GENOMICS 14, 959–964 (1992)

Conservation of a 23-kDa Human Transplantation Antigen in Mammalian Species

S. RUSS PRICE,[1] MARIA S. NIGHTINGALE, DAVID A. BOBAK,[2] MIKAKO TSUCHIYA,[3] JOEL MOSS,[4] AND MARTHA VAUGHAN

Laboratory of Cellular Metabolism, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892

Received May 26, 1992; revised August 28, 1992

A group of transplantation antigens, referred to as tum⁻ antigens, were identified in mouse tumor cells that had been mutagenized to produce variant cells and were recognized by clonal cytolytic T lymphocytes (CTL). Alterations in these variant cells that were recognized by CTL resulted from point mutations in the genes of specific proteins. We have isolated human and bovine cDNA clones that encode the homologs of the mouse tum⁻ antigen P198. This 23.6-kDa protein is highly basic with a predicted p*I* of 11.55. p23/P198 is highly conserved across mammalian species, with >94% identity (97% including conservative substitutions) among the human, bovine, and mouse deduced amino acid sequences. The nucleotide sequences of both the coding and 5′- and 3′-untranslated regions from human, bovine, and mouse are also highly conserved with >88% identity in the coding regions. Hybridization of poly(A)⁺ RNA from various mammalian sources with cDNA and oligonucleotides specific for the coding region identified two mRNAs of 1.2 and 0.8 kb, whereas probes specific for the 3′-untranslated region between two consensus polyadenylation signals hybridized with the 1.2-kb, but not the 0.8-kb, mRNA. The abundance of the 1.2-kb mRNA relative to that of the 0.8-kb species varied depending upon the cell type. A single predominant transcription initiation site was mapped by primer extension. These studies indicate that this highly basic 23.6-kDa protein is encoded by two major mRNA species that differ only in the length of their 3′-untranslated regions and that the mechanism that gives rise to these two mRNAs, utilization of alternative polyadenylation sites, is conserved across species. Moreover, it appears that the protein product of the p23/P198 gene is under considerable evolutionary pressure to maintain its structural features. © 1992 Academic Press, Inc.

INTRODUCTION

Tumor-specific transplantation antigens frequently elicit cytolytic T-lymphocyte responses but not B-cell antibody responses (Loveland and Simpson, 1986). The identity of many of these antigens remains unclear primarily because they cannot be isolated using immunoprecipitation techniques. Boon and co-workers developed a technique for the isolation of cytolytic T-lymphocyte clones that recognized specific mutant antigens present in mutagenized mouse tumor (tum⁻) cells but not untreated (tum⁺) cells (Uyttehove *et al.*, 1980). The term *tum⁻* refers to the absence of tumors in mice injected with mutagenized tumor cells. The rejection of the tumor cells presumably occurs due to the action of the cytolytic T lymphocytes that recognize an antigen in tum⁻, but not tum⁺, cells. Using this approach, several transplantation antigens referred to as tum⁻ antigens were identified in tum⁻ variant cells derived from the methylcholanthrene-induced mastocytoma P815 (Boon *et al.*, 1989). In these studies, tum⁺ cells were transfected with genomic DNA from tum⁻ cells and screened for expression of the tum⁻ antigen using specific CTL. One of these antigens, P198, was identified as a 23.5-kDa protein whose gene contained a point mutation that resulted in the substitution of a threonine for an alanine in the mutant protein, thus making the protein antigenic (Sibille *et al.*, 1990).

We report here the isolation of cDNA clones corresponding to its human and bovine homologs. In human and bovine, this protein is encoded by two mRNAs that differ in the lengths of their 3′-untranslated regions due to differences in the sites of polyadenylation. Moreover, the P198 gene, including the polyadenylation sites, is highly conserved across mammalian species consistent with the notion that the tum⁻ antigen P198 has an important role in cell function.

Sequence data from this article have been deposited with the EMBL/GenBank Data Libraries under Accession Nos. X56932 and X56933.

[1] Present address: Renal Division, Department of Medicine, Emory University School of Medicine, Atlanta, GA 30322.

