Human RNA-specific adenosine deaminase \textit{ADAR1} transcripts possess alternative exon 1 structures that initiate from different promoters, one constitutively active and the other interferon inducible

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\textbf{ABSTRACT} RNA-specific adenosine deaminase (ADAR1) catalyzes the deamination of adenosine to inosine in viral and cellular RNAs. Two size forms of the ADAR1 editing enzyme are known, an IFN-inducible 150-kDa protein and a constitutively expressed N-terminally truncated 110-kDa protein. We have now identified alternative exon 1 structures of human \textit{ADAR1} transcripts that initiate from unique promoters, one constitutively expressed and the other IFN inducible. Cloning and sequence analyses of 5'-rapid amplification of cdNA ends (RACE) cDNAs from human placenta established a linkage between exon 2 of \textit{ADAR1} and two alternative exon 1 structures, designated herein as exon 1A and exon 1B. Analysis of RNA isolated from untreated and IFN-treated human amnion cells demonstrated that exon 1B–exon 2 transcripts were synthesized in the absence of IFN and were not significantly altered in amount by IFN treatment. By contrast, exon 1A–exon 2 transcripts were IFN inducible. Transient transfection analysis with reporter constructs led to the identification of two functional promoters, designated P\textsubscript{C} and P\textsubscript{I}. Exon 1B transcripts were initiated from the P\textsubscript{C} promoter whose activity in transient transfection reporter assays was not increased by IFN treatment. The 107-nt exon 1B mapped 14.5 kb upstream of exon 2. The 201-nt exon 1A that mapped 5.4 kb upstream of exon 2 was initiated from the interferon-inducible P\textsubscript{I} promoter. These results suggest that two promoters, one IFN inducible and the other not, initiate transcription of the \textit{ADAR1} gene, and that alternative splicing of unique exon 1 structures to a common exon 2 junction generates RNA transcripts with the deduced coding capacity for either the constitutively expressed 110-kDa \textit{ADAR1} protein (exon 1B) or the interferon-induced 150-kDa \textit{ADAR1} protein (exon 1A).

RNA editing represents an important posttranscriptional process by which transcripts are covalently modified in a manner that has the potential to alter the coding capacity of the RNA and thus the function of the encoded product (1, 2). RNA-specific adenosine deaminase (ADAR) represents one such type of RNA editing enzyme. ADAR catalyzes the C-6 deamination of adenosine to generate inosine at sites within double-stranded structures present within cellular pre-mRNAs and viral RNAs as well as synthetic double-stranded (ds)RNA substrates (1–4).

Two classes of adenosine-to-inosine (A-to-I) editing processes have been defined in naturally occurring RNA substrates of ADAR. First, highly site-specific A-to-I modifications are found at one or a few sites in cellular RNA transcripts that encode receptors for two important neurotransmitters in the vertebrate central nervous system, glutamate and serotonin. A total of eight adenosine positions so far have been identified that undergo A-to-I editing in five different glutamate receptor (GluR) subunit mRNAs (2, 3). These editing events result in changed RNA coding and subsequently altered biophysical properties and physiological functions of the GluR proteins. The serotonin-2C receptor (5-HT\textsubscript{2C}-R), one of the three 5-HT\textsubscript{2} subtype receptors linked to phospholipase C via G-protein coupling, also is regulated by RNA editing (2). A-to-I editing at four sites within the third exon of 5-HT\textsubscript{2C}-R results in three amino acid substitutions that cause a 10- to 15-fold reduction in G-protein-mediated signaling (5). Second, multiple clustered modifications characteristic of adenosine deamination are found in viral RNAs. Such hypermutation deamination has most often been described in negative-stranded RNA virus genomes during lytic and persistent infections, as exemplified by measles virus RNA (2, 6), but extensive adenosine modifications are also present on the viral antiviral RNA late in polyoma virus infection (7).

