Induction of Cytidine to Uridine Editing on Cytoplasmic Apolipoprotein B mRNA by Overexpressing APOBEC-1*

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Post-transcriptional editing of apolipoprotein B (apoB) mRNA is regulated in hepatic cells to achieve a steady state proportion of edited and unedited RNA molecules. This activity is catalyzed by APOBEC-1 (apoB mRNA editing catalytic subunit 1) in what has been widely accepted as nuclear event occurring during or after mRNA splicing. Introns impair the efficiency of editing within an adjacent exon in a distance-dependent manner in reporter RNAs. We show here that this inhibition can be overcome by overexpressing APOBEC-1 and that the enhanced editing efficiency on these reporter RNAs occurred after splicing on cytoplasmic transcripts. Given the absolute requirement of auxiliary proteins in apoB mRNA editing, the data suggested that auxiliary proteins were distributed with APOBEC-1 in both the nucleus and cytoplasm of McArdle cells. In fact, immunolocalization of one such auxiliary protein, APOBEC-1 complementation factor (ACF) demonstrated a nuclear and cytoplasmic distribution. We also demonstrate that in the absence of alterations in APOBEC-1 expression, changes in edited apoB RNA induced by ethanol arise through the stimulation of nuclear editing activity. The finding that apoB mRNA editing can occur in the cytoplasm but normally does not suggests that under biological conditions, restricting editing activity to the nucleus must be an important step in regulating the proportion of the edited apoB mRNAs.

Apolipoprotein B (apoB) mRNA editing involves a site-specific deamination reaction wherein a cytidine at nucleotide 6666 is changed to a uridine thereby placing an in frame UAA stop codon within the 14-kilobase apoB mRNA (1, 2). Translation of the unedited and edited variants of apoB mRNA generates two isoforms of apoB proteins, apoB100 and apoB48, which behave differently in terms of lipoprotein secretion and uptake by peripheral cells (3). A tripartite RNA sequence motif, consisting of an 11-nucleotide mooring sequence, a spacer, and a regulatory element, is required for site-specific RNA editing (4–8). Recently, a stem-loop model of secondary structure involving the essential sequence required for editing has been proposed (9, 10). These cis-acting elements are required for the assembly of an editing complex, the C/U editosome (5, 11), and site-specific editing activity. APOBEC-1, the cytidine deaminase responsible for editing (12), is the catalytic subunit of the editosome (9) and may function in this capacity as a dimer (13, 14).

APOBEC-1 alone cannot edit apoB mRNA and requires multiple yet-to-be characterized proteins referred to collectively as the auxiliary proteins (15–20). Auxiliary proteins are broadly expressed in mammalian cell lines and tissues, independent of the expression of APOBEC-1 and apoB mRNA (12, 16–18, 21). Recently, APOBEC-1 complementation factor (ACF) has been cloned (40). ACF is 64.3-kDa RNA binding protein, it binds to apoB mRNA in vitro and in vivo. ACF and APOBEC-1 comprise the minimal protein requirements for specific and efficient editing of apoB mRNA in vitro. Other candidate auxiliary proteins such as an hnRNP A/B homolog ABBP-1, hnRNP D, and hnRNP C (22–24), mooring sequence-selective RNA-binding proteins of 100 and 55 kDa (15, 20, 25–27), and general RNA-binding proteins 40–44 kDa (15, 20, 25, 28) have been identified through their affinity for APOBEC-1 or apoB RNA. A complex of proteins (referred to as AUX240 for the 240-kDa antigenic protein it contains) identified with monoclonal antibodies raised against in vitro assembled 27 S editing complexes (editosomes) (29) has also been proposed to contain auxiliary proteins. The specific functional role that each candidate auxiliary protein may play in apoB mRNA editing remains to be further clarified.

