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Genetic Mapping in Human and Mouse of the Locus Encoding TRBP, a Protein That Binds the TAR Region of the Human Immunodeficiency Virus (HIV-1)

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Productive infection with HIV-1, the virus responsible for AIDS, requires the involvement of host cell factors for completion of the replicative cycle, but the identification of these factors and elucidation of their specific functions has been difficult. A human cDNA, TRBP, was recently cloned and characterized as a positive regulator of gene expression that binds to the TAR region of the HIV-1 genome. Here we demonstrate that this factor is encoded by a gene, TARBP2, that maps to human chromosome 12 and mouse chromosome 15, and we also identify and map one human pseudogene (TARBP2P) and two mouse TRBP-related sequences (*Tarbp2-rs1*, *Tarbp2-rs2*). The map location of the expressed gene identifies it as a candidate for the previously identified factor encoded on human chromosome 12 that has been shown to be important for expression of HIV-1 genes. Western blotting indicates that despite high sequence conservation in human and mouse, the TARBP2 protein differs in apparent size in primate and rodent cells.

INTRODUCTION

Human immunodeficiency virus-1 (HIV-1) is the etiological agent for AIDS (Barre-Sinoussi *et al.*, 1983; Gallo *et al.*, 1984). As with other retroviruses, the virion contains an RNA genome that produces a chromosomally integrated DNA intermediate during the replicative cycle. Experiments directed toward an understanding of the HIV-1 life cycle have revealed that host cell factors influence replication. In particular, a human gene located on chromosome 12 has been implicated as being critically important for optimal expression of HIV-1 genes (Hart *et al.*, 1989; Newstein *et al.*, 1990; Alonso *et al.*, 1992).

An intriguing aspect to regulatory mechanisms operative for HIV-1 is the role of RNA-binding proteins (see reviews, Jeang *et al.*, 1991; Jeang and Gatignol, 1994; Vaishnav *et al.*, 1991). Specifically, the virus encodes a transcriptional activating protein, *Tat*, and a post-transcriptional regulatory factor, *Rev*, that bind directly to RNAs with a high degree of secondary structure (TAR and RRE, respectively) within the viral genome (see reviews, Jeang *et al.*, 1991; Jeang and Gatignol, 1994; Vaishnav *et al.*, 1991). TAR and RRE, as RNA sequences, serve important regulatory functions and are recognized separately by many cellular RNA-binding proteins (Marciniak *et al.*, 1990; Sheline *et al.*, 1991; Wu *et al.*, 1991; McCormack *et al.*, 1992; Roy *et al.*, 1991; Rounseville and Kumar, 1992; Gatignol *et al.*, 1991; Vaishnav *et al.*, 1991). Although much descriptive information is known, our current understanding of the functional interplay between the virally encoded RNA-binding proteins and their cellular counterparts is limited.

In an attempt to better elucidate the identity and the function(s) of cellular proteins that bind HIV-1 RNA, we previously developed an affinity-binding-based screening procedure to isolate cDNAs that code for TAR-binding proteins (Gatignol *et al.*, 1993). One such human cDNA, TRBP, was isolated and characterized as a positive regulator of gene expression and has been classed in an RNA-binding protein family that includes the human P1/dsI kinase and *Drosophila* staufen (Gatignol *et al.*, 1991, 1993). Here, we provide genetic map locations for the expressed TRBP gene and related sequences in the human and mouse genomes. In the human genome, the expressed gene, TARBP2, was mapped to chromosome 12 and the mouse homolog maps to chromosome 15.

MATERIALS AND METHODS

TRBP probes. Three segments of TRBP cDNA or genomic clones were selected for use as hybridization probes. A 1.3-kb *Bam*HI cDNA fragment containing the entire coding region of TRBP was prepared from the cDNA clone described previously (Gatignol *et al.*, 1991). A second probe was an *Eco*RI fragment containing 637 nt of TRBP2 5′ end cDNA. TRBP2 is encoded by a cDNA from the same origin as TRBP and differs by a 61-nt extension at the 5′ terminus (GenBank Accession No. U08998). These fragments were purified by gel electro-

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phoresis and electroelution; they were labeled to high specific activity (i.e., $>10^9$ cpm/μg DNA) by random hexamer primed DNA synthesis.

