Two Proteins Essential for Apolipoprotein B mRNA Editing Are Expressed from a Single Gene through Alternative Splicing*

Received for publication, November 28, 2001, and in revised form, January 8, 2002
Published, JBC Papers in Press, January 28, 2002, DOI 10.1074/jbc.M111337200

Geoffrey S. C. Dance†‡§, Mark P. Sowden†¶**, Luca Cartegni‡, Ellen Cooper‡, Adrian R. Krainer‡, and Harold C. Smith†§***

From the Departments of †Biochemistry and Biophysics and ‡Pathology and the **Environmental Health Sciences and ¶James P. Wilmot Cancer Centers, University of Rochester, Rochester, New York 14642 and the †Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724

Apolipoprotein B (apoB) mRNA editing involves site-specific deamination of cytidine to form uridine, resulting in the production of an in-frame stop codon. Protein translated from edited mRNA is associated with a reduced risk of atherosclerosis, and hence the protein factors that regulate hepatic apoB mRNA editing are of interest. A human protein essential for apoB mRNA editing and an eight-amino acid-longer variant of known function have been recently cloned. We report that both proteins, henceforth referred to as ACF64 and ACF65, supported APOBEC-1 (the catalytic subunit of the editosome) equivalently in editing of apoB mRNA. They are encoded by a single 82-kb gene on chromosome 10. The transcripts are encoded by 15 exons that are expressed from a tissue-specific promoter minimally contained within the −0.33-kb DNA sequence. ACF64 and ACF65 mRNAs are expressed in both liver and intestinal cells in an approximate 1:4 ratio. Exon 11 is alternatively spliced to include or exclude 24 nucleotides of exon 12, thereby encoding ACF65 and ACF64, respectively. Recognition motifs for the serine/arginine-rich (SR) proteins SC35, SRp40, SRp55, and SF2/ASF involved in alternative RNA splicing were predicted in exon 12. Overexpression of these SR proteins in liver cells demonstrated that alternative splicing of a minigene-derived transcript to express ACF65 was enhanced 6-fold by SRp40. The data account for the expression of two editing factors and provide a possible explanation for their different levels of expression.

Mammalian mRNAs can be post-transcriptionally modified by site-specific adenosine or cytidine deaminases in a process known as mRNA editing (1). Editing of coding sequences or RNA splice sites can alter the primary sequence of a protein, and hence the protein function of the gene. ADARs (adenosine deaminases active on RNA) that recognize deamination are known as CDARs (cytidine deaminases active on RNA) and results in a change to uridine. Of the many CDAR-like proteins known to edit mRNA, the best characterized enzyme, APOBEC-1, catalyzes the deamination of nucleotide C^6666^ of apolipoprotein B mRNA, resulting in a codon change from glutamine to STOP (5). Thus, apoB expression generates two isoforms of the apoB-100 (unedited) and apoB-48 (edited) protein isoforms (5). Although apoB100 and apoB48 have similar lipid carrying capacities in blood, apoB48 containing lipoprotein particles are cleared more rapidly from the circulation and do not associate with the lipoprotein (Lp(a)). Hence, elevated levels of apoB48 containing lipoproteins reduce atherogenic risk (6, 7).

Unlike the ADARs, APOBEC-1 cannot edit mRNA without interactions with auxiliary proteins (4). A 64-kDa human protein, cloned and identified as either ACf (APOBEC-1 complementation factor) (8) or ASp (APOBEC-1 stimulating protein) (9), together with APOBEC-1 function as a minimal editosome in vitro. Several studies have suggested that apoB mRNA is edited by a multiprotein complex of 27 S called the C to U editosome (10, 11). The complexity of the editosome may be attributed to several identified proteins that associated with APOBEC-1 and/or the apoB mRNA editing site to modulate the efficiency of apoB mRNA editing (10–16). The interactions, functions, and regulation of the auxiliary proteins are of interest not only in the study of the mechanism of apoB mRNA editing but also because apoB mRNA editing is a regulated process during tissue development (17) and in response to metabolic or hormonal perturbation (18, 19).

