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Molecular cloning of a human small intestinal apolipoprotein B mRNA editing protein

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ABSTRACT

Mammalian small intestinal apolipoprotein B (apo B) mRNA undergoes posttranscriptional cytidine deamination with the production of an in frame stop codon and the translation of apo B48. We have isolated a cDNA from human jejunum which mediates *in vitro* editing of a synthetic apo B RNA template upon complementation with chicken intestinal S100 extracts. The cDNA specifies a 236 residue protein which is 69% identical to the apo B mRNA editing protein (REPR) cloned from rat small intestine [Teng, B., Burant, C. F. and Davidson, N.O. (1993) *Science* 260, 1816–1819] and which, by analogy, is referred to as HEPR. HEPR does not contain the carboxyl-terminus leucine zipper motif identified in REPR but contains consensus phosphorylation sites as well as the conserved histidine and both cysteine residues identified as a Zn^{2+} binding motif in other cytidine deaminases. The distribution of HEPR mRNA was predominantly confined to the adult small intestine with lower levels detectable by reverse-transcription polymerase chain reaction amplification in the stomach, colon and testis. These differences in the structure and distribution of the human as compared to the rat apo B mRNA editing protein suggest an important evolutionary adaptation in the mechanisms restricting apo B48 production to the small intestine.

INTRODUCTION

Apo B is an essential component of intestinal and hepatic triglyceride-rich lipoproteins and plays a central role in their receptor-mediated uptake (1). Mammalian apo B circulates in two distinct forms (apo B100 and B48), each the product of a single gene (2,3). Apo B48 is produced in the small intestine as a result of post-transcriptional cytidine deamination of codon 2153 in apo B100 mRNA which thereby alters a CAA codon, encoding glutamine, to a UAA or in-frame stop codon (2,3). Unlike mammalian intestinal apo B mRNA, which is essentially all edited, chicken intestinal apo B mRNA is not edited but chicken intestinal extracts were found to contain factor(s) which enhance the ability of mammalian intestinal extracts to edit a synthetic apo B RNA template *in vitro* (4). This finding facilitated the cloning of a rat intestinal apo B mRNA editing protein, REPR, which demonstrates the ability to perform *in vitro* editing of either a rat or human apo B RNA template, but only in the presence of chicken intestinal extracts (5). These findings suggest that REPR, a protein of 27 kD, may operate as part of an editing complex with an obligate requirement for other factor(s), a possibility consistent with previous estimates of the size of the editing activity, judged by gel filtration chromatography, of around 125 kD (6). Among other possibilities, this complementation factor(s) may operate by enhancing binding of the catalytic component of the editing complex and/or mediate site-specific RNA binding and target recognition. Support for this latter hypothesis comes from other studies in which proteins have been identified which can be specifically cross-linked to the apo B RNA region thought to be critical for effective *in vitro* editing (7–9). More recently, it has been established that REPR contains a conserved motif which delineates a zinc-binding region in the active site of other cytidine deaminases and that REPR, moreover, demonstrates cytidine deaminase activity (10). Taken together, the data suggest that REPR is the catalytic component of the rat intestinal apo B mRNA editing complex and support the hypothesis that apo B mRNA editing involves a site-specific cytidine deamination (11).

The molecular mechanisms by which REPR interacts with its substrate, the structural constraints on this process and the nature of the complementation factor(s) are still largely unknown. It was of interest, therefore, to identify and characterize the human homolog of REPR with a view to identifying structural adaptations in the catalytic component of the apo B mRNA editing complex and, ultimately, the molecular mechanisms responsible for apo B mRNA editing. The findings suggest that there is limited conservation of the predicted amino acid sequence of the rat and human editing proteins although the zinc-binding motif recently identified in REPR is conserved in the human homolog. Additionally, while REPR mRNA demonstrated a wide distribution among extraintestinal tissues, mRNA encoding the human homolog (HEPR) appears to be largely confined to the adult small intestine.

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MATERIALS AND METHODS

Isolation of cDNA encoding human small intestinal apo B mRNA editing protein

Samples of normal human adult tissues including small intestine were obtained from the National Disease Research Interchange or during the course of organ donor procurement, as approved by the Institutional Review Board of the University of Chicago Hospitals. Total or polyA+ RNA was prepared as previously described (12) and used for reverse transcription-polymerase chain reaction (RT-PCR) amplification with two degenerate oligonucleotide primers [downstream primer, BFH-5-R 5′-CC-ANAGRTGNGGRTANCGNGGCCARTG-3′ and upstream primer, BFH-3-F 5′-TGGTTYCTNWSNTGGWSNCCNTGY-3′ where R=A or G; N=A,C,G or T; Y=C or T; W=A or T; S=C or G] selected from a region spanning nucleotides 297−563 of the rat intestinal apo B mRNA editing protein (5).