A direct demonstration of the intracellular site of editing activity through immunolocalization of APOBEC-1 has not been possible due to the very low level of expression of the enzyme in cells and tissues. Evidence for a nuclear localization of the editing activity was first provided through the demonstration of editing on polyadenylated but unspliced apoB mRNA and that the level of editing of spliced nuclear apoB mRNA was equivalent to that of cytoplasmic or total cellular mRNA (30, 31). These findings were corroborated in studies in which chimeric splicing/editing RNA substrates were used to demonstrate that the close proximity of introns to the editing site within apoB encoding exon inhibited editing activity in McArdle cells until splicing had occurred (32). Taken together, the data suggested that editing of apoB mRNA took place in the cell nucleus; however, the possibility of cytoplasmic editing has not been ruled out.

The localization of APOBEC-1 has been evaluated by indirect immunofluorescence microscopy of HA-tagged APOBEC-1 in transiently transfected McArdle cells and HepG2 human hepatoma cells (33, 34). The enzyme was distributed in both the nucleus and cytoplasm of these cells but was localized in
the cytoplasm of cells that did not support editing activity (Chinese hamster ovary and COS-7 cells). The nuclear distribution of APOBEC-1 required an N-terminal sequence (residues 13–35) similar to a bipartite nuclear localization signal (NLS). This sequence, however, was not sufficient to direct chimeric proteins to the nucleus and hence did not satisfy the strict definition of an NLS (33). The data suggested that auxiliary proteins may play an important role in determining the intracellular distribution of APOBEC-1.

In contrast, residues 174–180 in the C terminus of APOBEC-1 behaved as a strong nuclear export signal (NES)/cytoplasmic retention signal (CRS) and were sufficient in directing chimeric proteins to the cytoplasm (33). These findings further suggested that the nuclear and cytoplasmic distribution may be due to the combined activities of interactions involving APOBEC-1's NLS and NES/CRS signals.

We report here that apoB mRNA editing can be experimentally induced in the cytoplasm of McArdle cells, suggesting that auxiliary proteins as well as APOBEC-1 are distributed in both the nucleus and cytoplasm of liver cells. Biological alterations in the proportion of edited apoB mRNA appear, however, to result primarily or exclusively from changes in nuclear editing activity.

**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—The construction of plasmids encoding His$_{10}$-HA-APOBEC-CMPK, His$_{10}$-HA-APOBEC-1, and CMPR, respectively (33), and the splicing/editing substrate IVS-apoB (32) has been described previously. Human ACF (40) was amplified from human total liver RNA by RT-PCR methodology with the following primers 5'-CTCGATACTGAGGATAACAACTACCGG and 5'-CTCTCTAGATCAGAAGGTCGATCATCACG and subcloned into EcoRI and XbaI sites downstream of HA and His$_{10}$ epitope tags in a modified pcDNA3 vector (33).

**Tissue Culture**—The rat hepatoma McArdle RH7777 cells were obtained from ATCC (Manassas, VA). Cells were transfected as reported previously (32). Stable cell lines transfected with apobec-cmpk were obtained by limiting dilution under 500 µg/ml G418 (Life Technologies, Inc.) selection 48 h after transfection. Cells were treated with ethanol by growth in a 96-well plate and centrifuged (150,000 × g for 1 h) and the resulting pellet was washed twice with ethanol and resuspended in CER-II buffer. APOBEC-CMPK proteins were incubated at 30 °C for 3 h under conditions described previously (19, 20, 33). Editing efficiency was evaluated by poisoned primer extension analysis, and the primer extension products were resolved on a 10% denaturing polyacrylamide gel and quantified by laser densitometric scanning (PhosphorImager 425E, Molecular Dynamics) as described previously (20, 32–34).

**Immunostaining**—Cells fixed with 2% paraformaldehyde and permeabilized with 0.4% Triton X-100 were incubated with anti-HA monoclonal antibody for 1 h at room temperature (Babco, Berkeley, CA; 1:500 in phosphate-buffered saline containing 3% bovine serum albumin), followed by incubation for 1 h at room temperature with fluorescein-conjugated goat anti-mouse antibody (ICN/Cappel; 1:25 in 3% bovine serum albumin/phosphate-buffered saline). Coverslips were mounted onto DAPI containing mounting medium. Slides were observed under an Olympus BH-2 fluorescence microscope using a 40× Olympus objective.