[2] Present address: Department of Medicine, University of Virginia School of Medicine, Charlottesville, VA 22908.

[3] Present address: Department of Biochemistry, Shimane Medical University, 89-1 Izumo, 693, Japan.

[4] To whom correspondence should be addressed at Laboratory of Cellular Metabolism, Building 10, Room 5N307, National Institutes of Health, Bethesda, MD 20892.

0888-7543/92 $5.00

MATERIALS AND METHODS

Materials. The cAMP-differentiated HL-60 Lambda ZAP cDNA library prepared using oligo(dT) and random primers was a gift from Dr. Harry Malech, NIAID, NIH, Bethesda, Maryland. The bovine retinal λgt10 cDNA library was purchased from Promega; [α-^{32}P]dATP (6000 Ci/mmol) and [α-^{35}S]dATP (600 Ci/mmol) were from New England Nuclear; terminal deoxynucleotidyltransferase, M-MLV reverse transcriptase, and RNA size standards were from Bethesda Research Laboratories; random-primed DNA labeling kits were from Boehringer-Mannheim; T4 polynucleotide kinase and all restriction endonucleases were from Promega; Sequenase (version 2) DNA sequencing kits were from United States Biochemicals; and GeneAmp polymerase chain reaction kits were from Perkin–Elmer Cetus.

Isolation of cDNA clones. During the characterization of cDNA clones for ADP-ribosylation factors (ARFs), ~20-kDa guanine nucleotide-binding proteins that enhance the ADP-ribosyltransferase activity of cholera toxin (Bobak *et al.*, 1989), a human cerebellar cDNA was isolated that was composed of two cDNA fragments, one of which contained a partial open reading frame (ORF) that encoded the human homolog of mouse tum$^-$ antigen P198 (Sibille *et al.*, 1990). This P198-like cDNA fragment was used to screen a bovine retinal λgt10 cDNA library. Replicate filters were hybridized at 42°C in 5× SSC (1× SSC = 0.15 *M* NaCl/0.015 *M* sodium citrate)/5× Denhardt's solution (1× Denhardt's = 0.02% bovine serum albumin/0.02% Ficoll/0.02% polyvinylpyrolidone)/10 m*M* Tris–HCl, pH 7.4/0.5% SDS/10% dextran sulfate (w/v) containing denatured salmon sperm DNA (100 μg/ml); hybridizations with cDNA fragments also contained 40% formamide. cDNA fragments were radiolabeled with ^{32}P using the random-primed method (Feinberg and Vogelstein, 1983). Oligonucleotide probes were radiolabeled with ^{32}P at the 5′ terminus using T4 polynucleotide kinase or at the 3′ terminus using terminal deoxynucleotidyltransferase. Filters were washed once with 2× SSC/0.5% SDS at 42°C for 20 min and twice with 0.5× SSC/0.5% SDS for 20 min each at 50°C for the oligonucleotide probes or 65°C for the cDNA. The cAMP-differentiated HL-60 Lambda ZAP cDNA library was screened using the cDNA insert from bovine retinal p23 clone 18A (1101 nucleotides) as described above.

cDNAs containing portions of the putative 5′-untranslated region of human p23 were amplified by the polymerase chain reaction (Frohman *et al.*, 1988) from single-stranded HL-60 cDNA prepared using a p23-specific primer (primer-ex: 5′ ACGATGGCCGCCAGGCGGCCCAGGAGATGGCCTCG 3′) complementary to the sense strand downstream from the initiation codon and tailed using dATP and terminal deoxynucleotidyltransferase. Amplifications were performed using primer-ex that was modified to include a *Not*I restriction site [5′ ACGTACGTGCGGCCGC(primer-ex) 3′] and *Not*I primer adapter (5′ AATTCGCGCCGGCG[dT]$_{15}$ 3′; Promega). Reaction mixtures were subjected to 35 cycles of 30 s at 95°C, 1 min at 48°C, and 2 min at 72°C using an ERICOMP thermal cycler. The amplified cDNAs were subcloned into the *Not*I site of pGEM5 and colonies were screened using a p23-specific nested primer (5′ CACCAGGACCTGCACCTCCGCCAT 3′). Four independent clones from two separate experiments were sequenced using the dideoxynucleotide chain termination method (Sanger *et al.*, 1977).