500 ng total RNA from normal human jejunum was annealed to 60 pmoles downstream primer (BFH-5-R) in 14.6 μl rTth buffer (10 mM Tris HCl, pH 8.3, 90 mM KCl) by heating to 75°C for 5 min, cooling to room temperature and incubation at 42°C for 10 min. RT was carried out at 60°C for 15 min in 20 μl final volume rTth buffer supplemented with 1 mM $MnCl_2$, 200 μM dNTP's and 5u rTth (#N808-0097, Perkin-Elmer Cetus). PCR amplification was conducted by adding 8 μl chelating buffer (50% glycerol, 100 mM Tris HCl, pH 8.3, 1 M KCl, 7.5 mM EGTA, 0.5% Tween-20), 6 μl 25 mM $MgCl_2$, 60 pmoles upstream primer (BFH-3-F) and water to a final volume of 100 μl. 40 cycles of PCR were conducted with denaturation for 30 seconds at 95°C, annealing at 45°C for 1 min and extension at 65°C for 1.5 min. A single PCR product of 267 bp was detected by agarose electrophoresis and cloned directly into pCRII (InVitrogen, San Diego, CA). Three independent clones were sequenced on both strands as previously described (4,5). Using the sequence so obtained, specific primers were then designed for selective amplification of the 5′ and 3′ ends of the cDNA with overlap at a convenient restriction site (see below).

The 5′ end of the human cDNA was obtained using 2 μg poly A+ RNA from human jejunum which was reverse transcribed at 42°C for 30 min using 12.5 U avian myeloblastosis virus reverse transcriptase and 10 μM downstream (specific) primer (DSP1) 5′-TCCTCCAGCAGTGATAATACTCTG-3′ (13). A modified olgonucleotide anchor 5′P-CACGAATTCACTATCG-ATTCTGGAACCTTCAGAGG-NH_3-3′(#K1800-1,Clontech, CA) was then ligated to the single stranded cDNA with 10 units T4 RNA ligase (12) at 22°C for 20h as described by the supplier (Clontech). PCR amplification was conducted using an anchor primer 5′-CTGGTTCGGCCCACCTCTGAAGGTTCCAG-AATCGATAG-3′ (Clontech) and a nested antisense (specific) primer (DSP2) 5′-GCTCGAGGAGCTACGTAGATCACTA-GAGTCA-3′. PCR was conducted using Hot Tub DNA polymerase (Amersham, Arlington Heights, IL) with 35 cycles of 45 seconds denaturation at 94°C, 45 seconds annealing at 60°C and 2 minutes extension at 72°C with a final 7 minute extension after the last cycle. A single 374 bp PCR product was cloned into pCRII yielding 8 clones with inserts of which 3 were sequenced on both strands and shown to have the identical sequence.

The 3′ end of the cDNA was independently obtained (14) using 2 μg poly A+ RNA from human jejunum to generate first strand cDNA synthesis which was primed using an adaptor primer 5′-GGCCACGCGTCGACTAGTAC-$(dT)_{17}$ (GIBCO-BRL, Gaithersburg, MD). PCR amplification used an upstream (specific) primer (USP1) 5′-AGTCGACGTGTGACTCTAGTG-ATCTACG-3′ and a downstream primer 5′-CUACUACUA-CUAGGCCACGCGTCGACTAGTAC-3′. PCR conditions (30 cycles) were: denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute and extension at 72°C for 1.5 minutes with a final extension time of 15 minutes. A single 537 bp PCR product was cloned into pCRII yielding 13 clones with inserts of which 5 were sequenced on both strands and found to have the identical sequence.

Both plasmids were digested with BamHI and SnaBI and a 410 bp fragment (369 bp of 5′ cDNA and 41 bp of polylinker) removed from the 5′ cDNA clone and ligated into the BamHI−SnaBI site of the 3′ cDNA to yield the full length cDNA clone, pHEPR.