Mehta et al. (8) reported ACf as a 586-amino acid protein in intestine, whereas Lellek et al. (9) reported an identical intestinal 586-amino acid protein, ASP (henceforth referred to as ACF64), and a 594-amino acid variant (ACF65) isolated from liver cDNAs. The role of ASP in apoB mRNA editing had not been determined, nor was it known how these virtually identical proteins were encoded. We show that human ACF64 and ACF65 mRNAs are encoded by the same gene through alternative splicing of exon 12 and that their protein products support equivalent levels of editing in cells. A promoter region was identified immediately upstream of exon 1, and several ESTs were identified that suggested that the ACf transcript may be subject to further alternative and nonexclusive alternative splicing events.

* This work was supported in part by United States Public Health Service Grants DK43739 (to H. C. S.) and GM42699 (to L. C. and A. R. K.) and by the Alcohol Beverage Medical Research Foundation (to H. C. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ These authors contributed equally to this study.

¶ The nucleotide sequence(s) reported in this paper has been submitted to the GenBank††‡ and EMBL Data Bank with accession number(s) AL512366.

* The abbreviations used are: apoB, apolipoprotein B; EST, expressed sequence tag; contig, group of overlapping clones; RT, reverse transcription; SR, serine/arginine-rich; CHO, Chinese hamster ovary; RRm, RNA recognition motif.
Materials and Methods

Genomic Mapping—cDNA sequences for ACF64 (AF209192) and ACF65 (AJ272079) were aligned to the Celera (www.celera.com) human genomic database by BLAST analysis and identified an incomplete 53-kb contig on chromosome 10 (identification number hCT18761). This contig was used to perform a BLAST search of the Public Chromosome 10 data base (www.sanger.ac.uk) and identified the bacterial artificial chromosome (BAC) ba449016, which contains the entire ACF64/ACF65 cDNA sequence. Sequence data reported in this manuscript were produced by the Chromosome 10 Sequencing Group at the Sanger Center (ftp.sanger.ac.uk/pub/human/sequences/Chr_10/unfinished_sequence/)

Southern Blotting—Restriction enzyme-digested human genomic DNA was resolved through 1% agarose and transferred to a nylon (ftp.sanger.ac.uk/pub/human/sequences/Chr_10/unfinished_sequence/)

Sequence Analysis—cDNA sequences for ACF64 (AF209192) and ACF65 (AJ272079) were aligned to the Celera (www.celera.com) human genomic database with this contig identified a single BAC clone (ba449016) that mapped to the identical region but contained all ACF64 and ACF65 cDNA sequences. The exon/intron structure of the ACF gene was determined by pairwise BLAST (www.ncbi.nlm.nih.gov/BLAST) of ba449016 sequence with ACF64/ACF65 cDNA sequences. The ACF gene spans 82 kb and comprises 15 exons (Fig. 1) ranging from 45 to 274 bp (Table I). The introns range from 997 bp to 21.4 kb, and all splice junctions follow the consensus GT-AG motif. The currently described functional domains of ACF are present in separate exons (Table I). ACF65 cDNA differs from ACF64 by insertion of 24 nucleotides at position 1140 with respect to the start codon (9). Significantly, this insertion is identical to the 3′ 24 nucleotides of intron 11, suggesting that ACF65 and ACF64 are alternatively spliced variants of exon 12 (Fig. 1).

A reporter minigene construct containing a 2.3-kb PCR product encompassing exons 11 and 12, and the intron was amplified from the BAC clone (ba449016) and subcloned into pCNAIII V5-His (Invitrogen). To ensure detection of the ACF64/65 spliced variant mRNAs encoded by the transfected minigene, an ACF-specific and a vector-specific primer (T7) were used in the PCR. Expression plasmids for SR proteins have been described (23).