Protein purification and molecular cloning studies established that the ADAR enzymes constitute a multigene family of enzymes (8). So far, cDNAs for functional deaminases encoded by two human ADAR genes, \textit{ADAR1} and \textit{ADAR2}, have been characterized (2, 3, 8). We isolated the \textit{ADAR1} cDNA as an interferon-inducible gene (9). The \textit{ADAR1} cDNA predicts a 1,226 amino acid protein possessing in the central region three functionally distinct copies of the highly conserved dsRNA-binding domain (dsRBD), designated as R\textsubscript{I}, R\textsubscript{II}, and R\textsubscript{III}; these dsRBD copies are implicated in the recognition of dsRNA structures within the substrate RNAs (9–11). A repeated domain present in the N-terminal region of \textit{ADAR1} homologous to the N-terminal region of the vaccinia virus E3L protein (9) corresponds to two Z-DNA binding domains of \textit{ADAR1}, designated Z\textalpha and Z\beta (12).

Two immunologically related forms of the human \textit{ADAR1} deaminase were demonstrated in a variety of human cell lines by Western immunoblot and immunofluorescence microscopy analyses, by using antisera prepared against three nonoverlapping regions of the human cDNA expressed in \textit{Escherichia coli} (9, 13). One, an interferon-inducible 150-kDa protein as estimated by SDS/PAGE, is present in both the cytoplasm and nucleus of human cells.

Abbreviations: ADAR1, the RNA-specific adenosine deaminase; dsRNA, double-stranded RNA; UTR, untranslated region; ISRE, IFN-stimulated response element; RACE, rapid amplification of cdNA ends; RT-PCR, reverse transcription—PCR; CAT, chloramphenicol acetyltransferase. Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos. AF084516–AF084518). To whom reprint requests should be addressed. e-mail: Samuel@lifesci.ucsb.edu.

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nucleus of IFN-treated human SY5Y and U cell lines. The other, a constitutively expressed ~110-kDa protein by SDS/PAGE, is present in comparable amounts in untreated and IFN-treated cells and is found predominantly if not exclusively in the nucleus. The natural ~150-kDa protein appears indistinguishable from the full-length recombinant protein produced from Met1 of the 1,226 ORF in transfected COS cells, and the natural ~110-kDa protein displays similar properties on SDS/PAGE to a truncated recombinant protein initiated from the second methionine, Met-296, of the 1,226 ORF (9, 14). The full-length and truncated recombinant ADAR1 proteins display similar deaminase activity when measured with synthetic dsRNA substrates (14). The proteins display similar deaminase activity when measured with synthetic dsRNA substrates (14). The ADAR1 gene encoding these proteins maps to human chromosome 1q21.1-21.2 (15).

Little is known regarding the regulation of the ADAR1 gene expression. A single ~6.7-kb RNA is the only major ADAR1 mRNA detected in cell lines and various organs, with hybridization probes corresponding to different regions of the 15-exon ADAR1 cDNA (9–11, 16). Although the level of ADAR1 transcript is increased by treatment with both IFN-α and IFN-γ (9, 13, 17), a significant basal level of ADAR1 transcript is observed in human cell lines and organs in the absence of IFN treatment (9–11). Because of the important role that ADAR1 plays in the editing of cellular neurotransmitter receptor pre-mRNAs in the apparent absence of IFN treatment (2, 18), the ID-1 protein was coupled with the unresolved origin of the constitutively expressed ~110-kDa form of ADAR1 protein ubiquitously observed at high levels in the nucleus of animal cells in the absence of IFN treatment (9, 20), and because of the potential role of the IFN-inducible ~150-kDa form of ADAR1 in the antiviral actions of IFN (9, 21), we have attempted to define the elements responsible for ADAR1 transcriptional control.