In vitro transcription/translation of HEPR

pHEPR was linearized in both sense and antisense orientations, transcribed and capped using T7 or SP6 RNA polymerase and the purified RNA dissolved in water. 600 ng of RNA was used in a 30 μl (final volume) *in vitro* translation reaction with nuclease-treated rabbit reticulocyte lysate (Gibco-BRL) as recommended by the manufacturer. Following *in vitro* translation, aliquots (5−10 μl) of the lysate were incubated in an *in vitro* editing assay using either rat or human synthetic apo B RNA templates in the presence of 20 μg of either chicken intestine or human liver S100 extracts (15), as indicated in the legend to Figure 4, and the products quantitated by primer extension (4,5). Separate aliquots of HEPR-RNA were translated *in vitro* using a methionine-deficient rabbit reticulocyte lysate in the presence of ^{35}S-methionine and the products resolved by denaturing SDS-PAGE and detected by fluorography (5).

Detection of HEPR mRNA

Human fetal and adult RNA samples (20 μg) were fractionated through formaldehyde-agarose gels and transferred to nylon membranes, as previously described (12) and probed with a ^{32}P-labeled 372 bp fragment corresponding to nucleotides 111−483 of pHEPR. Hybridization and washing stringencies were as previously described with the final wash at 42°C in 0.1×SSC/0.1%SDS (12). The blots were exposed to XAR film for 6 days at −80°C with intensifying screens and reprobed without stripping with a ^{32}P-labeled human glyceraldehyde-3-phosphate-dehydrogenase cDNA (ATCC #57090) under identical stringency.

Reverse transcription polymerase chain reaction (RT-PCR) amplification of HEPR

Aliquots of total RNA (2 to 20 μg) were treated with 0.5 units of DNAse RQ1 (Promega, WI), sequentially extracted with phenol-chloroform and chloroform, precipitated with ethanol and resuspended in H_2O. As an additional control for genomic DNA contamination, primers for both HEPR (Funahashi, T, Davidson, NO, unpublished observations) and $\beta_2$microglobulin (16), the latter used as a housekeeping control, were chosen to flank introns, resulting in cDNA products of 373 and 201 base pairs, respectively. HEPR and $\beta_2$microglobulin mRNA were reverse-transcribed and amplified in identical but separate tubes (16). RT-PCR was performed with 500 ng total RNA using rTth, as recently described (16). Reverse transcription was performed at 65°C for 15 minutes in a final volume of 20 μl RT buffer (see above), containing 60 pmoles reverse primer (see below) and

5u rTth. Following reverse transcription, 80 μl (final volume) of PCR buffer (see above) was added to each tube, containing 8 μl chelating buffer, 6 μl 25 mM $MgCl_2$ (1.5 mM final concentration), 60 pmoles forward primer in 66 μl of H_2O together with 0.3 μl ^{32}P dCTP (3000 Ci/mmol, 10 mCi/ml, Dupont). After 3 minutes at 95°C, PCR was performed for 20 ($\beta_2$microglobulin), 26 or 30 cycles (HEPR), as detailed in the results and legend to Figure 3, as follows: 30 seconds at 95°C, 1 minute at 55°C, 1.5 minutes at 72°C. A final 10 minute extension was added after the last cycle. Separate, negative controls for HEPR and $b_2$microglobulin were run to check for DNA contamination using rTth and leaving the tubes on ice during reverse transcription (RT−). 15 μl of the reaction material was analyzed by 2% agarose-1% NuSieve gel electrophoresis and bands of the expected size corresponding to HEPR and $\beta_2$microglobulin cDNA products excised and subjected to liquid scintillation counting (Packard 1500 LS, Downers Grove, IL). Negative controls were run in parallel and a gel slice corresponding to the size of the expected product excised and counted to check for DNA contamination. Background radioactivity was assessed by counting the adjacent upper and lower regions of the gel relative to the excised band and subtracting the averaged counts from those found in the positive band.