DNA sequencing and analysis. cDNA inserts were sequenced in both directions after either subcloning into pGEM3 (bovine retinal clones) or *in vivo* excision of insert-containing pBlueScript plasmids (HL-60 clones). Nucleotide sequences were analyzed using the PC/GENE software package (IntelliGenetics). Database comparisons were performed at the National Center for Biotechnology Information Network Service using the BLAST program (Altschul *et al.*, 1990).

Primer extension analysis. Primer extension analysis of the human p23 5′-untranslated region was performed using HL-60 poly(A)$^+$ RNA (5 μg) and [^{32}P]primer-ex. After an initial annealing step at 50°C for 20 min, the primer was extended using M-MLV reverse transcriptase. A sequencing ladder was prepared using primer-ex in conjunction with a partial human p23 cDNA containing the putative initiation codon. Both the primer extension reaction products and sequencing ladder were electrophoresed on a denaturing 6% acrylamide gel.

RNA blot analysis. Poly(A)$^+$ RNA was prepared using oligo(dT) cellulose chromatography following the isolation of total RNA according to Chirgwin *et al.* (1979). Poly(A)$^+$ RNA was fractionated in a 1% agarose/formaldehyde gel and transferred to a Nytran membrane. Hybridizations with ^{32}P-labeled cDNA or oligonucleotide probes as well as posthybridization washes were performed as described for the isolation of cDNA clones. Sequences of oligonucleotide probes were as follows: Hum p23-COD, 5′ GTACTTCCAGCCAACCTCGTGAGCCAGGCGCCCCAGATAGGCAAA 3′ (complementary to bases 420–464, Fig. 1A); Bov p23-UTR, 5′ ACATCCTAACAGCTGCAGAGTATAGTATCCGACCACGTGGGAAGCATG 3′ (complementary to bases 916–963, Fig. 1A); Hum p23-UTR, 5′ GAAGGGCAGGCAACGCATGAGGAA 3′ (complementary to bases 649–672, Fig. 1A).

RESULTS AND DISCUSSION

Using a human cDNA fragment that contained a partial ORF corresponding to the mouse tum$^-$ antigen P198 (Sibille *et al.*, 1990), 100,000 bovine retinal cDNA clones were screened; two positive clones were sequenced. Clone 15A contained 638 nucleotides and ended with a poly(A) tail (not included in the sequence numbering) at the 3′ terminus (Fig. 1A, beginning at base 1 of Bov p23). An incomplete ORF extended for 607 nucleotides from the 5′ end of the clone to end with a termination codon. The incomplete protein that was encoded by the ORF was highly homologous to the tum$^-$ antigen P198-deduced amino acid sequence and lacked only the initiator methionine. A polyadenylation signal (AATAAA) was located five bases after the termination codon; the poly(A) tail started 19 nucleotides (nucleotide 639, Fig. 1A) downstream from the polyadenylation signal. Similarly, clone 18A lacked an initiation codon and overlapped clone 15A from nucleotides 13 to 639. Instead of ending with a poly(A) tail at a position corresponding to base 639 as was the case with clone 15A, it continued for an additional 462 nucleotides, ending in a poly(A) tail (included in the nucleotide numbering, Fig. 1A). A second polyadenylation signal was present in the extended 3′-untranslated region 15 nucleotides upstream from the start of the poly(A) tail.

Because of the apparently incomplete nature of the bovine clones, a cAMP-differentiated HL-60 cDNA library was screened using the bovine retinal clone 18A cDNA insert. The human clones appeared to contain some artifact that probably arose during library construction because one end of each clone contained nucleotide sequence highly homologous to those of the bovine p23 and mouse P198, whereas the sequence at the opposite end was unrelated to the mammalian p23/P198 sequences. A composite was constructed from overlapping sequences that corresponded to the bovine p23 and mouse P198 sequences (bases 8–672 in Hum p23, Fig. 1A). The first seven nucleotides reported for the human p23 sequence were obtained using the polymerase chain reaction to amplify the 5′-untranslated region; the reaction products terminated 17 nucleotides upstream from the initiation codon.