The third probe was developed from a partially sequenced phage isolated from a human genomic library from placenta DNA (Stratagene) screened with TRBP cDNA. The clone was partially sequenced and shown to be 82% identical to TRBP cDNA but with frameshifts and stop codons in all possible reading frames, indicating that it was a pseudogene (Graham and Gatignol, unpublished data). To generate a pseudogene-specific probe, 54- and 56-nt oligomers were synthesized corresponding to a segment located 15 kb 3′ of the TRBP sequences and containing 50 bp complementarity at their 3′ ends. These oligonucleotides were permitted to anneal by slow cooling after denaturation at 100°C. The recessed 3′ ends were then labeled with [^{32}P]dCTP using the Klenow fragment of DNA polymerase and unlabeled dATP, dGTP, and dTTP.

Human gene mapping using somatic cell hybrids. Isolation and characterization of a panel of human/rodent somatic cell hybrids has been described (McBride *et al.,* 1982). Hybrid cells were analyzed for the presence of all human chromosomes except Y by standard isoenzyme analyses, by Southern analysis with probes from previously localized genes, and frequently, by cytogenetic analysis. Southern blots of DNA restriction digests of hybrid cells on positively charged nylon membranes were prepared after 0.7% agarose gel electrophoresis (Olson *et al.,* 1991) and hybridized at high stringency with ^{32}P-labeled probes under conditions allowing no more than 10% divergence of hybridizing sequences.

Hybridizing sequences were sublocalized by analysis of previously described human/rodent hybrid cell lines containing specific breaks or translocations involving human chromosome 8 (McBride *et al.,* 1987) and chromosome 12 (McBride *et al.,* 1983, 1990; Park *et al.,* 1992). Loci on chromosome 8 used for regional localizations included AAC1 (8pter–q11), GSR (8p21.1), POLB (8p12–p11), J protein retropseudogene (8q13–q21), MOS (8q11), MYC (8q24.12–q24.3), nine anonymous DNA fragments and pseudogenes, and two additional recently mapped genes. Loci on chromosome 12 used for regional mapping included TPI1 (12p13.3), LDHB (12p12.2–p12.1), KRAS2 (12p12.1), DDIT3 (12q13.1–q13.2), PEPB (12q21), D12S7 (12q14.3–qter), DCN (12q21.2–q23), and ATP2B1 (12q21–q23).

Mouse genetic mapping. Chinese hamster × mouse somatic cell hybrids were produced and characterized as described previously (Hoggan *et al.,* 1988). For this study, 24 hybrids were selected from a larger panel of 76 hybrids; 15 of these hybrids were karyotyped, and the remainder were typed for markers on specific mouse chromosomes.

To position the TRBP genes on specific chromosomes, the progeny of three genetic crosses were typed: (NFS/N or C58/J × *Mus mus musculus*) × *M. m. musculus* (NMM cross; Kozak *et al.,* 1990), (NFS/N × *Mus spretus*) × *M. spretus* (NSS; Adamson *et al.,* 1991), and (NFS/N × *M. spretus*) × C58/J (NSC; Adamson *et al.,* 1991). Progeny of these crosses have been typed for over 700 markers distributed over the 19 autosomes and the X chromosome. These markers include the Chr 6 markers *Tcrb* (T-cell receptor-β), *Cbl1* (Cas-Br-M virus oncogene 1), *Cycs* (cytochrome C, somatic), and *Mtv8* (mammary tumor virus 8) and the Chr 7 markers *Tyr* (tyrosinase), *Zp2* (zona pellucida protein 2), *Oat* (ornithine aminotransferase), *Cyp2e1* (cytochrome P450, 2e1), and *Hras1* (Harvey ras oncogene 1). Probes and restriction enzymes for these markers were described previously (Hake *et al.,* 1994; Majors and Varmus, 1983; Danciger *et al.,* 1993; Lunsford *et al.,* 1990; Ramesh *et al.,* 1992). *Pcp1* was typed in the *M. m. musculus* cross following digestion with *Xba*I and in the *M. spretus* crosses following digestion with *Pvu*II.