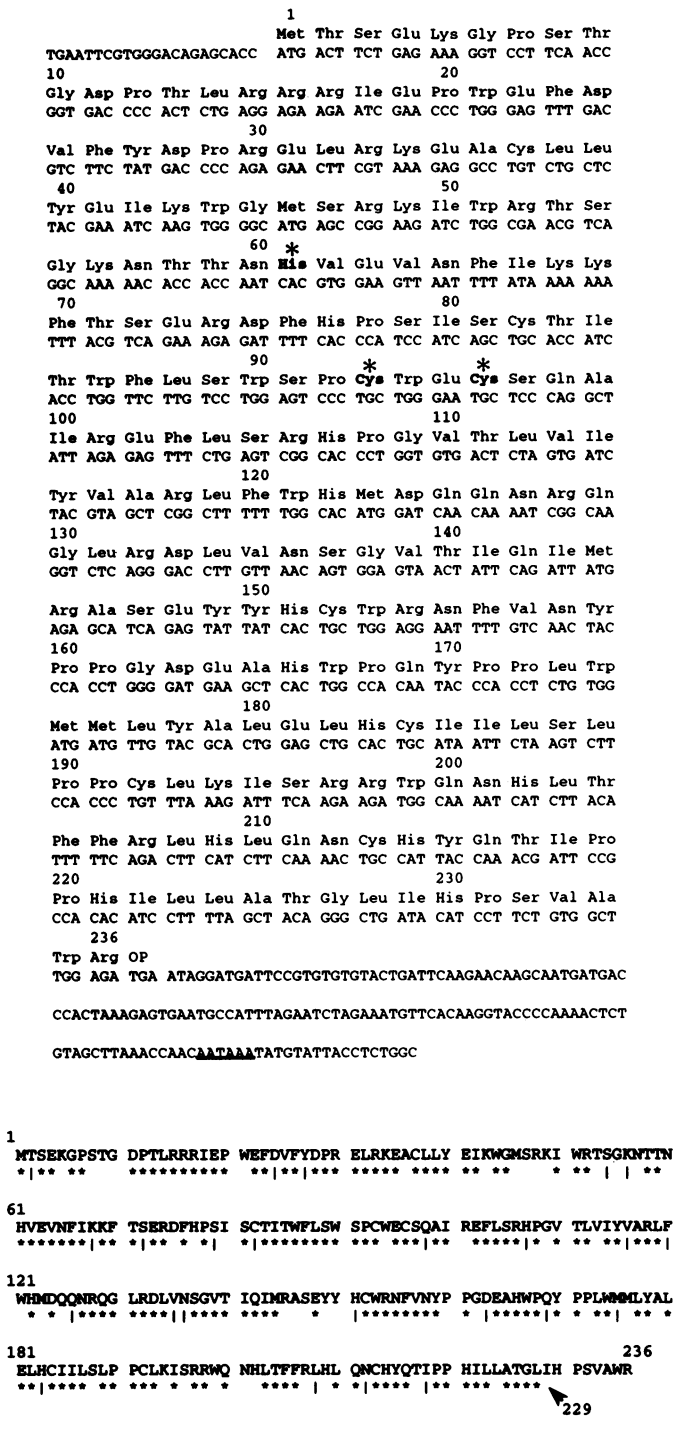

Figure 1. Upper panel. Nucleotide and deduced amino acid sequence of HEPR. The conserved His^{61}, Cys^{93} and Cys^{96} are indicated in bold type and highlighted with an asterisk. The polyadenylation signal is underlined. Lower panel. Aligned amino acid sequence of HEPR and REPR. The single letter code is used, with identity indicated by an asterisk and similarity by a vertical line.

Oligonucleotides

HE1-F (5′-GAGAACTTCGTAAAGAGGCC-3′, 5′ at 89*)
HE2-R (5′-CTCCAGCAGTGATAATACTC-3′, 5′ at 461*)
[*considered from the first codon].
hβ_2m/F (5′-GTGGAGCATTCAGACTTGTCTTTCAGCA-3′, 5′ at 1477).
hβ_2m/R(5′-TTCACTCAATCCAAATGCGGCATCTTC-3′, 5′ at 3537).

RESULTS

Cloning of a human intestinal apo B mRNA editing protein

Using degenerate oligonucleotides from a region of the rat apo B RNA editing protein (REPR), a fragment of the human homolog was obtained which showed 75% nucleotide homology