As a step toward this goal, we isolated and characterized genomic clones of the human ADAR1 gene (14, 15). The ADAR1 gene spans about 30 kilobases and consists of 15 exons (14). Characterization of the 5′-flanking region immediately adjacent to the exon 1 that contains Met1 of the ADAR1 cDNA (herein designated as exon 1A) led to the identification of an IFN-inducible ADAR1 promoter (P) that possesses an IFN-stimulated response element (ISRE) responsible for IFN inducibility (16). We now have identified an alternative exon 1 structure of the human ADAR1 transcript, designated as exon 1B, that initiates from a second promoter not regulated by IFN, designated Pβ. These results reveal a surprisingly complex organization of the ADAR1 gene and provide insights regarding the regulation of expression of ADAR1 in human cells.

**EXPERIMENTAL PROCEDURES**

**ADAR1 Promoter Cloning.** Two kinds of libraries, a λ-phage library and a PI-phage library, were screened to obtain genomic clones of human ADAR1 (14). Clone 176 was isolated from a genomic library in the λ phage vector EMBL-3 SP6/T7 prepared from human placenta DNA (CLONTECH); clones P1–249, P1–652, and P1–959 were isolated from a genomic library in the PI phage vector pAD10SacBII vector prepared from human foreskin fibroblast DNA (22). Genomic inserts were characterized by restriction mapping and Southern blot analysis (14, 23, 24). Restriction fragments of genomic clones were subcloned into the plBluescript SK plasmid (Stratagene) according to the manufacturer’s recommendation.

**Southern Gel-Blot Analysis.** Southern gel-blots were performed as described (28, 29). RNA isolated from U cells either untreated or IFN-treated by using the TRI-Reagent (Molecular Research Center, Cincinnati) according to the manufacturer’s recommendation was reverse transcribed by using random hexamer oligonucleotides as the primer and Moloney murine leukemia virus reverse transcriptase (New England Biolabs) at 37°C. PCR reactions (29) were performed by using native Taq DNA polymerase (Fisher). The random-primed U cell cDNA was amplified with the following two primer pairs: exon 1A plus 170 (nucleotides 170–193), 5′-ATAATGCTCGGGGGCGGCGCAATGAATC-3′; exon 2 minus 273 (nucleotides 290–273); exon 1B plus 12 (nucleotides 12–27), 5′-GAGAAGGCTACGTGGTGG-3′; and exon 2 minus 273 (nucleotides 290–273). Exon 1A and 1B numbering is as in Fig. 1.

**Northern Gel-Blot Analysis.** Northern gel-blot analysis of total RNA isolated from human amnion U cells was performed as described previously (9, 13, 16).

**Construction of Reporter Gene Plasmids.** The pCAT-Basic promoter-less plasmid (Promega) containing the chloramphenicol acetyltransferase (CAT) gene was used for construction of the reporter gene plasmids for analysis of the ADAR1 gene promoter function following standard cloning procedures (23) as previously described (16, 24, 30). Deletions (Fig. 5)

**FIG. 1.** Nucleotide sequence of the ADAR1 5′-RACE-derived cDNAs and comparison to corresponding genomic DNA sequences. Sequences of the two unique 5′-RACE-derived cDNAs are designated 5′-RACE-1A and 5′-RACE-1B. The previously described exon 1 (14) corresponds to the 5′-RACE-1A sequence, with the ATG translation initiation codon in bold font. The newly identified exon 1 corresponds to the 5′-RACE-1B sequence. The bold sequence shown by the lower lines corresponds to the 5′ portion of exon 2; the position of the exon 2 nested minus primer at nucleotides 48 to 67 is indicated by the underlined sequence. Nucleotide numbering begins with 1 at the 5′ end of each exon. Genomic DNA sequence was obtained from a subclone of the 176 genomic clone for exon 1A and exon 2, and from a subclone of the P1-249 genomic clone for exon 1B (see Fig. 2B).
were subsequently made from the parent 5.8-kb BamHI-BamHI plasmid by using appropriate restriction enzymes; the structures of ADARI promoter deletion constructions were confirmed by restriction analysis and by sequencing. The pCAT-0.6-kb P1 S/X plasmid was the previously described 591-bp Sac/Xho construct (16).