Western blot analysis. Mouse NIH 3T3 (ATCC CRL 1658), Chinese hamster ovary (CHO, ATCC CCL 61), Chinese hamster E36 cells, monkey Cos (ATCC CRL 1651), and human HeLa and Jurkat (ATCC TIB 152) cells were grown to 50% confluency, washed twice with PBS, resuspended in SDS lysis buffer, and boiled for 10 min. A similar amount of each cell lysate (estimated by Coomassie staining) was loaded on two identical gels. One was stained by Coomassie blue, and the second one was transferred to a polyvinylidene difluoride membrane (Immobilon P, Millipore). Western blot analysis was performed using the antibody already described (Gatignol *et al.,* 1993). The reactions were developed with enhanced chemiluminescence (Tropix). Filters were exposed 1 to 2 h after the reaction for about 3 min for autoradiography.

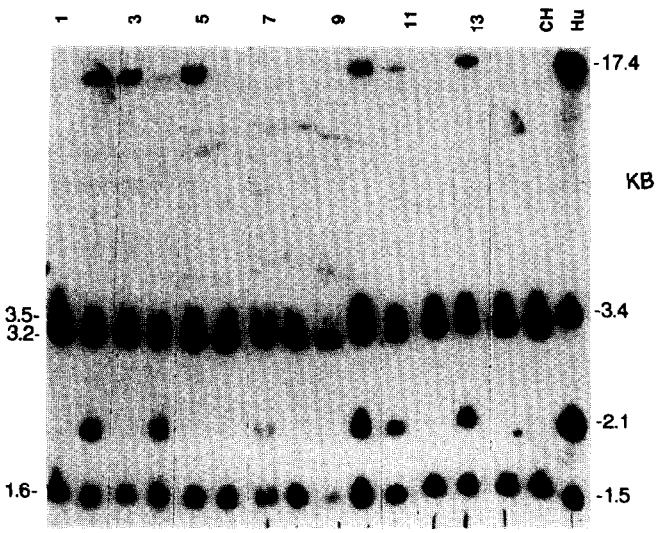

FIG. 1. Southern blot analysis of representative human/hamster somatic cell hybrid DNAs. Aliquots (10 μg) of independent hybrid cell DNAs (lanes **1**–**14**) and parental Chinese hamster (CH) and human placental (Hu) DNAs were digested with *Eco*RI and transferred to nylon membranes after agarose gel electrophoresis. The blot was hybridized with a 1.3-kb human TRBP2 cDNA insert probe and washed at high stringency (see Materials and Methods). A 2.1-kb hybridizing human band was detected in hybrids retaining human chromosome 12 (lanes **2, 4, 7, 10, 11,** and **13**), whereas a 17.4-kb hybridizing band was present in hybrids retaining human chromosome 8 (lanes **2–5, 10, 11,** and **13**). Hybridizing 1.5 and 3.4 human bands were not resolved from cross-hybridizing hamster bands. Sizes of human and hamster bands are shown to the right and left, respectively.

RESULTS

Genetic Mapping of TRBP-Related Sequences in the Human

Genes containing TRBP-related sequences were identified on two different human chromosomes by Southern analysis of a panel of human/rodent somatic cell hybrid DNAs digested with *Eco*RI using the 1.3-kb cDNA probe (Fig. 1). Four hybridizing bands were detected in human DNAs under these conditions, and a 2.1-kb band clearly segregated with human chromosome 12 (Table 1), while a 17.4-kb band could be assigned to human chromosome 8. Digestion of human DNA with the enzymes *Hin*dIII and *Xba*I produced only 2 TRBP-related fragments in each case (data not shown). Examination of the same panel of hybrids digested with these enzymes confirmed the assignment to chromosomes 8 and 12. The chromosome 8 locus was identified by a 3.9-kb *Hin*dIII fragment and a 6.8-kb *Xba*I fragment and the chromosome 12 locus by a 10-kb *Hin*dIII fragment and a 15.4-kb *Xba*I fragment.

The *Eco*RI blots were reprobed with a 60-bp oligonucleotide specific for the 3′ flank of the cloned TRBP pseudogene. A single 2.1-kb band that segregated with human chromosome 8 was detected, and no cross-hybridizing rodent bands were found. These results

TABLE 1

Segregation of the TRBP2 Gene and Pseudogene with Human Chromosomes 12 and 8, Respectively

Human chromosome	% Discordancy	
	Gene	Pseudogene
1	24	33
2	25	35
3	28	41
4	39	46
5	20	30
6	26	29
7	40	48
8	28	0
9	24	35
10	26	20
11	16	26
12	0	26
13	30	35
14	35	34
15	36	53
16	43	22
17	35	46
18	40	47
19	29	33
20	18	23
21	40	53
22	27	38
X	39	51