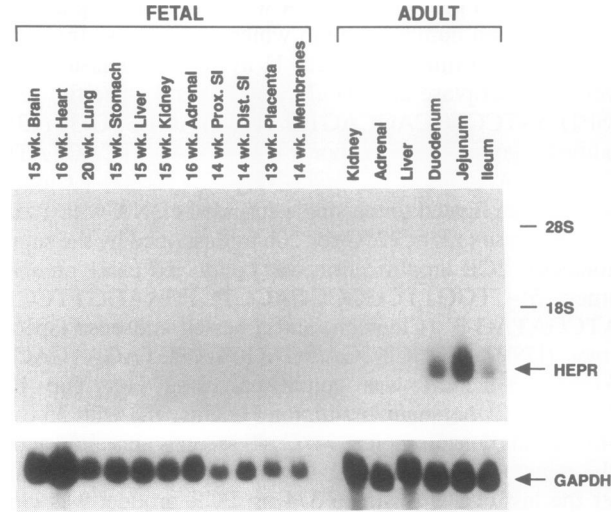

Figure 2. Tissue distribution of HEPR mRNA. 20 μg total RNA from the indicated sources of fetal or adult tissue were separated by formaldehyde-agarose electrophoresis and transferred to nylon membranes for sequential hybridization with either a [^{32}P] labelled HEPR cDNA probe or a constitutive marker (GAPDH) as described in Methods. The blots were exposed for 6 days and 18h, respectively and the mobility of 28S and 18S rRNA is shown.

to REPR. The 5′ and 3′ ends of the human cDNA were subsequently obtained using sequence-specific primers and anchor-PCR. The DNA and deduced amino acid sequence are shown in Figure 1 (upper panel). The nucleotide sequence is 879 bp with a single open reading frame encoding a protein (HEPR) of 236 amino acids with a calculated molecular mass of 28206 daltons. *In vitro* transcription/translation of the full length HEPR cDNA demonstrated a protein of approximately 27 kD in contrast to a smaller species (25kD) translated from REPR cDNA (5), findings consistent with the relative predicted size of the human and rat apo B mRNA editing proteins (data not shown). The 3′ untranslated region contains a consensus polyadenylation signal at nucleotides 858−863. The nucleotide sequence demonstrates 76% overall identity to the rat (5). A search of the National Center for Biotechnology Information peptide database showed only one protein with significant sequence similarity, namely REPR, which demonstrated 69% amino acid identity and 79% similarity (Figure 1, lower panel) (5). HEPR was found to have a consensus N-glycosylation site (residue 57), phosphorylation sites for cAMP-dependent kinase (residue 68), casein kinase (residues 8 and 72) and protein kinase C (residues 3, 13, 47, 54, 72 and 196). The functional importance of these phosphorylation sites, however, remains to be determined experimentally in both REPR and HEPR. Of significance, however, as demonstrated in figure 1, HEPR contains the zinc-binding motif His^{61}, Cys^{93}, Cys^{96} previously identified in the active site of other cytidine deaminases including REPR (10). Thus it is likely that HEPR is a member of this family. By contrast, while the carboxyl-terminus of REPR (residues 182−210) was found to contain a series of leucine residues in a heptad repeat suggestive of a leucine-zipper, this region was only 65% identical in HEPR and did not contain the same pattern of heptad leucine repeats.