Protein extracts were made from approximately equal number of transfected cells and subsequently resolved and transferred to nitrocellulose membrane, probed with anti-HA monoclonal antibody as described previously (33).

**RESULTS**

**The Inhibitory Effect of Introns on ApoB mRNA Editing Is Reduced by Overexpressing APOBEC-1 in McArdle Cells**—When the mRNA splicing cassette from the adenovirus late leader sequence was placed either 5' or 3' of a 492-nucleotide apoB exon containing the editing site, editing efficiency was markedly reduced in wild type McArdle cells (32). In these studies, the number of edited apoB mRNA molecules at steady state was demonstrated to be dependent on the level of APOBEC-1 expression and was independent of the abundance of apoB mRNA or the presence of competing RNA editing substrates (32, 34). Taken together, the data suggested the “gating hypothesis” wherein editing was proposed to occur at a temporal and/or a spatial point (a gate) through which all apoB pre-mRNAs pass during their maturation. The number of apoB mRNA molecules that are edited at the gate was determined by the probability of assembling functional editosomes on each mRNA, which in turn is determined by the number of APOBEC-1 molecules available for assembly into these editosomes. As a further test of the gating hypothesis, wild type McArdle cells and a stable transfected McArdle cell line expressing APOBEC-1 (McAPOBEC cells, Ref. 20) were transfected with plasmids encoding a splicing-editing RNA (IVS-apoB RNA) and the editing efficiency evaluated by poisoned primer extension analysis of RT-PCR product as described under “Experimental Procedures.”

As determined previously (32), the presence of an intron severely impaired editing efficiency of IVS-apoB RNA (Fig. 1A) in wild type McArdle cells 2.7% (n = 3, S.E. = 1.0%) (Fig. 1B, first lane). Overexpression of APOBEC-1 in McAPOBEC cells markedly stimulated the editing of IVS-apoB 44% (n = 3, S.E. = 1.0%) (second lane). Additional editing of C$_{6661}$ due to overexpression of APOBEC-1 (promiscuous editing Refs. 34–37) was also observed.

To evaluate whether the regulation of editing activity had occurred at a “gate” localized within the cell nucleus, the proportion of edited IVS-apoB RNA was determined in subcellular fractions of wild type McArdle and McAPOBEC cells. The quality of the nuclear preparation was evaluated by phase microscopy (Fig. 1C), the gross morphological integrity of the isolated nuclei, and the virtual absence of cytoplasmic materials was apparent.

The amount of edited nuclear IVS-apoB was low in wild type
McArdle cells 2.1% (n = 3, S.E. = 0.5%) but may have been slightly higher than that determined in the cytoplasm 1.7% (n = 3, S.E. = 0.3%) (compare the first two lanes of Fig. 1D). In contrast, an elevated proportion of edited IVS-apoB RNA was observed in the nucleus and cytoplasm 48% (n = 3, S.E. = 2.3%) of McAPOBEC cells compared with that measured on nuclear IVS-apoB RNA 7.8% (n = 3, S.E. = 4.4%, the last two lanes of Fig. 1D). The markedly higher proportion of edited RNA in the cytoplasm of McAPOBEC cells compared with that found in the nucleus suggested the unprecedented possibility that additional editing of spliced IVS-apoB RNA had occurred in the cytoplasm. To evaluate this possibility, an APOBEC-1 chimera was designed that would retain the enzyme in the cytoplasm through the addition of full-length CMPK (chicken muscle pyruvate kinase, a well characterized neutral cargo protein in the nuclear-cytoplastmic trafficking field) as bulk protein sequence (33).