The nucleotide sequences of human and bovine p23 and mouse P198 were >88% identical in their coding regions. The 5′- and 3′-untranslated region sequences

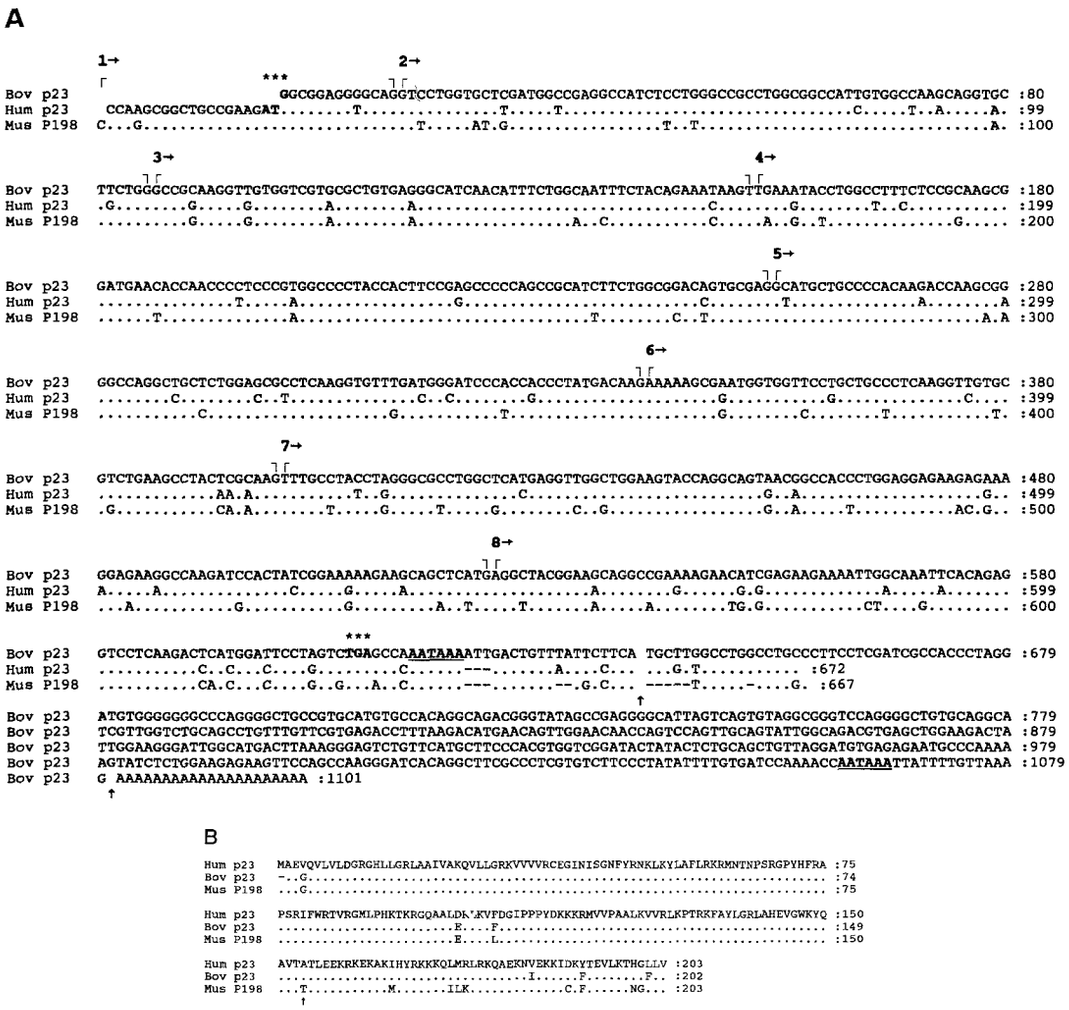

FIG. 1. Comparison of human and bovine p23 and mouse P198 nucleotide and deduced amino acid sequences. (A) Nucleotide sequences from overlapping human/bovine p23 cDNAs and the corresponding mouse tum⁻ P198 antigen genomic sequence (Sibille *et al.*, 1990) were aligned using CLUSTAL (PC/GENE) with gap penalties of 20, *K*-tuple value of 2, and a window size of 10. Gaps introduced for optimal alignment are indicated by hyphens. Nucleotides in the human and mouse sequences that are identical to those in the bovine sequence are indicated by periods. The initiation (ATG) and termination (TGA) codons are indicated by asterisks above the sequences, polyadenylation signals (AATAAA) are underlined, and sites of polyadenylation are indicated by arrows. Positions and numbering of the mouse exons are indicated above the bovine sequence; exon 8 was reported to terminate at the first polyadenylation site. The G in position 458 of the mouse P198 sequence corresponds to the normal genomic DNA sequence and was mutated to an A in the mutant P198 gene (Sibille *et al.*, 1990). (B) Shown are the deduced amino acid sequences of the human/bovine p23 and mouse P198 antigen aligned using CLUSTAL as described in A. The methionione missing from the bovine sequence is indicated by a hyphen. Amino acids in the bovine p23 and mouse P198 deduced sequences that are identical to those in the human deduced sequence are represent by periods in the alignment. An arrow indicates the position (amino acid 154) reported to be altered in the mouse tum⁻ mutant protein; the normal allele encodes an alanine in this position. The human and bovine p23 nucleotide sequences were submitted to the EMBL/GenBank Data Library and have the accession numbers X56932 and X56933, respectively; corresponding mouse P198 sequence is from EMBL/GenBank Accession No. X51528.