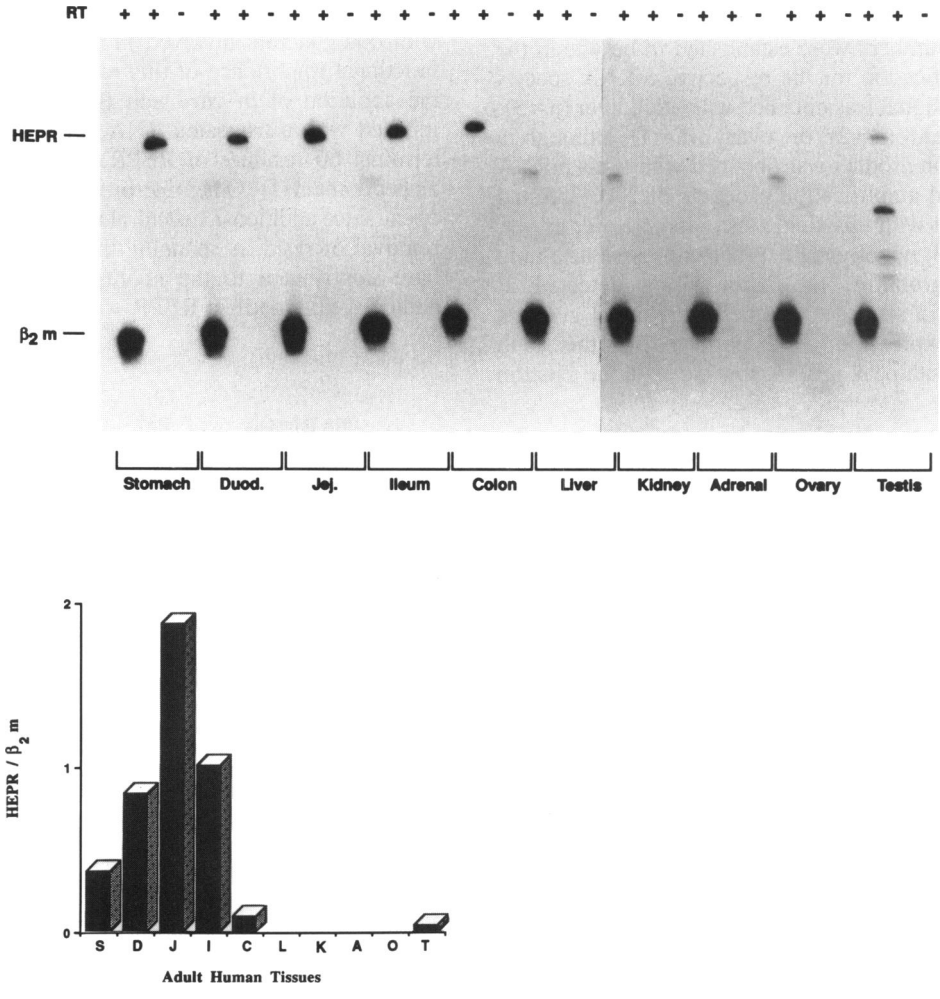

Figure 3. Reverse transcription polymerase chain reaction (RT-PCR) amplification of HEPR from multiple human tissues. Upper panel: 500 ng total RNA was subjected to RT-PCR using primers specific for either HEPR or β_2 microglobulin (β_2m) as described above (methods). 20 cycles of PCR were used for β_2m in all samples. For HEPR, 30 cycles of PCR were used for all tissues except duodenum (duod), jejunum (jej) and ileum, where, for ease of visual presentation, 26 cycles were used. Control reactions with each primer pair were conducted without reverse transcription (RT−) and, for ease of presentation, aliquots of the reaction products were combined into a single lane for analysis in parallel with RT+ samples using 5% nondenaturing acrylamide electrophoresis. The additional bands present in the testis sample following amplification of HEPR are unidentified. Lower panel: For the accurate quantitation of HEPR mRNA abundance, bands corresponding to HEPR (373 bp) and β_2m (201 bp) were identified following 30 cycles of PCR for HEPR (all tissues) and 20 cycles for β_2m.The ratio of HEPR/β_2m was determined by scintillation counting of the excised bands from a 2% agarose-1% NuSieve gel, and the average results from two representative assays illustrated. S= stomach [0.37], D= duodenum [0.84], J= jejunum [1.87], I= ileum [1.01], C= colon [0.1], L= liver [0], K= kidney [0], A= adrenal [0], O= ovary [0], T= testis [0.04].

Tissue distribution of human apo B mRNA editing protein (HEPR)

RNA blots prepared from a variety of human fetal and adult tissues demonstrated a single transcript detectable only in the adult human small intestine (Fig.2). At this level of sensitivity, HEPR transcript abundance appeared greater in the jejunum than in either duodenum or ileum, as evidenced by the comparable hybridization signal with GAPDH, suggesting the possibility that HEPR mRNA abundance may be regionally heterogeneous within the adult small intestine. Extending the sensitivity of the methodology by means of RT-PCR, HEPR was detectable in adult stomach and colon in addition to the small intestine (Fig.3, upper panel). It should be noted that, for the purposes of presentation, PCR amplification of the small intestinal RNA samples was restricted to 26 cycles (versus 30 cycles for other tissues) so as to produce amplification signals of comparable intensity (Fig.3, upper panel). Quantitative analysis of HEPR mRNA abundance was conducted with 30 cycles of PCR for all tissues and was normalized to the amplification product obtained with 20 cycles of PCR for β_2 microglobulin (Fig.3, lower panel). These cycle numbers were established to be within the linear range of amplification for the respective mRNA species (data not shown). No signal was obtained with adult liver (n=3), kidney (n=2), adrenal (n=2) or ovary (n=1) although a detectable amplification product was obtained with testis mRNA (n=1). The additional amplification products of 290, 255 and 235 base pairs evident with this single testis sample are, as yet, uncharacterized (Fig.3, upper panel). When expressed as a ratio of HEPR/β_2 microglobulin, the latter being detected at comparable intensity in all tissues studied, a regional gradient of HEPR mRNA expression was confirmed in the adult gastrointestinal tract with peak mRNA abundance in the jejunum (Fig.3, lower panel).