**APOBEC-CMPK Fusion Protein Edits ApoB RNA in Vitro**—To evaluate whether editing could occur in the cytoplasm, we targeted APOBEC-1 to the cytoplasm by taking advantage of our previous finding that the addition of protein mass to APO-BEC-1 impaired the ability of the chimeric enzyme to localize within the nucleus (33, 34). The chimeric enzyme retained editing activity as evident from the ability of HA- and His-tagged APOBEC-CMPK fusion protein, purified from *E. coli* to stimulate in vitro apoB editing in a concentration-dependent manner when added to wild type McArdle cell extracts (Fig. 2).

**APOBEC-CMPK Is Localized in the Cytoplasm**—Having demonstrated that APOBEC-CMPK was functional in apoB mRNA editing, McArdle cells were transfected with plasmid encoding APOBEC-CMPK and evaluated for the intracellular distribution of this chimeric protein using antibodies specific for the HA tag in indirect immunofluorescence analysis. The data suggested that most, if not all, of APOBEC-CMPK was localized in the cytoplasm of transiently transfected McArdle cells (Fig. 3).

**APOBEC-CMPK Expression in McArdle Cells Increases Editing Activity**—If apoB mRNA editing was primarily a nuclear event, overexpression of APOBEC-CMPK should cause little or no change in the editing activity of McArdle cells due to its cytoplasmic distribution. To evaluate editing of the endogenous, McArdle cell apoB mRNA, cells were transiently transfected with plasmids encoding either APOBEC-1, CMPK, or APOBEC-CMPK cDNAs, and total cellular RNAs were isolated and analyzed. The expression of full-length wild type and chimeric proteins was evident on Western blots of cell extracts from an equivalent number of transfected cells (10⁶) that were reacted with HA-specific antibody (Fig. 4A). APOBEC-1 expression elevated apoB mRNA editing in McArdle cells to 48% (n = 3, S.E. = 3.9%), whereas overexpression of CMPK as a negative control did not significantly change editing activity from that observed in untransfected cells, 14% (n = 3, S.E. = 1.9%) (Fig. 4B). In contrast, overexpression of APOBEC-CMPK increased apoB mRNA editing efficiency to 50% (n = 3, S.E. = 4.8%).

To evaluate whether the editing efficiency observed in cells expressing APOBEC-CMPK was dependent on the expression level of the enzyme, McArdle cell lines were established by clonal selection in the presence of G418, and cell lines expressing low, medium, and high levels of APOBEC-CMPK fusion protein were identified by Western blotting (Fig. 4C). Primer extension analysis of RT-PCR product specific to endogenous McArdle cell apoB mRNA demonstrated that the editing efficiency in three cell lines expressing low, medium, or high levels of APOBEC-CMPK was 22% (n = 3, S.E. = 1.2%), 50% (n = 3, S.E. = 1.8%), and 93% (n = 3, S.E. = 2.4%), respectively.
that the cell in the bottom left autofluorescence.

Body. BEC-1, CMPK, or APOBEC-CMPK were analyzed for the expression of McArdle cells transiently transfected with plasmids encoding APOBEC-CMPK to increase editing activity.

A

B

C

D

Fig. 3. Subcellular localization of APOBEC-CMPK in transiently transfected McArdle cells. The DAPI and anti HA-APOBEC-CMPK stained images of the same high magnification field are shown (left and right panels). Arrowheads indicate the cell nucleus (Nu). Note that the cell in the bottom left of the field was not transiently transfected and serves as a background control for antibody reactivity and autofluorescence.

Fig. 4. Expression of APOBEC-CMPK in McArdle cells increases editing activity. A, proteins from an equivalent number of McArdle cells transiently transfected with plasmids encoding APOBEC-1, CMPK, or APOBEC-CMPK were analyzed for the expression of full-length protein by Western blotting using anti-HA monoclonal antibody. B, poisoned primer extension analyses were performed upon RT-PCR-amplified apoB cDNA templates from each transfection. The editing efficiency of the corresponding transfections in B from left to right was: 48 ± 3.9, 14 ± 1.9, and 50 ± 4.6 (n = 3). C, proteins from an equivalent number of cells from three clonal McArdle cell lines, each expressing a different level of APOBEC-CMPK, were analyzed by Western blotting and reacted with anti-HA antibody. D, editing of apoB mRNA isolated from the cell lines in C was analyzed by poisoned primer extension and quantified as described in the legend to Fig. 1B. Editing efficiency was: 22 ± 1.2, 50 ± 1.8, and 93 ± 2.4 (n = 3) for the low to high APOBEC-CMPK-expressing cell lines.