Note. The human TRBP2 gene and pseudogene were detected as 2.1- and 17.4-kb hybridizing bands, respectively, by Southern blotting of a panel of *Eco*RI-digested human/rodent somatic cell hybrid DNAs with a 1.3-kb *Bam*HI cDNA fragment as probe (see Fig. 1). These bands were well resolved from 1.6-, 3.2-, and 3.5-kb or 1.8-, 2.7-, 4.0-, and 16.4-kb cross-hybridizing bands in hamster and mouse DNAs, respectively. Discordancy indicates the presence of the gene in the absence of the chromosome or the absence of the gene despite the presence of the chromosome, and the sum of these numbers divided by total hybrids examined (×100) represents the percentage discordancy. The human/hamster hybrids consisted of 29 primary hybrids and 14 subclones (20 and 18 positive for gene and pseudogene, respectively, of 43 total) and the human/mouse hybrids represented 19 primary hybrids and 30 subclones (14 and 8 positive for gene and pseudogene, respectively, of 49 total).

clearly indicate that the functional gene, designated TARBP2, is located on human chromosome 12, whereas the sequence on chromosome 8, TARBP2P, is a processed pseudogene.

It was possible to sublocalize each of these sequences by examination of hybrids containing breaks and translocations involving both of these chromosomes. Several subclones of a human/mouse hybrid contained a deletion of distal 12p but retained KRAS (12p12.1) and all 12q markers, and TARBP2 was retained in these subclones. Several subclones of another human/mouse hybrid retained no 12p markers including KRAS, but all 12q markers including DDIT3 (12q13.1) were present, as was TARBP2. Two independent human/hamster and one human/mouse hybrid retained most of chromosome 12p including KRAS, but all 12q markers were absent; TARBP2 was present in one of these hybrids and absent in the other two. These results allow assignment of TARBP2 to the centromeric region between KRAS (12p12.1) and DDIT3 (12q13.1).

One human/hamster hybrid isolated after fusion of human fibroblasts (GM3501) containing the reciprocal translocation t(1;8)(p34;q22) retained the 8pter–q22 translocation chromosome in the absence of the reciprocal translocation chromosome or normal chromosome 8; the TRBP pseudogene was not present in this hybrid. Hence, the pseudogene, TARBP2P, can be assigned to 8q22–qter.

The cDNA probe was also used to examine DNAs from 10 unrelated individuals for restriction fragment length polymorphisms. No RFLPs were found in digests with 12 different enzymes including *Bam*HI, *Hin*dIII, *Eco*RI, *Taq*I, *Msp*I, *Xba*I, *Sac*I, *Pvu*II, *Pst*I, *Bgl*II, *Eco*RV, and *Kpn*I.

Genetic Mapping of TRBP-Related Sequences in the Mouse

Southern blot analysis of BALB/c and NFS/N mouse and Chinese hamster DNAs identified *Bam*HI fragments of 19.4, 8.6, 2.8, and 2.2 kb in mouse and 15.5, 2.1, and 0.7 kb in hamster (Fig. 2). Analysis of 24 somatic cell hybrids for the presence or absence of each of the 4 fragments demonstrated that these TARBP-related sequences could be assigned to chromosome (Chr) 6 (the 8.6-kb fragment), Chr 7 (19.4 kb), and Chr 15 (2.8 and 2.2 kb) (data not shown). Since Chr 15 contains a region of linkage homology with human chromosome 12, we conclude that this chromosome contains the expressed gene, *Tarbp2,* and that related sequences map to Chr 6 and Chr 7.

To position these 3 TRBP-related loci on the linkage map, progeny of three multilocus crosses were typed for variant restriction enzyme fragments using the 1.3-kb TRBP probe. The Chr 7 locus, *Tarbp2-rs1,* was identified in the NMM cross as a 6.0-kb *Sca*I NFS/N fragment, in the NSC cross as a 6.7-kb *Bam*HI *spretus* fragment, and in the NSS cross as a 19.4-kb *Bam*HI NFS/N fragment (Fig. 2). Comparison of the inheritance of this locus with markers already typed in these 3 crosses positioned this gene on distal Chr 7 between *Zp2* and *Oat* as shown in Fig. 3.