In vitro editing of a synthetic apo B RNA template

HEPR cDNA was transcribed and translated *in vitro* and aliquots of the translation reaction used in an *in vitro* apo B RNA editing assay (Fig.4). The data show that HEPR will edit a synthetic apo B RNA template, prepared from either rat or human apo B cDNA, when complemented by chicken intestinal S100 extracts. The activity of HEPR is comparable to that of REPR studied under the same conditions with differences in the extent of conversion noted with different *in vitro* translation reactions (Fig.4). S100 extracts prepared from human liver also complemented the ability of HEPR to edit an apo B RNA template, although the editing activity of this mixture was lower (~2% UAA) than in mixtures supplemented with chicken intestinal S100 extracts (3–37% UAA), (Fig.4). The ability of HEPR to edit an apo B RNA template varied in a dose-dependent manner but was strictly dependent upon the presence of a source of complementation activity, either chicken intestine or human liver S100 extracts (Fig.4). No editing was observed in reaction mixtures lacking a source of complementation activity (data not shown).

DISCUSSION

Little is currently known about the molecular mechanisms underlying the tissue- and species-specific regulation of apo B mRNA editing. The present report, in addressing some of these issues, demonstrates several important differences between the rat apo B mRNA editing protein and the human homolog. First, the overall species conservation between both the cDNA and predicted amino acid sequence is less than 70%. This is in contrast to the nucleotide conservation flanking the edited base in apo B mRNA where there is only one difference in 29 nucleotides between the rat and human sequence (4). However, the conservation of the zinc-binding residues which coordinate to the active site of other cytidine deaminases (10) suggests that the active site of REPR and HEPR are likely to be similar. Recent studies have demonstrated that zinc chelation irreversibly abolishes the ability of rat intestinal S100 extracts to edit a synthetic apo B RNA template, suggesting that REPR indeed functions as a zinc-dependent cytidine deaminase (10). A more rigorous analysis of the nature and number of zinc atoms within REPR and HEPR and the precise functional correlates of zinc occupancy will await the production of recombinant bacterial protein for such examinations.

A second distinguishing feature of HEPR was the absence of a carboxyl terminal leucine zipper, previously identified in REPR and postulated to be important in mediating potential interactions with other factors involved in apo B mRNA editing (5). The functional importance of this region was established following the abolition of *in vitro* apo B mRNA editing from oocytes injected with a truncated RNA which eliminated the carboxy-terminal 60 residues of REPR, including the putative leucine zipper domain (5). Other interpretations of this experiment remain open, since additional structural alterations to REPR, beyond the removal of residues spanning the putative leucine zipper, may have contributed to the abolition of editing activity (5). In addition, this motif in REPR would be considered atypical for

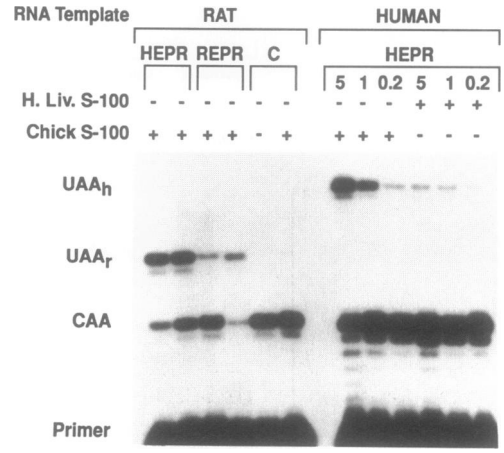

Figure 4. *In vitro* editing of a synthetic apo B RNA template with HEPR. Coupled *in vitro* transcription/translation reactions using cDNA encoding either HEPR, REPR or nuclease-treated rabbit reticulocyte lysate alone (C) were used in an *in vitro* apo B RNA editing assay with either human or rat apo B RNA templates. Left panel: Aliquots (5–10 μl) of the translation mixture were incubated in the presence of 20 μg of chicken intestine S100 extract using a rat apo B RNA template. Right panel: Aliquots (5,1 or 0.2 μl) were incubated in the presence of either 20 μg chicken intestine or human liver S100 extract using a human apo B RNA template. Following incubation, RNA was extracted and the extent of apo B RNA editing determined by primer extension. The mobility of the primer, unedited (CAA) and edited (UAAr or UAAh) RNA species are indicated. Values for % UAA were [left panel] 76,52 (HEPR); 12,46 (REPR); 0,0 (C). Values for % UAA in the right panel were 37, 20, 3, 2, 2, 0. This experiment is a representative of three independent preparations assayed separately.