Editing Analyses—ApoB RNA editing efficiency was determined upon RT-PCR-amplified human apoB reporter RNA transcripts by poisons primer extension analysis (24). An ACF64 cDNA was PCR-amplified from a rat liver cDNA library (Stratagene) and subcloned into pcDNAIII V5-V (Invitrogen). ACF65 cDNA was derived from ACF64 by run-around PCR (25).

Promoter Analysis—A 1.8-kb PCR-amplified human genomic DNA fragment containing exon 1 and 2 as well as promoter subfragments were subcloned into pGL3Basic (Promega). The cells were transfected in triplicate in 24-well cluster dishes with 0.5 μg of reporter DNA and 0.2 ng of DNA encoding Renilla luciferase (pRL-CMV; Promega) as a transfection control and harvested after 48 h. Luciferase activity was determined using the dual luciferase assay kit (Promega).

Results

Sequence Analysis—BLAST analyses using cDNAs for ACF64 (AF209192) and ACF65 (AJ272079) of the public and private genomic data bases were performed. The Celera data base contained a 53-kb aligned contig (identification number hCT18761) that mapped to chromosome 10, position 10q11.21 at 47.76–47.85 M but that lacked 135 nucleotides of mRNA sequence. BLAST analysis of the Sanger Center’s unpublished chromosome 10 data base with this contig identified a single BAC clone (ba449016) that mapped to the identical region but contained all ACF64 and ACF65 cDNA sequences. The exon/intron structure of the ACF gene was determined by pairwise BLAST (www.ncbi.nlm.nih.gov/BLAST) of ba449016 sequence with ACF64/ACF65 cDNA sequences. The ACF gene spans 82 kb and comprises 15 exons (Fig. 1) ranging from 45 to 274 bp (Table I). The introns range from 997 bp to 21.4 kb, and all splice junctions follow the consensus GT-AG motif. The currently described functional domains of ACF are present in separate exons (Table I). ACF65 cDNA differs from ACF64 by insertion of 24 nucleotides at position 1140 with respect to the start codon (9). Significantly, this insertion is identical to the 3′ 24 nucleotides of intron 11, suggesting that ACF65 and ACF64 are alternatively spliced variants of exon 12 (Fig. 1).

ACF64 and ACF65 Are Encoded by One Gene—An alternative origin for ACF64 and ACF65 mRNAs would be gene duplication. To investigate this possibility, human genomic DNA was analyzed by Southern blotting using diagnostic restriction sites located within intronic sequences flanking exons 11 and 12 (Fig. 2). The exon 11 probe hybridized to only the predicted 2.6-, 4.3-, and 5.5-kb restriction fragments. Similarly, the exon 12 probe, containing the additional 24 nucleotides, hybridized to only the predicted 5.1- and 4.3-kb fragments and two fragments of 2.35 and 0.8 kb. The lack of hybridization of either probe to other restriction fragments indicated that there is only one gene that encoded both ACF64 and ACF65. Moreover, searches of the public and private human genomic DNA data bases with cDNA or identified genomic sequence failed to identify other loci that encode complete cDNA sequences.

Promoter Analysis—Despite extensive searching of GenBank's human EST data base, no indication of the transcribed sequence upstream of exon 1 was found. To prove that the identified ACF gene is functional and not an inactive pseudogene, the upstream sequence was evaluated for promoter activity. Exon 1 and the −1.73-kb region were subcloned into the promoter assay plasmid, pGL3Basic (Promega). The cells were transfected in triplicate in 24-well cluster dishes with 0.5 μg of reporter DNA and 0.2 ng of DNA encoding Renilla luciferase (pRL-CMV; Promega) as a transfection control and harvested after 48 h. Luciferase activity was determined using the dual luciferase assay kit (Promega).
Alternative Splicing of ACF mRNA

The exons and their sizes, coding capacity, and sequences at their 5' and 3' termini are listed. Nucleotides shown in bold indicate the reading frame of each exon.