The data demonstrated that the level of APOBEC-CMPK expression determined the proportion of edited apoB mRNA despite the enzyme’s distribution in the cytoplasm and thereby support the possibility of cytoplasmic editing.

The Editing of IVS-ApoB RNA Is Restored by APOBEC-CMPK—The data raise the question of whether cytoplasmic APOBEC-CMPK was active on cytoplasmic apoB mRNA or whether a small amount of APOBEC-CMPK, which was below the detection limit of immunofluorescence microscopy, was in the nucleus for a period of time long enough to edit nuclear apoB. If APOBEC-CMPK’s editing activity was cytoplasmic, then the proportion of edited apoB should not be inhibited by the placement of introns adjacent to the editing site, and the proportion of edited apoB RNAs in the cytoplasm should be substantially higher than that observed with nuclear apoB RNA. In contrast, if APOBEC-CMPK editing activity occurred exclusively or predominantly in the nucleus, then nuclear apoB RNAs should reflect elevated levels of editing activity equivalent to that determined on total cellular apoB RNAs.

The cellular site of editing was evaluated in the APOBEC-CMPK high level expression McArdle cell line and wild type McArdle cells as negative control following transient transfection of a plasmid encoding IVS-apoB. The proportion of edited apoB RNA was assayed 24 h after transfection to ensure an early enough time point where maximal detection of nuclear editing would theoretically occur before a steady state cytoplasmic pool of edited RNA was achieved. RT-PCR amplification of IVS-apoB RNA transcripts at different stages in RNA processing was performed using the indicated primer pairs (Fig. 1A) as described under “Experimental Procedures.” The data demonstrated that IVS-apoB was poorly edited in McArdle cells regardless of the stage of splicing, unspliced IVS-apoB RNA, 2.4% (n = 3, S.E. = 0.5%) and apoB exon RNA-specific, 2.7% (n = 3, S.E. = 1.0%) (Fig. 5, first two lanes). Unspliced IVS-apoB RNA was marginally edited 3% (n = 3, S.E. = 0.7%) in the APOBEC-1-CMPK high expression cell line; however, editing of IVS-apoB after splicing was markedly elevated 64% (n = 3, S.E. = 3.9%) (Fig. 5, the last two lanes), and promiscuous editing was also observed.

To further confirm the occurrence of cytoplasmic editing, nuclear and cytoplasmic fractions were prepared from APOBEC-1-CMPK high expression cell line transiently transfected with IVS-apoB. The exons corresponding to human apoB sequence in IVS-apoB RNA was specifically amplified from RNA in both cellular subfractions using MS2/MS2 amplimers. Nuclear editing of IVS-apoB in APOBEC-1-CMPK high expression cell line was low, 8.0% (n = 3, S.E. = 2.9%) compared with 69% (n = 3, S.E. = 4.6%) editing in the cytoplasmic fraction (Fig. 6). These data strongly suggest that although it is possible that a small amount of nuclear editing activity had occurred in the APOBEC-CMPK overexpressing cell line after splicing but before RNA export (3% versus 8%, compare Figs. 5 and 6), most of the editing activity had to have taken place in the cytoplasm. Taken together, the data indicate that the interference from adjacent introns on editing can be overcome if the editing enzyme was specifically targeted to the cytoplasm as in the case of APOBEC-CMPK cell line or when APOBEC-1 itself is overexpressed.