The Chr 6 locus, *Tarbp-rs2,* was identified as a 13.8-kb *Sca*I NFS/N fragment in the NMM cross and as an 8.6 kb *Bam*HI NFS/N fragment in the NSS cross. No fragment corresponding to this locus was identified in *M. spretus* DNA; following *Bam*HI digestion, *spretus* DNA contains only the 6.7-kb Chr 7 *Tarbp-rs1* fragment and the 2.8- and 2.2-kb fragments that map to Chr 15. This Chr 6 locus, present in inbred mice but not in *M. spretus,* was positioned in the proximal region of Chr 6 near *Tcrb* (Fig. 4).

Analysis of the NMM cross following digestion with *Sca*I and *Bgl*II and the *spretus* crosses following digestion with *Bam*HI and *Sac*I failed to identify polymorphic fragments corresponding to the Chr 15 locus using as hybridization probes the 1.3-kb TRBP probe and the 637-bp Trbp2 probe. Digestion of the parental DNAs

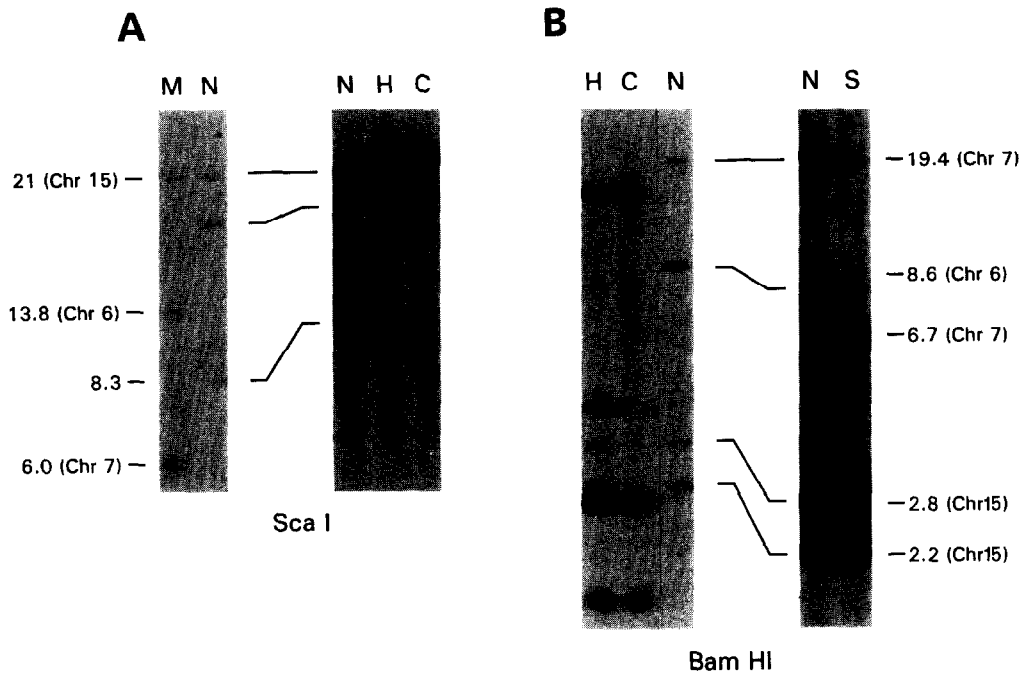

FIG. 2. Southern blot analysis of mouse DNAs using TRBP2 as hybridization probe following digestion with *Sca*I (**A**) and *Bam*HI (**B**). Lane **M,** *M. m. musculus;* lane **N,** NFS/N; lane **H,** Chinese hamster × mouse somatic cell hybrid containing Chr 15; **C,** Chinese hamster; lane **S,** *M. spretus.* Fragment sizes are indicated to the left in **A** and to the right in **B** along with chromosome locations for the fragments that could be mapped.

of the *M. spretus* crosses with 8 additional restriction enzymes and those of the NMM cross with 12 additional enzymes failed to identify a cross/enzyme combination for which all fragments could be typed in the progeny. In an effort to determine whether any of the observed polymorphic fragments were derived from the Chr 15 locus, DNAs from hamster, mouse, and a somatic cell hybrid containing mouse Chr 15, but not Chr 6 or Chr 7, were analyzed by Southern blotting. These DNAs were digested with *Bam*HI, *Sca*I, and 6 other enzymes that produced polymorphic fragments among parental DNAs. In all cases, however, the only mouse TRBP-related fragment present in the Chr 15 hybrid was the fragment identified as nonpolymorphic in the