<table>
<thead>
<tr>
<th>Intron 3' sequence</th>
<th>Exon termini</th>
<th>Intron 5' sequence and size (bp)</th>
<th>Exon number and size (bp)</th>
<th>Codon at splice junction</th>
<th>Protein domain</th>
</tr>
</thead>
<tbody>
<tr>
<td>cttag</td>
<td>ATTACCA</td>
<td>1/47</td>
<td>Q</td>
<td>5'-Untranslated region</td>
<td></td>
</tr>
<tr>
<td>tcag</td>
<td>ATACAGA</td>
<td>2/48</td>
<td>K</td>
<td>5'-Untranslated region</td>
<td></td>
</tr>
<tr>
<td>ttag</td>
<td>CATGACT</td>
<td>3/93</td>
<td>G</td>
<td>5'-Untranslated region</td>
<td></td>
</tr>
<tr>
<td>aoag</td>
<td>AAATTTT</td>
<td>4/144</td>
<td>None defined</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gaac</td>
<td>GAAATGG</td>
<td>5'/143 Internal STOP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gcag</td>
<td>GAATAAGA</td>
<td>6/135</td>
<td>K</td>
<td>RNP2 of RRM1</td>
<td></td>
</tr>
<tr>
<td>gcag</td>
<td>ATACGGT</td>
<td>7/131</td>
<td>R</td>
<td>RNP1 of RRM1</td>
<td></td>
</tr>
<tr>
<td>gcag</td>
<td>AAAATGGG</td>
<td>8/239</td>
<td>G</td>
<td>RRM2</td>
<td></td>
</tr>
<tr>
<td>accag</td>
<td>GAAGAACG</td>
<td>9/165</td>
<td>G</td>
<td>RNP2 of RRM3</td>
<td></td>
</tr>
<tr>
<td>acag</td>
<td>GTGCTTGT</td>
<td>10/98</td>
<td>K</td>
<td>RNP1 of RRM3</td>
<td></td>
</tr>
<tr>
<td>acag</td>
<td>GTGCTGAG</td>
<td>11/274</td>
<td>G</td>
<td>None defined</td>
<td></td>
</tr>
<tr>
<td>ctag</td>
<td>AAATTTA</td>
<td>12 ASP variant/210</td>
<td>ASP variant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>gttag</td>
<td>GGGGTCG</td>
<td>12 ASP variant/182</td>
<td>Q</td>
<td>ASP variant</td>
<td></td>
</tr>
<tr>
<td>gttag</td>
<td>ATATTAG</td>
<td>13/137</td>
<td>Q</td>
<td>ACS variant</td>
<td></td>
</tr>
<tr>
<td>ccag</td>
<td>CCACCCCT</td>
<td>14/149</td>
<td>I</td>
<td>Double-stranded RNA</td>
<td></td>
</tr>
<tr>
<td>ccag</td>
<td>CAAATGC</td>
<td>15/211</td>
<td>G</td>
<td>None defined</td>
<td></td>
</tr>
<tr>
<td>nyag</td>
<td>G/A-Ac consensus/C/AAG</td>
<td>16/211</td>
<td>None defined</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Alternatively spliced exons located in EST clones only.

Fig. 2. ACF is expressed from a single gene. Shown is a Southern blot of human DNA digested with the indicated restriction enzymes and probed with exon 11- or 12-specific probes. The sizes of the ACF specific restriction fragments are shown in kb.

but in which the exon 1-proximal -330 bp had been deleted. The -330-bp region proximal to exon 1 retained significant promoter activity in both HepG2 and Caco2 cells (Fig. 3B). However, deletion of the upstream 1.4-kb sequence resulted in a 2-fold less active promoter in Caco2 cells (180-fold induction). These data indicate that a 330-bp region proximal to exon 1 retained significant promoter activity in HepG2 cells (1/47), whereas Mehta et al. (8) and others had reported the presence of ACF64 and ACF65 in intestinal and kidney cDNA libraries, respectively. ACF64 cDNAs from intestinal and kidney libraries were detected in primary human hepatocytes, HepG2, hepatoma cells, and Caco2 intestinal cells at an approximate ratio of 4:1 (Fig. 4). These data are the first demonstration that the spliced variants are simultaneously expressed in liver and intestinal cell types.