were also highly conserved, although gaps were required for optimal alignment of the 3′-untranslated regions (Fig. 1A). In addition to the high degree of overall interspecies nucleotide conservation, the nucleotides adjacent to the intron/exon boundaries reported for the mouse genomic sequence and the corresponding nucleotides in the human and bovine cDNAs are identical, suggesting that the splice junctions may be conserved in mammals also (Fig. 1A). Two of the isolated human clones terminated in a poly(A) tail at a site (beginning at nucleotide 655 of the Hum p23 sequence, Fig. 1A) that corresponds to the first polyadenylation site in the bovine clones; a polyadenylation signal was present 16 bases upstream from this site. One other clone continued beyond this site for an additional 17 nucleotides, suggesting that the human p23 mRNA, like its bovine homolog, contains an extended 3′-untranslated region. The site of polyadenylation reported for the mouse P198 mRNA corresponds to the first site present in both human and bovine (Fig. 1A). The fact that the reported genomic nucleotide sequence 3′ to this site in mouse (Sibille *et al.*, 1990) is highly homologous to the corresponding region in the human and bovine sequences supports our contention that the mammalian p23/P198

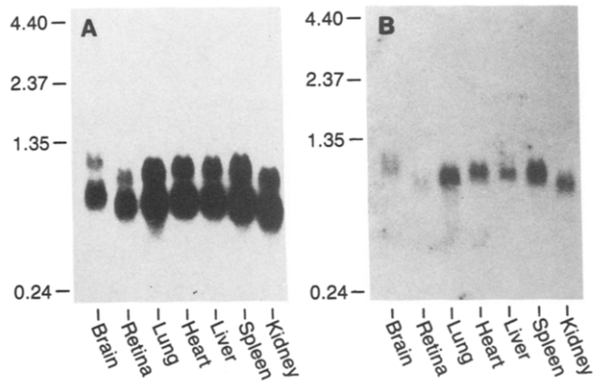

FIG. 2. Hybridization of bovine poly(A)$^+$ RNA with p23 cDNA and oligonucleotide probes. Poly(A)$^+$ RNA (10 μg) from the indicated bovine tissues was hybridized with (A) a bovine p23 cDNA (clone 18A) or (B) a bovine p23 oligonucleotide probe (Bov p23-UTR) specific for the 3′-untranslated region between the two polyadenylation signals as described under Materials and Methods. Films were exposed to the blots for 6 h (A) or overnight (B). The positions of RNA standards (kb) are shown on the left of each figure. The same blot was used for hybridization in A and B.

genes contain extended 3′-untranslated regions with multiple polyadenylation sites.