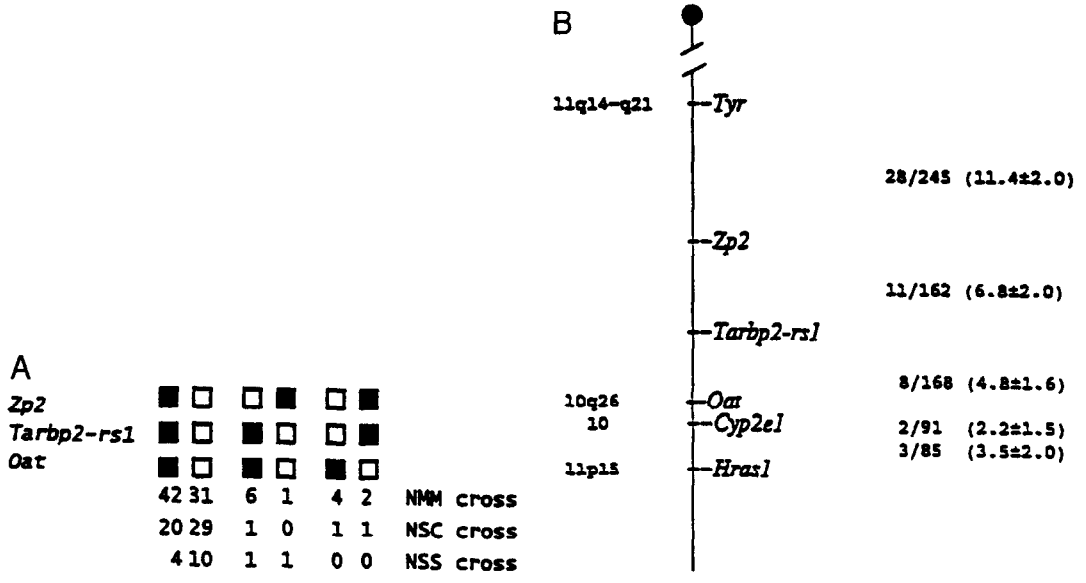

FIG. 3. (**A**) Segregation of *Tarbp2-rs1* and the flanking genes *Zp2* and *Oat* in the progeny of three genetic crosses. Filled squares represent heterozygous mice; open squares are homozygotes. Numbers under each column represent the number of mice with each genotype. (**B**) An abbreviated map showing the map location of *Tarbp2-rs1* with *Zp2, Oat,* and other markers on distal Chr 7 mapped in these crosses. For each adjacent locus pair the recombination fraction is given along with percentage recombination and standard error calculated according to Green (1981).

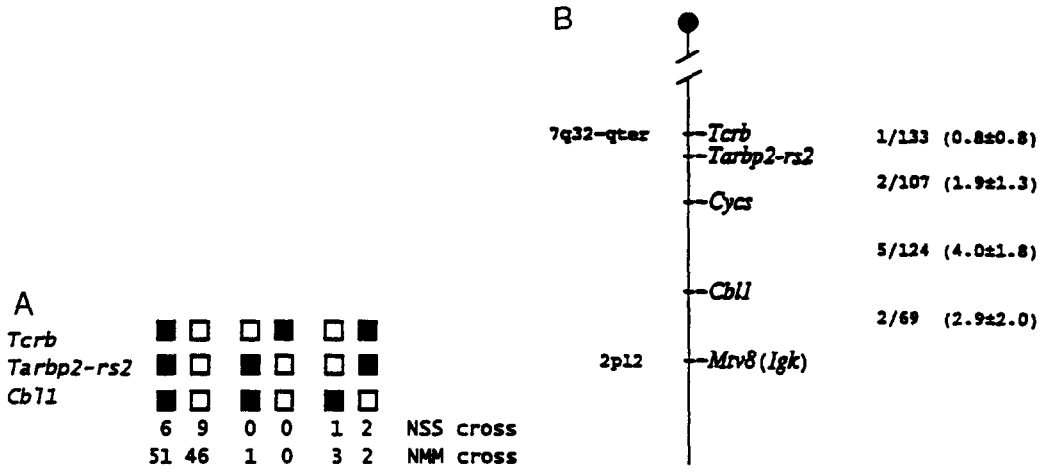

FIG. 4. (**A**) Segregation of *Tarbp2-rs2* and the flanking genes *Tcrb* and *Cbl1* in the progeny of two genetic crosses. Filled squares represent heterozygous mice; open squares are homozygotes. Numbers under each column represent the number of mice with each genotype. (**B**) An abbreviated map showing the map location of *Tarbp2-rs2* with *Tcrb, Cbl1,* and the additional Chr 6 markers *Cycs Mtv8.* For each adjacent locus pair the recombination fraction is given along with percentage recombination and standard error calculated according to Green (1981). *Cycs* and *Mtv8* were not typed in the NSS cross, and therefore the recombinant fractions for locus pairs distal to *Tarbp2-rs2* were taken from the NMM cross only.