**Cell Maintenance and Interferon Treatment.** Human amnion U cells were maintained as previously described (13, 30). IFN-α treatment was with 1,000 units per ml by using Sendai virus-induced leukocyte IFN generously provided by K. Cantell (Helsinki, Finland) or recombinant IFN-αA/D (PBL Biomedical Laboratories, New Brunswick). Parallel cultures were left untreated as controls.

**Transfection and Reporter Assays.** U cells were transfected by the DEAE-dextran-chloroquine phosphate transfection method by using 10 μg of the CAT reporter gene construct and 5 μg of the internal reference plasmid pRSV2-βgal as previously described (16, 30, 31). Treatment with IFN-α, cell harvest, extract preparation, and CAT and β-galactosidase assays were performed as previously described (16, 30). CAT activity was quantified after thin-layer chromatography by use of a Bio-Rad GS-525 molecular image system. CAT activity values, normalized by β-galactosidase activity to control for variation in transfection efficiency, were calculated as percent conversion of [14C]chloramphenicol to the 14C-acetylated derivatives. Transfections were repeated three to five times in independent experiments to permit calculation of a mean value and standard deviation.

**Materials.** Unless otherwise specified, materials and reagents were as described previously (9, 14–16).

**RESULTS**

**Isolation and Characterization of 5’-RACE cDNA Clones of Human ADARI.** To obtain additional 5’-UTR sequence for human ADARI, 5’-RACE was made with nested primers and an adaptor-ligated cDNA library prepared from human placenta. The ADARI minus primer corresponding to antisense nucleotides 290 to 273 of exon 2 (E2 minus 273) and the plus AP1 primer were first used, followed by the nested primer pair ADAR1 antisense nucleotides 67 to 48 of exon 2 (E2 minus 48) or E2 minus 273 and the plus AP2 primer. The 5’-RACE cDNAs were subcloned and sequenced. Unexpectedly, two kinds of sequences were obtained for the 5’-RACE cDNA clones that diverged from each other exactly at the exon 1–exon 2 junction (Fig. 1). In addition to the 5’-RACE-1A sequence that matches exactly the previously identified exon 1 (herein designated as exon 1A) sequence obtained for cDNA prepared from human kidney and human U cells (14, 16), a second 5’-RACE cDNA sequence was obtained. This 5’-RACE cDNA sequence was designated as exon 1B; the longest 5’-RACE-1B cDNA clone extended 107 nucleotides upstream of the exon 2 junction (Fig. 1).

**Physical Map Position of Exon 1B.** Overlapping phase λ and P1 clones containing the human ADARI gene were characterized by restriction mapping and Southern blot analysis to map the position of exon 1B (14). A composite map of the ADARI gene was determined (Fig. 2A). The newly identified exon 1B fragment hybridized to a 5.8-kb BamHI fragment present within the P1–249 genomic clone but not in the P1–652 or λ176 genomic clones (Fig. 2B). The precise exon 1B-intron junction was determined by sequencing plasmid subclones. The 210 nt exon 1A was previously shown to be separated from exon 2 by a 5.4-kb intron (14, 32). Exon 1B was positioned ≈9-κb upstream of exon 1A. The 5’-end cDNA sequences of ADARI obtained from the 5’-RACE-1A and -1B clones isolated from the human placenta library corresponded exactly to the genomic sequence obtained from human placenta and human foreskin fibroblast DNA subclones (Fig. 1).