ACF Is Localized in Both Nuclear and Cytoplasmic Compartments of McArdle Cells—We have taken advantage of the recent findings by Mehta et al. (40) that ACF and APOBEC-1 comprise the minimal protein requirements for apoB mRNA editing in vitro to probe the intracellular distribution of an auxiliary protein in McArdle cells (Fig. 7). Given ACF’s important role in apoB mRNA editing, the subcellular distribution of ACF would provide more complete proof for the subcellular
localization of the editing activity. To this end, cells were stained 16 h after transfection with full-length HA-tagged ACF to ensure minimal level of expression and avoid potential artifacts due to overexpression and saturation of nuclear transport process. Indirect immunofluorescence microscopy revealed that HA-tagged ACF had both a nuclear and cytoplasmic distribution in McArdle cells, thus supporting the data suggesting that editosomes could be assembled in the cytoplasm.

Ethanol Stimulates Predominantly Nuclear ApoB RNA Editing in McArdle Cells—The ability of experimentally manipulated cells to support cytoplasmic mRNA editing suggests that constraints must be present in wild type cells that regulate the proportion of edited mRNAs such that only a fraction of the total cytoplasmic apoB mRNA is edited for any given metabolic condition (3). To evaluate this possibility in the context of wild type cells, we have taken advantage of the ability of ethanol to rapidly (39) stimulate editing activity in liver cells without inducing the expression of APOBEC-1 (38). It is anticipated that if ethanol increased editing by the induction of the cytoplasmic editing, the increase in editing would only be observed in the cytoplasm. In contrast, a rapid induction of nuclear editing by ethanol followed by an increase in edited cytoplasmic apoB mRNA would suggest that under these conditions alterations in edited cytoplasmic mRNA are secondary to those in the nucleus and the result of the nuclear export of mRNA to the cytoplasm.

McArdle cells were treated with ethanol as described previously (39) and subsequently fractionated into nuclei and cytoplasm at the indicated time points after treatment, and the proportion of edited apoB mRNA was determined as described above (Fig. 8A). The editing efficiency over the course of treatment is summarized (Fig. 8B), and the ratio of edited apoB mRNA in treated versus untreated cell fractions is plotted (Fig. 8C). The relative proportion of edited mRNA in the nuclear compartment of ethanol-treated cells increased rapidly, showing a 1.8-fold increase over that in untreated cells within 15 min. Following slight fluctuations at 30 min and 1-h time points, the proportion of edited nuclear apoB mRNA remained increased 1.4-fold in ethanol-treated cells relative to that in untreated cells up to 14 h after treatment. Increased edited apoB mRNA was not apparent in the cytoplasmic compartment until 30 min after ethanol treatment, after which point it followed the trend of the nuclear compartment and achieved a 1.4-fold increase over that measured in untreated cells by 14 h. The increase in editing efficiency in the cytoplasm lagged that observed in the nucleus, suggesting that ethanol increased editing of nuclear apoB mRNAs, which were subsequently exported to the cytoplasm, where they contributed to an increase in the proportion of edited cytoplasmic RNA at a later time point. Taken together, the data suggest that ethanol increased apoB mRNA editing through the selective induction of nuclear editing activity.

DISCUSSION

This is the first description of cytoplasmic editing of apoB mRNA. The expression of APOBEC-1 in rat liver or McArdle cells is below the detection limit of most conventional immunohistoassays, and hence localization studies have had to rely on epitope-tagged proteins to study the enzyme’s subcellular distribution. In these studies, immunofluorescence microscopy demonstrated that HA-tagged or green fluorescent protein-tagged APOBEC-1 was distributed in both the nucleus and cytoplasm of transiently or stably transfected McArdle cells (33, 34). Recent studies with a variety of epitope-tagged proteins involved in RNA processing demonstrated biologically relevant localization of these proteins even under conditions of their overexpression (41). The dual intracellular distribution has been proposed to be biologically significant as it was observed regardless of the level of APOBEC-1 overexpression (33, 34) and could be converted into 100% nuclear distribution, even at the highest level of APOBEC-1 overexpression, by deleting residues 174–180 encoding APOBEC-1’s CR5/NES (33).