ACF64 and ACF65 Are Essential Auxiliary Proteins—ACF64 is necessary and sufficient as an auxiliary factor for complementing APOBEC-1 to edit apoB mRNA (8). However, the function of ACF65 has not been determined (9). The eight-amino acid insert (EIYMNVPV) constitutes a tyrosine phosphorylation site (NETPHOS; www.cbs.dtu.dk/services/NetPhos/) that might alter complementation activity and/or subcellular localization of ACF65. CHO cells were transfected with expression vectors encoding epitope-tagged ACF64 or ACF65, together with APOBEC-1 and apoB mRNA expression vectors. CHO cells were selected for these analyses because they do not express detectable levels of ACF by Western blot compared with HepG2 or Caco2 cells (data not shown), nor are they capable of supporting high levels of APOBEC-1-dependent editing in the absence of exogenous ACF64 or ACF65 (4% editing; Fig. 5A). ACF64 and ACF65 increased the editing efficiency of the apoB reporter mRNA 17-fold (Fig. 5A). Furthermore, an equivalent level of promiscuous editing (Fig. 5A, bands labeled 1 and 2) was promoted by both proteins. Western blotting demonstrated that the epitope-tagged ACF64 and ACF65 were expressed at equivalent levels and that the expression of APOBEC-1 was also comparable between all transfectants (Fig. 5B). Editing efficiency is not affected by apoB mRNA abundance (20), and therefore the expression level of the apoB reporter RNA was not determined. The subcellular localization of epitope-tagged ACF65 was determined by immunocytochemistry in editing competent (McArdle RH7777) and incompetent (CHO) cell lines (data not shown) and showed equivalent nuclear and cytoplasmic localization to that of ACF64 (26).

SR Proteins Involved in Alternative Splicing of Exon 12—The SR family of proteins consists of highly conserved serine/arginine-rich RNA-binding proteins that regulate alternative splice site selection (reviewed in Ref. 27). Sequence analysis (21, 22)
Alternative Splicing of ACF mRNA

predicted several high score motifs for the SR proteins SC35, SRp40, SRp55, and SF2/ASF in exon 12 (Fig. 6A). To evaluate whether SR proteins are involved in regulating exon 12 alternative splicing, HepG2 cells were cotransfected with the exon 11–12 minigene (Fig. 6A) and an expression plasmid for each of the four SR proteins. RT-PCR was performed using primers specific for the ACF64/65 spliced variant mRNAs encoded by the transfected minigene. The PCR products differ by only 24 nucleotides; therefore, it is reasonable to assume that each mRNA was amplified with equal efficiency; hence the ratio of the two bands is a good semi-quantitative means of evaluating the level of alternative splicing within each sample (Fig. 6B).

Compared with the vector control, SRp40 had the most significant effect on alternative splicing. Its expression promoted the inclusion of the 24 nucleotides at the 5’ end of exon 12. SRp55 increased the abundance of ACF65 mRNA slightly. SC35 marginally increased ACF64 mRNA. SF2/ASF had no effect on the ratio of ACF65/ACF64. SR proteins affect alternative splicing in vivo in a concentration-dependent manner (27), and thus it is likely that the alteration of the ratio of endogenous SR proteins to mRNA transcript will affect the level of alternative splicing. The reduced ratio of ACF65/ACF64 mRNA expressed from the minigene construct compared with that observed on the endogenous transcript (4:1 ratio; Fig. 4) is likely due, therefore, to overexpression of the ACF reporter RNA.