a leucine zipper by virtue of the presence of proline residues and the overall absence of alpha helicity (17). Thus, the demonstration that leucine periodicity in the homologous region of HEPR is not conserved leaves open to speculation the function of this region in REPR. Nevertheless, a fundamental objective to emerge from the current study is the identification of regions in both REPR and HEPR which are important in mediating interaction with the requisite complementation factor(s). It has been proposed that the zinc-binding regions of other regulatory proteins may be involved in protein−protein interactions and one testable hypothesis to emerge from the present studies will be to determine whether a similar function may reside in this region of REPR and HEPR (18,19).

A further distinguishing feature to emerge between REPR and HEPR concerns the tissue distribution of these gene products. As assessed by Northern blotting, REPR mRNA was demonstrated as a variable sized transcript in several tissues in addition to the small intestine and liver, including spleen, lung, kidney and colon (5). The present results, using methodology of comparable sensitivity to this earlier report, demonstrates the presence of a single transcript detectable only in adult small intestine. When examined using RT-PCR, HEPR mRNA was detectable, albeit at lower levels than found in the small intestine, in the stomach, colon and testis. Previous studies demonstrated the presence of low levels, as determined by RT-PCR, of apo B mRNA in both fetal and adult human stomach and colon, a substantial portion of which was edited, leading to the suggestion that the editing machinery was widely distributed (12). By contrast, despite the concomitant demonstration of low levels of edited apo B mRNA in both fetal and adult kidney, no amplification product for HEPR was detectable in either of two independent adult kidney RNA samples. This apparent discrepancy suggests either that HEPR is present at extremely low levels in kidney or that other factor(s) mediate apo B mRNA editing in this location. The demonstration that HEPR mRNA is most abundantly expressed in the adult small intestine is generally consistent with the previous finding that human fetal small intestinal apo B mRNA editing is developmentally regulated (12). The temporal and cell-specific pattern of HEPR expression in the developing human fetal small intestine is currently under investigation. Such information will be important in resolving the precise role of HEPR versus complementation factor(s) in regulating the developmental profile of small intestinal apo B mRNA editing. Immunocytochemical analysis of HEPR distribution within the small intestinal enterocyte will also be of interest in view of studies demonstrating that apo B mRNA editing in rat hepatocytes is an intranuclear event (20), although considerable evidence exists for shuttling of proteins between the nucleus and cytoplasm (21).

HEPR mRNA was not detectable by RT-PCR in three separate samples of adult human liver, suggesting a plausible explanation for the absence of apo B mRNA editing in this location. Preliminary studies indicated that S100 extracts prepared from HepG2 cells, when mixed with oocyte homogenates expressing REPR, were competent to edit a synthetic apo B RNA template (5). This result suggested that HepG2 extracts acquire editing competence through complementation with REPR. Support for this hypothesis has been recently obtained in experiments in which normal adult and fetal human liver S100 extracts demonstrated complementation activity for apo B RNA editing activity when mixed with REPR (15). Furthermore, HepG2 cells, transfected with REPR, demonstrated endogenous apo B mRNA editing and secreted apo B48 (15). Taken together, the evidence points to an absence of HEPR from human liver as a major component of the observation that this tissue does not edit endogenous apo B mRNA and fails to synthesize and secrete apo B48. Extending this suggestion, it is tempting to speculate that the targeted introduction of HEPR into liver cells may be a feasible alternative to current strategies of gene therapy for hypercholesterolemia, since the production of apo B48 by the human liver would obviate the requirement for LDL receptor expression as a means to effect clearance of hepatic lipoproteins.

The evolutionary advantage underlying the differences in the structure and distribution of REPR and HEPR are unknown. Among other possibilities, these differences may reflect alterations in the distribution and composition of the complementation factor(s) between the rat and human small intestine thereby imposing structural constraints on the orientation of the catalytic component of the editing complex for optimal alignment with the substrate. Study of the promoter elements which operate to restrict the expression of HEPR to the adult small intestine may help resolve some of these complexities and these as well as other issues will be the focus of future reports.

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