The human p23 ORF encodes a protein of 203 amino acids (23.6 kDa) of which 50 are either lysines or arginines (Fig. 1B). The predicted p*I* of the protein is 11.55. The deduced amino acid sequences of human and bovine p23 and mouse P198 are highly conserved, exhibiting 94% identity; if conservative substitutions are counted, they are 97% identical. The alanine reported in position 154 of the normal mouse P198 protein is present in both the human and bovine deduced amino acid sequences (Sibille *et al.*, 1990). Point mutation of the G to A in the codon for amino acid 154 (base 458, Fig. 1A) of the mouse tum$^-$ antigen P198 resulted in an alanine to threonine change that was apparently responsible for the antigenicity of this mutant cell line (Sibille *et al.*, 1990).

To characterize the size, number, and distribution of p23 mRNAs, a blot containing size-fractionated poly(A)$^+$ RNA from several bovine tissues was hybridized with the bovine clone 18A cDNA insert (corresponding to nucleotides 13–1101, Fig. 1A) under moderate stringency conditions (Fig. 2A). In all tissues surveyed, two mRNAs of ~1.2 and 0.8 kb hybridized with this probe. Based on the short exposure times (6 h) required to detect hybridization, these mRNAs appear to be abundant. The quantity of the 1.2-kb mRNA, relative to the 0.8-kb mRNA, was lower in brain and retina than in the other tissues, suggesting that expression of the 1.2-kb mRNA might be regulated in a cell-specific manner whereas expression of the 0.8-kb mRNA was relatively uniform among different cell types. To determine whether the 1.2-kb mRNA differs from the 0.8-kb mRNA in the length of its 3′-untranslated region, the blot in Fig. 2A was stripped and rehybridized with an oligonucleotide probe specific for the portion of the putative bovine 3′-untranslated region (nucleotides 916–963) between the two polyadenylation sites (Fig. 2B). This oligonucleotide hybridized with the 1.2-kb, but not the 0.8-kb, mRNA, consistent with the conclusion that the 1.2-kb mRNA results from the use of the downstream site for polyadenylation.

To determine whether the two p23 mRNAs might also differ in the length of their 5′-untranslated regions, primer extension was performed using an oligonucleotide primer 30 nucleotides 3′ to the putative human p23 initiation codon (Fig. 3A). The predominant product extended 75 nucleotides upstream from the start codon. On a shorter autoradiographic exposure, this extension product corresponded to a single band on the sequencing ladder (data not shown). The primer used for these experiments hybridized only with the 1.2- and 0.8-kb mRNAs detected with other p23 probes (Fig. 3B). Assuming that the poly(A) tail consists of 50 to 100 nucleotides, the predicted lengths of the two p23 mRNAs are consistent with the sizes determined by Northern blot hybridization (Fig. 2). Several less abundant, shorter primer extension products were consistently observed in several experiments (Fig. 3A). The smallest of the extension products mapped to the approximate location in the 5′-untranslated region where the human p23 PCR amplification products consistently terminated. This position in the mouse P198 nucleotide sequence (Sibille

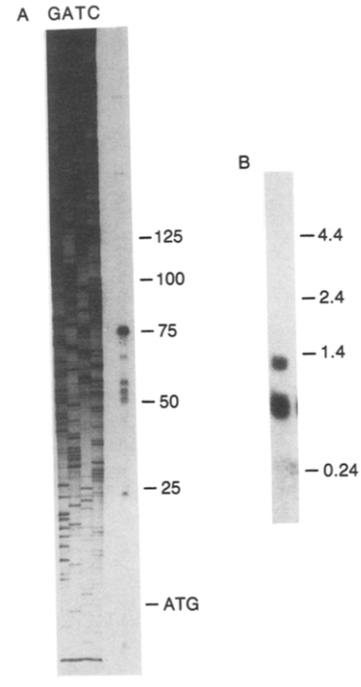

FIG. 3. Analysis of human p23 5′-untranslated region by primer extension. (A) HL-60 poly(A)$^+$ RNA (5 μg) was used as a template to extend ^{32}P 5′-end-labeled primer-ex with M-MLV reverse transcriptase (right lane) as described under Materials and Methods. A sequencing ladder was also prepared using primer-ex in conjunction with a partial human p23 cDNA clone. The terminating dideoxynucleotide used in each reaction is shown above each lane. The distances in nucleotides from the initiation codon (ATG) are shown to the right. (B) HL-60 poly(A)$^+$ RNA (10 μg) was hybridized with primer-ex as described under Materials and Methods. The film was exposed to the blot for 3 h. The positions of RNA standards (kb) are shown to the right.