Other Splice Variants of ACF mRNA—Recently, a deletion variant of ACF (ACFdel55) was reported (28) for which neither a function nor an origin was described. From our described exon structure ACFdel55 lacks exon 9 and therefore likely results from alternative splicing of the ACF primary transcript. To evaluate this possibility, mRNA from primary human hepatocytes, HepG2 cells, and Caco2 cells was analyzed by RT-PCR. Primers specific for exons 8 (E4/H9004) and 10 (ACF/H11032) generated a 292-bp product indicative of exon 9 inclusion and, although at much lower abundance, a product of 127 bp, the expected size for an mRNA in which exon 9 had been skipped (Fig. 7A). The size difference between the two PCR products precludes an accurate determination of their relative abundance, but it is likely that the ACFdel55 variant is very poorly expressed. A primer (5E6) specific for the exon 8/10 junction created in ACFdel55 mRNA together with ACF65WT (specific for the alternatively spliced 24 nucleotides of exon 12) yielded a PCR product of 408 bp (Fig. 7B). The presence of another ACF variant that lacked exon 9 but included the 24 nucleotides at the 5’ end of exon 12 demonstrates that the two identified alternative splicing events of ACF RNA are not mutually exclusive. We propose that the exon 9-skipped ACF64 and ACF65 mRNAs be named ACF58 and ACF59 based upon their theoretical molecular masses.

Human EST data base analyses revealed further ACF splice variants (Fig. 8). Some have an altered exon composition upstream of the ATG initiator codon in exon 4. AV655933 is a unique variant that includes an additional exon (exon 3), whereas AK000324, AV687201, AV698948, AV684607, and AV688806 all lack exon 2. The role of these alternative splicing
Fig. 6. SRp40 is a major determinant of exon 12 alternative splicing. A, schematic of the minigene construct used to analyze exon 12 alternative splicing. Beneath is the partial sequence for exon 12 and calculated high score motifs for the indicated SR proteins. Several other high score motifs for each SR protein were present in the remainder of the exon (data not shown). ▼ indicates the 3′ end of the alternatively spliced 24 nucleotides. B, RT-PCR analysis of minigene-specific transcripts in the presence of the indicated SR protein. The ratio of abundance of ACF65 to ACF64 transcripts from two separate experiments is shown beneath. Lanes labeled + and − are as described in Fig. 4.

Fig. 7. Other splice variants are expressed in liver and intestinal cells. Total RNA from the indicated cell lines was analyzed by RT-PCR with primers specific for exons 8–10 and exon 12. The expected size of the PCR products is indicated. A, a splice variant that includes exon 9 yields a 292-bp product, whereas exon 9 exclusion yields a 127-bp product. B, primers specific for the exon 8/10 junction and the ACF65-specific exon 12 sequence were used to detect the ACF65/α exon 9 splice variant referred to as ACF59. Lanes labeled + and − are reactions with and without reverse transcriptase, respectively.

DISCUSSION

The cloning of APOBEC-1 demonstrated that cytidine to uridine editing of apoB mRNA editing requires a cytidine deaminase whose activity on RNA depends on auxiliary protein factors (4). The inability of APOBEC-1 alone to edit apoB mRNA can be explained, in part, by its low affinity and nonselective binding to the editing site (29). Consequently, multiple auxiliary proteins have been implicated in apoB mRNA editing through their ability to interact with the apoB mRNA editing site and/or APOBEC-1 and in some cases, modulate editing activity (8, 9, 11, 14–16). Recently, ACF was shown to comprise, together with APOBEC-1, the minimal functional editingosome in vitro (8). Simultaneously, two variants referred to as ASP were described, the shorter of which is identical to ACF in primary sequence and function (9). The origin, tissue-specific expression, and/or function of the eight-amino acid-larger variant were unknown.

Under conditions of equivalent APOBEC-1 expression, ACF64 and ACF65 supported editing of apoB RNA equally well. Neither ACF64 nor ACF65 prevented promiscuous editing (Fig. 5A), a phenomenon observed when APOBEC-1 is highly overexpressed (30), suggesting that although these proteins directed the activity of APOBEC-1 to the editing site, they could not limit its activity to a single cytidine. Regulating the relative levels of APOBEC-1 and ACF64/ACF65 may be important to ensure that promiscuous editing does not occur in intestines wherein high levels of editing activity are observed (31). It is possible that an auxiliary protein other than ACF64/ACF65 is necessary to prevent promiscuous editing.