parental mouse DNAs (Fig. 1). Therefore, this locus, *Tarbp2,* could not be positioned in our crosses.

Expression of TRBP-Specific Proteins in Murine and Primate Cells

The cDNA for the mouse homologue of TRBP has been isolated (R. E. Braun, University of Washington, pers. comm., 1993). A comparison between human and mouse revealed >95% identity in coding amino acid sequences and identified a message of 1.6 kb in both species (Gatignol, unpublished data). To determine whether rodent TRBP and primate TRBP were processed identically within cells, we examined protein expression in mouse (NIH 3T3), Chinese hamster (CHO and E36), monkey (Cos), and human (HeLa and Jurkat) cells (Fig. 5). With an antibody raised against two TRBP-specific peptides (Gatignol *et al.,* 1993), immunoblotting analysis revealed qualitative and quantitative differences among these cells (Fig. 5). Interestingly, in mouse cells, despite the high conservation with human seen at the cDNA level, two TRBP bands were detected with molecular masses of 90 and 37 kDa (Fig. 5, lane 1). In hamster cells, only the 37-kDa protein was detected (Fig. 5, lanes 2 and 3). In comparison, monkey and human TRBP migrated with an apparent molecular mass of 55 kDa (Fig. 5, lanes 4–6). These findings suggest that the regulated expression of TRBP differs from cell to cell and that there are species-specific differences in post-translational events.

DISCUSSION

RNA-binding proteins play important roles in many cellular metabolic processes (see reviews, Bandziulius *et al.,* 1989; Kenan *et al.,* 1991; Mattaj, 1993; Zamore *et al.,* 1990). In the case of HIV-1, they contribute critically to transcriptional, post-transcriptional, and virion packaging functions (see reviews, Jeang *et al.,* 1991; Jeang and Gatignol, 1994; Vaishnav *et al.,* 1991). TRBP is one of many cellular proteins that bind to the HIV-1 leader RNA, TAR (see review Jeang *et al.,* 1991). It associates with the double-stranded stem of the TAR RNA hairpin. In contrast, other cellular proteins that bind to the loop and the bulge of TAR have been described (Sheline *et al.,* 1991; Wu *et al.,* 1991). Mutational analyses of HIV-1 TAR RNA have shown that the stem, the bulge, and the loop elements are all important for function (Berkhout and Jeang, 1989, 1991; see references below). This suggests that intimate contacts between the many TAR RNA-binding proteins are responsible for overall TAR function. Because TRBP is the only TAR-binding protein that has been molecularly cloned to date, characterization of the interactive biology of TAR-binding proteins is not possible.

TRBP was originally isolated as an RNA-binding protein that bound with great affinity to the double-stranded stem of TAR RNA (Gatignol *et al.,* 1991, 1993). Although the physiological function of TRBP in mammalian cells is unknown, current observations suggest that it serves as a cellular antagonist of the interferon-induced double-stranded RNA-activated protein kinase (Jeang, unpublished observation). We had previously observed that TRBP could augment the expression from the HIV-1 promoter, as well as that from certain other promoters (Gatignol *et al.,* 1991). Because a gene mapping to human chromosome 12 has been implicated as being important for HIV-1 gene expression in human cells (Hart *et al.,* 1989; Newstein *et al.,* 1990; Alonso *et al.,* 1992), and because our data map the expressed TRBP gene to human chromosome 12, it is possible that the TARBP2 gene product either is responsible for or contributes to this phenomenon. Direct confirmation awaits further studies.