et al., 1990) was reported to be the transcription start site based on polymerase chain reaction amplification studies similar to those reported here as well as S1 nuclease mapping (Sibille *et al.,* 1990) and ribonuclease protection assays (Sibille *et al.,* 1990). There are several plausible explanations for the apparent differences in the human and mouse transcription start sites. The position of the start site may differ in different species. Alternatively, the shorter extension products may represent products that terminated prematurely due to the inability of reverse transcriptase to read through regions of extensive secondary structure. Third, as the mRNA used for these studies was isolated from human HL-60 cells which are transformed, the structure of the mRNA may not reflect the structure of the p23 mRNA in normal cells.

The extensive identity among the mouse P198 and human and bovine p23 nucleotide and deduced amino acid sequences indicates that the p23 mRNA sequences are highly conserved among mammalian species. Hybridization of poly(A)$^+$ RNA from several mammals with the cDNA insert from bovine clone 18A identified a 0.8-kb mRNA in bovine, rat, mouse, rabbit, and human brain (Fig. 4). The ~1.2-kb mRNA was also weakly detected although it was considerably less abundant than the 0.8-kb mRNA. Hybridization of the rabbit 0.8-kb mRNA was notably weaker than that of the other species. This may reflect a lesser degree of p23 nucleotide conservation between rabbit and bovine than bovine and other mammals. There were also small differences in the sizes of the p23 mRNA from mammals. As noted previously, these size differences may be due to variations between species in the location of the transcription initiation site or the length of the poly(A) tail. These data, however, are consistent with the conclusion that the p23 genes are conserved in mammals.

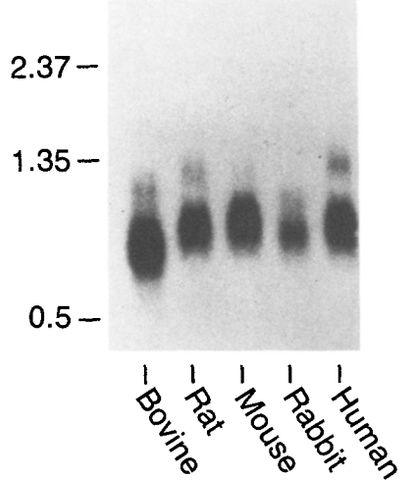

FIG. 4. Hybridization of mammalian brain poly(A)$^+$ RNA with a p23 cDNA Probe. Brain poly(A)$^+$ RNA (10 μg) from the indicated mammalian species was hybridized with a bovine p23 cDNA (clone 18A) as described under Materials and Methods. The film was exposed to the blot for 6 h. The positions of RNA standards (kb) are shown on the left.

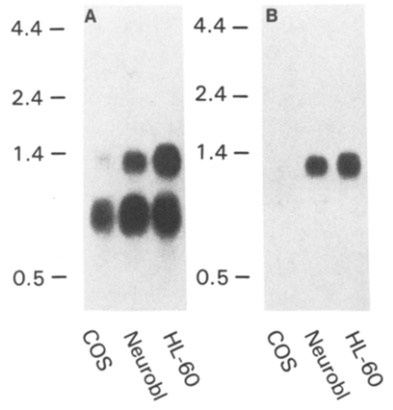

FIG. 5. Hybridization of mammalian poly(A)$^+$ RNA with human p23-specific oligonucleotide probes. Poly(A)$^+$ RNA (10 μg) from monkey kidney (COS), human neuroblastoma (Neurobl), or HL-60 cells was hybridized with oligonucleotide probes specific for the human p23 (A) coding (Hum p23-COD) or (B) extended 3′-untranslated region (Hum p23-UTR) as described under Materials and Methods. Films were exposed to the blots overnight. The positions of RNA standards (kb) are indicated to the left of each figure. The same blot was used for hybridization in A and B.