Given the equivalent levels to which ACF64 and ACF65 supported editing and their similar subcellular localization, the function of the alternatively spliced eight amino acids is unclear. Their inclusion creates a likely tyrosine phosphorylation site whose regulation by appropriate protein kinases and/or phosphatases might affect the RNA and/or APOBEC-1 binding affinities of ACF. Given that ACF is expressed in a wide variety of human tissues (8), not all of which express apoB mRNA, a role for ACF in other RNA processing events may be possible, and it is in this role that the functional differences between ACF64 and ACF65 may become apparent.

A search of the public human genome data base for regions encoding ACF64 and ACF65 cDNAs revealed only one chromosomal locus at 10q11.21 capable of encoding the entire cDNA. Southern blotting analysis established that ACF64 and ACF65 mRNAs are transcribed from a single gene on human chromosome 10 and are likely generated through alternative processing of exon 12. Clues as to alternative roles for ACF, alluded to earlier because of its widespread tissue expression, were not apparent from disease markers already mapped to this region.

Identification of the ACF gene was further confirmed by the isolation of a tissue-specific promoter in the 1.4-kb region upstream of exon 1. A promoter highly active in liver and intestinal cells was delimited to the −0.33-kb region. The larger promoter region was twice as active in intestinal cells compared with liver cells but inactive in HeLa cells. This is in contrast to Northern blot data (8, 9) that revealed highest expression of ACF in liver, with some transcripts detectable in kidney, whereas intestinal ACF was detectable only by RT-PCR (8). The explanation for this paradox is unknown, but there may be additional positive acting liver-specific transcription-factor-binding sites upstream of the 1.4-kb fragment. Compared with the 1.4-kb fragment, the −0.33-kb fragment displayed equivalent activity in HepG2 cells but 2-fold less in Caco2 cells, suggesting that the intestinal regulatory elements...
extend over a greater distance than those for hepatic regulation. In this regard, Fig. 3B shows predicted binding sites for TF-LF-1, a liver-specific factor; HNF-3, a liver and intestinally enriched factor; and cAMP-response element-binding protein, which is active in intestine. There are also putative sites for the general transcription factors SP1 and AP-1 and for the CAAT-binding factors CP1/CTF/NF1 and CP2, the identification of which is not unexpected because ACF64 mRNA was widely expressed albeit at low levels (8).

There is no consensus TATA box within the −330-bp promoter region, but the prediction of a weak initiator sequence (CCATAAT) and an A/T poor motif in the −110 to −135 region suggested that this is a TATA-less promoter (32). EST data base and primer extension analysis (data not shown) indicated the utilization of multiple clustered transcription start sites for ACF, a characteristic of many TATA-less promoters. The presence of several liver- and intestine-specific transcription factor-binding sites explains the strength of the promoter in HepG2 and Caco2 cells and its poor use in HeLa cells.

The ACF65 and ACF64 transcripts were expressed at an approximate ratio of 4:1 in human primary hepatocytes, HepG2 cells, and Caco2 cells, suggesting either preferential splicing of ACF65 mRNA and/or differential degradation of ACF64 mRNA. Cotransfection of HepG2 cells with a minigene reporter construct and each of SC35, SRp40, SRp55, and SF2/ASF (22) identified SRp40 as the predominant factor in determining the relative ratio of ACF64 and ACF65, although it remains to be proven that SRp40 determines alternative splicing of exon 12 in liver and intestine in vivo. Similarly, the other SR proteins may be predominant in determining alternative splicing of other exons, e.g. exon 9, in the ACF transcript. Given the presence of high score motifs for each of the four SR proteins in exon 12, it is not clear why only SRp40 had a pronounced effect on alternative splicing. Different SR proteins have distinct abilities to regulate alternative splicing of various mRNAs and regulate the selection of alternative splice sites in a concentration-dependent manner (27). Differences in the abundance of endogenous SR proteins in HepG2 cells, differences in expression levels of the transfected SR protein, or context effects caused by the sequences surrounding each motif may have determined the regulatory effect of SRp40. Given the role of SRp40 in alternative RNA splicing of ACF65, it is of interest that both apoB mRNA editing and SRp40 expression are induced late during liver development (17, 33) and are regulated by insulin (33–35).