**Effect of Interferon Treatment on the Expression of Exon 1A- and Exon 1B-Containing ADARI Transcripts.** The effect of IFN-α treatment on the expression of ADARI mRNA species was examined by quantitative RT-PCR to distinguish between exon 1A- and exon 1B-containing transcripts. cDNAs prepared from U cell RNA preparations were used as templates for amplification by PCR, either with the E1B plus 12 and E2 minus 273 primer pair for detection of transcripts possessing the exon 1B–exon 2 junction or with the E1A plus 170 and E2 minus 273 primer pair for detection of the transcripts with the exon 1A–exon 2 junction (Fig. 3A). Amplification of templates was determined after increasing cycle number, ranging from 10 to 35, by directly measuring incorporation of 32P-labeled primer into reaction products that were resolved by electrophoresis (Fig. 3B). Comparable amounts of the exon 1B–exon 2 product were detectable by PCR with samples prepared from untreated and IFN-α-treated cells as the amplification cycle number was increased (Fig. 3B Top). By contrast, the amount of exon 1A–exon 2 specific product obtained was very low with template cDNA prepared with RNA from untreated relative to IFN-α-treated human U cells (Fig. 3B Middle). As a control, when the same cDNA preparations were examined by using β-actin specific primers, the amount of actin product obtained was similar for samples prepared from untreated as compared with IFN-α-treated cells (Fig. 3B Bottom). The observed sizes of the ADARI and β-actin products were consistent with sizes predicted from their cDNA sequences.

Northern gel-blot analyses with hybridization probes specific to either exon 1A or exon 1B were performed to estimate the size of the corresponding transcripts. Both the exon 1A and exon 1B probes detected a single major transcript of about ≈6.7-kb with RNA isolated from human U cells (Fig. 4). The transcript detected with the exon-1A probe was IFN-inducible as predicted (Fig. 3B; 14, 16). By contrast, ≈6.7-kb transcript detected with the exon-1B probe was present in comparable amounts in untreated and interferon-treated cells.
shown by the results in Fig. 5, the 2.2-kb X fragment were fused upstream of a CAT reporter gene. As of exon 1B was capable of functioning as a promoter, restriction in human amnion U cells. Details are as described in human amnion U cells. Northern gel-blot analysis of RNA from untreated or treated with IFN-α for 24 h was analyzed by RT-PCR as described in Experimental Procedures. (A) Schematic showing the locations of the alternative exons 1A and 1B and exon 2, with exons denoted by boxes and introns by solid lines; the exon-specific oligomer primers are denoted by arrows. (B) PCR products fractionated by gel electrophoresis after the indicated number of cycles. cDNA templates were prepared by using RNA prepared from either IFN-α treated or untreated U cells. PCR reactions contained 0.25 μM of primer, by using the primer pairs E1B plus 12 and 32P-labeled E2 minus 273, E1A plus 170 and 32P-labeled E2 minus 273, or β-actin plus and minus primers as indicated.

Identification of a Constitutively Active Promoter Flanking Exon 1B Unique from the Interferon-Inducible Promoter Flanking Exon 1A. To examine whether the 5′-flanking region of exon 1B was capable of functioning as a promoter, restriction fragments derived from the 5.8-kb BamHI genomic DNA fragment were fused upstream of a CAT reporter gene. As shown by the results in Fig. 5, the 2.2-kb X/X reporter construct carrying the 2.2-kb XhoI genomic fragment fused upstream of the CAT gene exhibited significant CAT activity in transfected U cells; the activity was not further increased by IFN treatment. In contrast, the same 2.2-kb XhoI genomic fragment in reverse orientation showed very low CAT activity. Reporter constructs prepared with genomic fragments flanking the 2.2-kb XhoI fragment, either the 1.3-kb X/B or the 2.3-kb B/X fragment, lacked promoter activity. As a negative control, the pCAT-Basic promoter-less plasmid without inserted human genomic DNA; pCAT-0.6Pi (S/X), the IFN-inducible P1 promoter present within the 591-bp Sac-Xho genomic DNA fragment inserted into the CAT-Basic plasmid (16).