Although we do not know whether or not natively expressed APOBEC-1 has a cytoplasmic and nuclear distribution, the data suggest that auxiliary proteins are distributed in both cellular compartments. Given the absolute requirement of auxiliary proteins for APOBEC-1 mRNA editing activity, the observation that McArdle cells could carry out editing in the nucleus and cytoplasm suggests the novel concept that auxiliary proteins may also be distributed in both cellular compartments. We cannot rule out the trivial explanation that nuclear auxiliary factors were recruited to the cytoplasm by APOBEC-1 upregulation. This argument in favor of a bipartite distribution of auxiliary factors was strongly supported by the data showing a nuclear and cytoplasmic distribution of HA-tagged ACF, a 64.3-kDa apoB RNA-binding auxiliary protein (40) in McArdle cells.

Our findings clarify a long standing question of why nuclear and whole cell S100 extracts both contain editing activity. Previous studies have demonstrated that efficient in vitro RNA
FIG. 8. Ethanol predominantly induces editing in the nucleus. Wild type McArdle cells were treated with 2.5% ethanol for 5 min, 15 min, 30 min, 1 h, or 14 h. After the indicated duration of treatment, cells were harvested, fractionated as nuclei and cytoplasm, and RNAs from both fractions were extracted. ND1/ND2 amplimer pairs were used to generate RT-PCR product specific for the endogenous apoB mRNA isolated from ethanol-treated (+) and untreated control McArdle cells (-) grown in parallel. A. poisoned primer extension assays of apoB mRNA in nuclear and cytoplasmic subfractions from each time point. N, nuclear; C, cytoplasmic. B, the editing efficiency assayed in “A” was calculated as described in the legend to Fig. 1B, and the ratio of editing efficiencies of treated versus untreated (+/−) determined for both the nuclear and cytoplasmic apoB mRNAs. C, the ratio of edited apoB mRNA in treated versus untreated cell fractions is plotted over the time course of ethanol treatment.

ing activity can be assayed with both nuclear and cytoplasmic mRNAs from rat liver (30). Although an analysis of apoB mRNA editing at various stages of apoB mRNA maturation suggested that apoB mRNA editing occurred coincident or immediately subsequent to pre-mRNA splicing, the authors reasoned that at least part of the cytosolic editing activity in vitro could have resulted from leakage from the nuclear compartment during the preparation of extracts. A biological role for cytoplasmic apoB mRNA editing has been suggested from mathematical modeling of steady state proportion of edited apoB RNA in the cell and its regulation (31).

Wild type McArdle cells edit 8–20% of the endogenous apoB mRNA that they transcribe (32, 33, 36, 42). The proportion of RNA that is edited is related to the level of enzyme activity in the cell and is not affected by the number of total RNAs in the cell or the expression of competing RNA editing substrates. Therefore the inability of wild type McArdle cells to efficiently edit IVS-apoB RNA is not due to competition with the endogenous apoB mRNA substrate, but rather, the data suggested that the intron in IVS-apoB had impaired editing. As the only opportunity for this to occur is with the nucleus, the data suggested that IVS-apoB mRNA editing had to occur in the nucleus and that there was no further opportunity for editing to take place once spliced IVS-apoB entered the cytoplasm. Taken together with the data showing that ethanol altered the proportion of edited apoB mRNA by predominately increasing nuclear editing activity, we propose that under biological conditions apoB mRNA editing may be restricted to the nucleus.