We also identified several other ACF alternatively spliced mRNA variants. It is unknown whether they are all translated. Significantly, variants AF271790 and AK000324 include an additional exon (exon 5), which, if translation initiates at the ATG in exon 4, introduces a number of in-frame STOP codons. This would theoretically produce a 55-amino acid protein that may have a dominant negative effect on ACF function or a role in signaling. Alternatively, the mRNAs containing premature stop codons are degraded by nonsense-mediated decay (reviewed in Ref. 36). By analogy, exon 2 skipping in APOBEC-1 pre-mRNA results in a novel 36-amino acid peptide (37), suggesting that alternative splicing of the catalytic subunit and an auxiliary factor may be important mechanisms for regulating apoB mRNA editing (38). Another possibility is the use of an internal start codon in exon 5 that would replace the first 33 amino acids of ACF with 16 amino acids from exon 5 but leave the remainder of the protein identical to ACF. An increase in the 5′-untranslated region from 141 to 343 nucleotides may represent a regulatory mechanism to impose a level of translational control upon ACF expression.

ACF58 and ACF59 mRNAs, by virtue of the skipping of exon 5, lack the RNPF-2 submotif of RRM3. Modulation of the number of RRMs in an RNA-binding protein can result in altered substrate specificity (39), suggesting an altered binding affinity of ACF58/ACF59 for apoB or other mRNAs. The RRM can also direct protein-protein interactions (40). Therefore, deletion of the RNPF-2 submotif, which would likely preclude correct folding of an RRM, could alter the affinity of ACF58/ACF59 for APOBEC-1 and other auxiliary proteins and represent another mechanism for regulating the activity of ACF in apoB mRNA editing.

In conclusion, a single gene on human chromosome 10 has been identified that through multiple and nonexclusive alternative splicing events encodes at least two auxiliary proteins, each with equivalent capacity for APOBEC-1 complementation in apoB mRNA editing. They are expressed in both liver and intestine. Other alternative splicing events may play a role in regulating ACF expression and/or activity and thus be key determinants in the apoB mRNA editing activity. The relation-
Alternative Splicing of ACF mRNA

ship between the different alternative splicing events and ACF function in cell types that do not edit apoB mRNA will be the focus of future studies.

Acknowledgments—We thank Amanda Haefele for human genomic DNA, Mesut Muyan for advice in performing luciferase assays, and Jenny M. L. Smith for the figure preparation.

Note Added in Proof—While this manuscript was under review, another report of the alternative splicing of ACF mRNA was published (Blanc, V., Henderson, J. O., Kennedy, S., and Davidson, N. O. (2001) J. Biol. Chem. 276, 46386–46393).

REFERENCES


Note Added in Proof—While this manuscript was under review, another report of the alternative splicing of ACF mRNA was published (Blanc, V., Henderson, J. O., Kennedy, S., and Davidson, N. O. (2001) J. Biol. Chem. 276, 46386–46393).

Two Proteins Essential for Apolipoprotein B mRNA Editing Are Expressed from a Single Gene through Alternative Splicing
Geoffrey S. C. Dance, Mark P. Sowden, Luca Cartegni, Ellen Cooper, Adrian R. Krainer and Harold C. Smith

doi: 10.1074/jbc.M111337200 originally published online January 28, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M111337200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 25 of which can be accessed free at http://www.jbc.org/content/277/15/12703.full.html#ref-list-1