A series of deletions of the 2.2-kb XhoI construct was generated by using internal AvaI restriction sites and was analyzed for CAT reporter expression. The internal 0.9-kb AvaI fragment, tested as the 0.9-kb A/A construct, lacked promoter activity. However, the 1.3-kb X/X (A/A del) construct obtained by deletion of the 0.9-kb AvaI fragment showed strong reporter activity. The activity of the 1.3-kb X/X (A/A del) construct was comparable in the absence and presence of IFN treatment. The 1.05-kb X/X (A/A del) plasmid, generated by further deletion of a 0.25-kb AvaI fragment from the 1.3-kb X/X (A/A del) construct, showed greatly reduced activity. Although the 1.3-kb X/X (A/A del) construct was not IFN-inducible, the pCAT-0.6-kb P1 S/X reference plasmid that contains the 591-bp Sac/Xho genomic fragment flanking exon 1A into pCAT-Basic (16) showed promoter activity that was increased 4- to 5-fold by IFN treatment (Fig. 5). The pCAT-0.6-kb P1 S/X construct includes an ISRE element (16). Finally, neither pCAT-Basic nor pCAT-Control showed IFN inducibility of the CAT reporter (data not shown).

DNA Sequence of the ADAR1 Constitutive Promoter Region. The genomic DNA flanking exon 1B that possessed the necessary functional elements to support constitutive transcription was sequenced; 395 nucleotides of the sequence are...
shown in Fig. 6. Comparison of the sequence obtained for the ADARI P1–249 genomic DNA subclone to that for the 5′-RACE-1B cDNA revealed an exact match over the 107-nt region that constitutes exon 1B (Figs. 1 and 6). Intron 1B was not to the GT–AG rule. Computer analysis of the promoter region sequence revealed three canonical CAAT boxes in the immediate vicinity of the transcription initiation sites determined by 5′-RACE and primer extension analyses. A consensus initiator positioning sequence Inr and a consensus TATA box were also present, but not at the standard positions (34, 37). Additional motifs identified (35) in the flanking region include PEA3 of the Ets-1 family, AP-1, AP-2, MRE, Cap-box, purine box, and cAMP CRE-like motif (Fig. 6). The promoter region upstream of exon 1B lacked elements corresponding to the interferon responsive ISRE and GAS elements, and the KCS element (30, 31); the interferon-inducible promoter upstream of exon 1A includes an ISRE- and KCS-like element (16).

DISCUSSION

Two important points emerge from our results reported herein on the transcription-control regions of the ADARI gene encoding the RNA-specific adenosine deaminase. First, human ADARI transcripts possess alternative exon 1 structures that predict the synthesis of two differently sized forms of ADARI, one 1,226 amino acids and the other 931 amino acids. Second, the alternative exon 1A and exon 1B structures of the ADARI transcripts initiate from different promoters, one interferon inducible (P1) and the other constitutively active (P2). These findings now provide a molecular explanation for the earlier observation that two forms of ADARI protein occur in mammalian cells, one inducible by interferon and the other constitutively expressed (9, 14). The schematic diagram shown in Fig. 7A provides a summary of the relative organization of the 5'-region of the human ADARI gene. The ADARI gene structure was previously determined to include 15 exons that span ~30-kb (14) on chromosome 1q (15). With the identification of the alternative exon 1B position about ~9 kb upstream of the previously identified exon 1, now designated exon 1A, the size of the human ADARI gene increases to ~39 kb and spans 16 exons.

Human ADARI gene expression is complex. At least two mature similarly sized ~6.7-kb ADARI mRNAs are produced from the ADARI gene that can be distinguished from each other by the nature of the exon 1 at their 5'-ends and by their IFN inducibility. Although the level of ADARI expression is increased by IFN treatment (9, 17), significant basal amounts of ADARI transcripts are routinely observed in human cell lines and organs in the absence of IFN treatment (9–11). A single major ~6.7-kb ADARI mRNA is detectable in human cell lines in the absence of exogenous cytokine treatment as well as in various organs with hybridization probes corresponding to different regions of the ADARI cDNA (9–11, 16). Our findings establish that the basal level of mature ADARI RNA corresponds at least in part to the newly identified exon 1B-containing transcripts that initiate from the constitutively active P2 promoter (Figs. 3 and 4). By contrast, the increased level of the similarly sized ~6.7-kb ADARI RNA observed in IFN-treated cells corresponds to the exon 1A-containing transcripts that initiate from the IFN-inducible P1 promoter (Figs. 3 and 4). The P1 promoter of ADARI possesses ISRE- and KCS-like elements associated with IFN responsiveness (16).