The fact that several human p23 clones terminated in apparent poly(A) tails at position 655 (Fig. 1A) indicated that the human 0.8-kb p23 mRNA arises from polyadenylation just downstream from the first AATAAA sequence. Although sequence data suggested that the human 1.2-kb mRNA differs from the 0.8-kb mRNA in the length of its 3′-untranslated region, as is the case in bovine, direct evidence was lacking. To confirm that the 1.2-kb mRNA contained an extended 3′-untranslated region, poly(A)$^+$ RNA from monkey COS, human neuroblastoma, and HL-60 cells was hybridized with oligonucleotide probes specific for either the human p23 coding (nucleotides 420–464, Fig. 1A) or extended 3′-untranslated (nucleotides 649–672, Fig. 1A) regions (Fig. 5). The coding region probe hybridized with two mRNAs of 1.2 and 0.8 kb in each cell line; however, the 1.2-kb mRNA was barely detectable in the COS cells. The 3′-untranslated region probe detected the 1.2-kb mRNA in the neuroblastoma and HL-60 cells but not in the COS cells. The failure to detect a 1.2-kb mRNA in COS cells may be due to a failure of the probe which is based on the human sequence to hybridize with the monkey mRNA. Alternatively, the 1.2-kb mRNA may not be expressed in COS cells. Nevertheless, it appears that in addition to the coding region, two alternative sites of polyadenylation may also be conserved among mammals.

In summary, a highly basic 23.6-kDa protein, p23, that was previously identified in mouse as a transplantation antigen is coded for by two mRNAs that differ only in their sites of polyadenylation. From a evolutionary standpoint, this protein has been well conserved among mammals, perhaps indicating a functional pressure to maintain its structure. From a physiological standpoint, the p23 protein appears to play an important role in cell function based on the fact that a single point mutation in the mouse gene resulted in a protein capable of elicit-

ing a cytotoxic T-lymphocyte response, thus causing cell destruction. Further studies are required to elucidate the function of this protein or the extended 3′-untranslated region in cells.

ACKNOWLEDGMENT

We thank Dr. Warren J. Leonard for his critical review of the manuscript.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W., and Lipman, D. J. (1990). Basic local alignment search tool. *J. Mol. Biol.* **215:** 403–410.

Bobak, D. A., Nightingale, M. S., Murtagh, J. J., Price, S. R., Moss, J., and Vaughan, M. (1989). Molecular cloning, characterization, and expression of human ADP-ribosylation factors: Two guanine nucleotide-dependent activators of cholera toxin. *Proc. Natl. Acad. Sci. USA* **86:** 6101–6105.

Boon, T., Van Pel, A., and De Plaen, E. (1989). Tum$^-$ transplantation antigens, point mutations, and antigenic peptides: A model for tumor-specific transplantation antigens? *Cancer Cells* **1:** 25–28.

Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979). Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18:** 5294–5299.

Feinberg, A. P., and Vogelstein, B. (1983). A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132:** 6–13.

Frohman, M. A., Dush, M. K., and Martin, G. R. (1988). Rapid production of full-length cDNAs from rare transcripts: Amplification using a single gene-specific oligonucleotide primer. *Proc. Natl. Acad. Sci. USA* **85:** 8998–9002.

Loveland, B., and Simpson, E. (1986). The non-MHC transplantation antigens: Neither weak nor minor. *Immunol. Today* **7:** 223–229.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74:** 5463–5467.

Sibille, C., Chomez, P., Wildmann, C., Van Pel, A., De Plaen, E., Maryanski, J. L., de Bergeyck, V., and Boon, T. (1990). Structure of the gene of tum$^-$ transplantation antigen P198: A point mutation generates a new antigenic peptide. *J. Exp. Med.* **172:** 35–45.

Uyttehove, C., Van Snick, J., and Boon, T. (1980). Immunogenic variants obtained by mutagenesis of mouse mastocytoma P815. *J. Exp. Med.* **152:** 1175–1183.