Our findings raise an important question concerning the regulation of the proportion of edited mRNAs in cells. If all the factors required for editing have a nuclear and cytoplasmic distribution, why does additional editing not take place on cytoplasmic mRNAs under biological conditions? If there are regulatory molecules in the cytoplasm, then overexpression of APOBEC-1 may have induced cytoplasmic editing by exceeding the capacity of these factors to block the enzyme’s activity in the nucleus. Proteins have been identified through a variety of affinity approaches that map in vitro editing activity and may therefore serve this regulatory role in cells. For example, hnRNP C1 protein (24) and variants of hnRNP D protein (23) inhibit APOBEC-1 editing activity in vitro. Cytosolic extracts have been shown to contain auxiliary factors and APOBEC-1 as 60 S aggregates (15). These aggregates could be biochemically disaggregated in vitro to form 27 S complexes with high levels of editing activity (15). Auxiliary proteins have been identified in the 60 S complexes association with a 240-kDa antigen (29). Whether the 60 S complexes or AUX240 play a role in down-regulating editing activity in the cytoplasm remains to be determined.

APOBEC-1 abundance is maintained at very low levels in wild type cells. Regulation of APOBEC-1 expression occurs under some forms of stimuli (42). Our data demonstrated that experimental overexpression of APOBEC-1 increased both nuclear and cytoplasmic editing. Very high levels of enzyme must, however, be induced experimentally to affect relatively small increases in cellular editing activity (34). We interpreted our data therefore as suggesting that in wild type cells, interactions between APOBEC-1 and auxiliary proteins in the cytoplasm may be kinetically unfavorable. Such a situation could arise if cytoplasmic auxiliary proteins did not have appropriate post-translational modifications and/or were involved in more favorable interactions with other cytoplasmic proteins. Overexpression of APOBEC-1 or APOBEC-CMPK may have provided sufficient number of enzyme molecules to overcome these kinetic barriers and to allow functional editosomes to assemble on cytoplasmic apoB mRNA.

Finally, it was of great interest to see if cytoplasmic editing can be induced by physiological/pharmacological stimuli when APOBEC-1 is not overexpressed. Ethanol feeding was chosen to test this possibility, because ethanol has been shown to up-regulate apoB mRNA editing without increasing apoB 1 mRNA levels (38). Our previous data showed that in rat primary hepatocytes, editing efficiency increased rapidly after treatment with 0.2–2.5% ethanol. The induction occurred rapidly and reached maximum levels within 2 h and remained elevated for 18 h after treatment (39). In the present study, the kinetics of ethanol stimulated editing activity in McArdle cells was evaluated at the level of which subcellular compartment was maximally affected. The abundance of edited apoB mRNA in the cytoplasm was slightly higher than that observed in the nucleus in all cells regardless of time or ethanol treatment. Similar differences in editing efficiency between completely processed nuclear apoB mRNA and polysomal apoB mRNA has been reported (30). Significantly, however, in ethanol-treated cells an increase in edited apoB mRNA appeared first in the nucleus, followed by an increase in the cytoplasm. The simplest explanation for these observations is that ethanol increased editing activity on nuclear apoB mRNA, which then exported to the cytoplasm, where it subsequently contributed to a larger pool of edited mRNAs. Our data suggested that under condi-
tions where APOBEC-1 abundance is not altered, there is little or no cytoplasmic editing of apoB mRNA. The data leave open the possibility that alterations in edited apoB mRNA due to an increased expression of APOBEC-1 may involve cytoplasmic editing activity.

In conclusion, we have demonstrated that apoB mRNA editing can occur in the cytoplasm of McArdle cells when APOBEC-1 is overexpressed. The ability of McArdle cells to carry out apoB mRNA editing in both nuclear and cytoplasmic locations suggests that the auxiliary proteins are distributed along with APOBEC-1 in both compartments as further demonstrated by the dual nuclear/cytoplasmic subcellular distribution of ACF in McArdle cells. Ethanol treatment stimulates nuclear apoB mRNA editing with rapid onset without affecting APOBEC-1 expression level and does not induce cytoplasmic editing. The inability of wild type or ethanol-treated McArdle cells to carry out cytoplasmic editing suggests that negative regulatory mechanism(s) must be in place to prevent it from occurring. Our results, therefore, have important implications for how editing might be metabolically and developmentally controlled and experimentally manipulated.

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