ADARI transcripts containing exon 1A posses an ORF of 1,226 amino acids (Fig. 7B Upper). This ORF sequence is in agreement with the previously characterized human ADARI cDNA clones that were isolated in screens for IFN-regulated cDNAs (11, 22). Interestingly, the sequence of the exon 1A form of ADARI was also obtained by screening cDNA libraries prepared by using RNA from human natural killer cells (10) or HeLa cells (11) not known to be treated exogenously with IFN, possibly indicating P1 promoter activity in response to autocrine IFN action or some other inducer present under the culture conditions preceding RNA isolation. Exon 1A includes the initiator methionine codon, AUG1, whose flanking +3 and +4 nucleotides GcaAUG4 are purines, characteristic of a strong translational start codon (36). The next methionine in the 1,226 amino acid ORF is nearly 300 amino acid residues downstream, AUG296, and occurs within the unusually large 1,586 nt exon 2 (14). The newly identified exon 1B lacks AUG codons and when spliced to exon 2, a longer 5'-untranslated region is predicted than with exon 1A-containing transcripts. The long ORF of the ADARI transcripts possessing exon 1B linked to exon 2 is predicted to
ADAR1 protein lacks the Z-DNA binding domain Z activity, the C-terminal 931 residues of the 1,226 residue ORF conceivably affect ADAR1 mRNA stability or translational the central and C-terminal regions but not the N-terminal kDa protein is detected only by the antisera prepared against exon 1 rather than by differential proteolysis of the larger ADAR1 proteins are caused at least in part by the origin of differences in apparent molecular weight observed for residues in the same frame. These findings suggest that the different ORFs, one of 1,226 residues and the other of 931 residues in the same frame. These findings suggest that the differences in apparent molecular weight observed for ADAR1 proteins are caused at least in part by the origin of exon 1 rather than by differential proteolysis of the larger ~150-kDa protein to yield the ~110-kDa protein. Consistent with these conclusions, antisera prepared against three non-overlapping regions of the ADAR1 cDNA expressed in Escherichia coli including the N-terminal, central, and C-terminal regions of the 1,226 residue ORF all detect the IFN-inducible ~150-kDa protein, whereas the constitutively expressed ~110-kDa protein is detected only by the antisera prepared against the central and C-terminal regions but not the N-terminal region of the ORF (9). Although the alternative 5′-UTR may conceivably affect ADAR1 mRNA stability or translational activity, the C-terminal 931 residues of the 1,226 residue ORF includes the three dsRNA-binding domain motifs and the catalytic region. However, the shortened 931 amino acid ADAR1 protein lacks the Z-DNA binding domain Za (33). The biological significance of this deletion of Z-DNA binding activity is unknown.

It is now apparent from the results described herein that the ADAR1 RNA-specific adenosine deaminases constitute a family of enzymes specified by alternative exon 1 splicing of transcripts initiated from at least two promoters. In eukaryotes, alternative promoters are known to be associated with gene expression that is developmentally regulated or tissue specific (37). This is exemplified by the Pax genes, regulators in the developing nervous system (38, 39), and by the Apobec1 gene encoding the apolipoprotein B RNA-editing protein (40). Cytokines including interferons modulate the expression of a number of cellular genes; both the basal level of expression and the fold induction after cytokine treatment vary markedly (19). It is conceivable that alternative promoters may be used by other cytokine-regulated genes in addition to ADAR1. For ADAR1, it is now of utmost importance to define the tissue-specific utilization of the Pε- constitutive and Pγ-inducible promoters that drive transcription of the ADAR1 gene and the possible effect of cytokines other than interferons as well as growth factors on their activities in uninfected and virus-infected cells and tissues.

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