron (intron 25) immediately upstream of exon 26, the giant exon that contains the editing site. Using the sequence information, we developed a sensitive PCR-based method that enabled us to differentially examine the spliced versus the unspliced (with respect to intron 25) apoB mRNAs. The results indicate that the site-specific editing of codon 2153 in exon 26 is coincident with splicing of intron 25.

In the processing of pre-mRNAs, the order of intron splicing follows more than one pathway, and polyadenylation is not coupled to the splicing events (26). To obtain insight into the chronology of events relating editing to apoB mRNA splicing and polyadenylation, we assayed for the extent of apoB mRNA editing in the poly(A-) and poly(A+) nuclear mRNAs which have been further fractionated into unspliced and spliced subpopulations (Fig. 5). It is evident that poly(A-) unspliced apoB pre-mRNA is essentially totally unedited. Significant low level editing (7.4%) is present in nonpolyadenylated but spliced mRNA; an even higher degree of
Topography of ApoB mRNA Editing

... editing (25%) occurs with polyadenylation, even if the mRNA is still unspliced. When the apoB mRNA is both spliced and polyadenylated, the level of editing (53%) approaches the value found in polysomal RNA.

In conclusion, we have documented the topography of apoB mRNA editing in the rat liver. Taken together, our data are consistent with the following sequence of events: (i) apoB mRNA editing is still unspliced. When the apoB mRNA is both spliced and polyadenylated, the level of editing (53%) approaches the value found in polysomal RNA. (ii) cytoplasmic apoB mRNA is normally inaccessible to or protected from the cytoplasmic editing activity.

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