TRBP has an RNA binding domain that is conserved

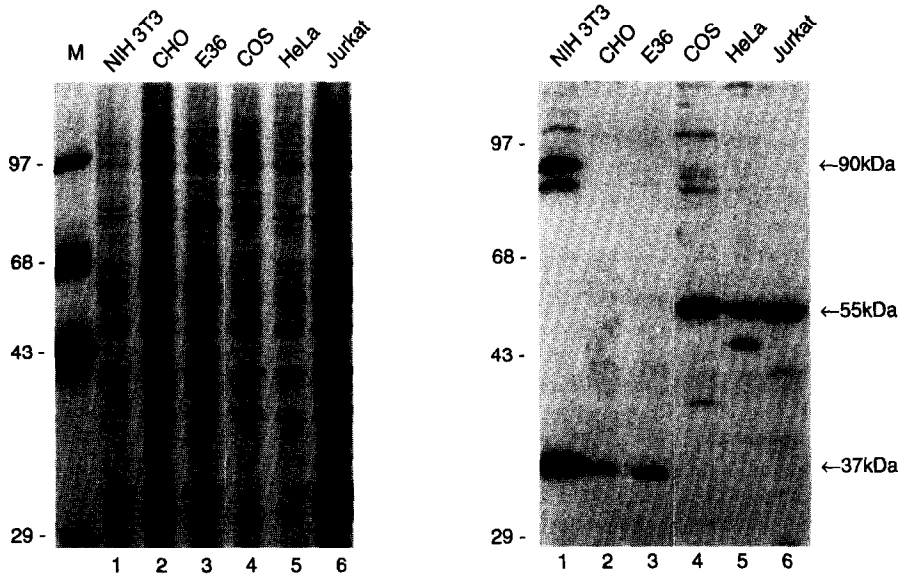

FIG. 5. Characterization of TRBP in rodent and primate cell lines. Coomassie staining (**left**) and Western blot analysis (**right**) of TRBP in murine (NIH 3T3, lane **1**), Chinese hamster (CHO, lane **2,** and E36, lane **3**), simian (COS, lane **4**), and human (HeLa, lane **5**, and Jurkat, lane **6**) cells. **M** represents molecular weight markers. The apparent molecular weight of simian and human TRBP is 55 kDa, while the rodent counterparts are 90 and 37 kDa for the mouse and 37 kDa for the hamster.

in the human P1/dsI protein kinase and in *Drosophila* staufen. Although the true role of TRBP in cells has not been defined, this homology with other RNA-binding proteins offers a functional clue. Ongoing experiments indicate that P1/dsI protein kinase can potently inhibit translation of HIV-1 proteins; however, overexpression of TRBP reverses this inhibition (Jeang, unpublished observation). Thus, TRBP might be a natural cellular regulator of P1/dsI kinase.

Genetic mapping indicates that mouse and human genomes contain multiple loci with sequences related to TRBP—two in human and three in mouse. The expressed gene in human, TARBP2, was mapped to chromosome 12p12.1–q13.1. Since mouse homologs of genes in this region of the human map have been localized to distal mouse Chr 15, this identifies the TRBP-related sequences assigned to mouse Chr 15 using somatic cell hybrids as the expressed gene and also suggests a regional assignment for this locus in the mouse chromosome. Although both human and mouse genomes contain a functional TARBP2 locus with a high degree of sequence conservation, Western blot analysis identifies gene products of different molecular mass in primate (human and monkey) and rodent (mouse and hamster) cells (Fig. 5). Whether this difference, presumably due to post-translational modification, is responsible for a functional difference in mouse vs primate cells and thereby contributes to the failure of HIV-1 to replicate in mouse cells remains to be determined.

In the human, the TRBP pseudogene maps to chromosome 8 and was clearly defined as a pseudogene by use of a 60-bp pseudogene-specific probe. The mouse contains two additional loci, one of which, *Tarbp2-rs1,* was identified in inbred laboratory mice as well as the wild mouse species, *M. spretus.* The second additional locus, *Tarbp2-rs2,* was not present in all mice; it was detected in the laboratory mice analyzed in genetic crosses (NFS/N and C58/J) and in somatic cell hybrids (NFS/N, BALB/c, and A/HeJ), but was not present in the cross designed to follow inheritance of *M. spretus* alleles. We can conclude that *M. m. musculus* also lacks this locus since many restriction enzymes generated identical TRBP fragment patterns in *M. spretus* and *M. m. musculus,* indicating that the two contain a comparable complement of TRBP-related loci. This Chr 6 locus therefore appears to represent a TRBP sequence present in inbred mice but not common to other species of *Mus.*

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