

A LOW POLYMORPHIC MOUSE H-2 CLASS I GENE FROM THE *Tla* COMPLEX IS EXPRESSED IN A BROAD VARIETY OF CELL TYPES

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Class I antigens are heterodimers that usually consist of a 40–45-kD glycosylated heavy chain often anchored in the cell membrane, in association with a 12-kD light chain, β_2 -microglobulin (β_2 -m). In the mouse, β_2 -m is encoded by a single gene located on chromosome 2, whereas heavy chains are encoded by multiple class I genes in the H-2 complex on chromosome 17 (reviewed in reference 1).

The major transplantation antigens, encoded at the H-2K, H-2D, and H-2L loci, are highly polymorphic and are expressed in most somatic cells (reviewed in references 2 and 3). They function as recognition elements for CTLs during the interaction and elimination of virus-infected and neoplastically transformed cells. In contrast, the known Qa and TL antigens encoded by genes of the *Tla* complex are poorly polymorphic and are expressed in a few cellular subsets of the hematopoietic lineage (reviewed in reference 4). Their biological function is, as yet, unknown.

The molecular analysis of the H-2 complex of C57BL/10 (or B10) and BALB/c mice has shown that the mouse genome contains 26–35 class I genes (5, 6), the majority of which have been assigned to the *Tla* complex (5, 7, 8). The number of serologically defined Qa and *Tla* region antigens is much lower, implying that many *Tla* complex genes have no identified protein product at the moment.

While investigating the diversity of the H-2-related mRNAs present in the liver of the DBA/2 (H-2^d) mice, we have previously isolated a clone (pH-2^d-37) that contains a full-length cDNA, potentially coding for a new class I antigen (9). Using a probe derived from its unusual 2nd domain coding sequence (the 37 probe), we found that most of the mouse strains studied contain two hybridizing genes. One, as judged by restriction site polymorphism, is poorly polymorphic, and corresponds to the 37 gene. The other displays two allelic forms. The allele

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present in H-2^d haplotype corresponds to the BALB/c *T10^c* gene, which has been cloned by Steinmetz et al. (6) and has been assigned to the *Tla* region (7). Finally, this 37 probe detected RNAs from DBA/2 mouse liver, spleen, and kidney, suggesting that at least one of the two crosshybridizing genes is expressed in each tissue (9).

We report here the isolation and mapping of the 37 gene in BALB/c mouse, its entire nucleotide sequence, as well as the precise tissue distribution of its mRNA. We show here that the 37 gene is located in the *Tla* region and is linked to the previously cloned *T17^c* gene (10). Its nucleotide sequence reveals several unusual features, such as the lack of a conventional TATA box in the promoter region. Finally, despite its location in the *Tla* region, which is usually correlated with expression in a tissue-specific manner, the 37 gene is transcribed in a large variety of cell types, including fibroblasts and brain. We discuss the possible functions of the 37 gene product, consistent with both a low polymorphism and a broad tissue expression.

Materials and Methods

Enzymes and Reagents. Restriction enzymes were purchased from Appligène (Illkirch, France); calf alkaline phosphatase, T4 polynucleotide kinase, and the large fragment of DNA polymerase I were from Boehringer Mannheim, Federal Republic of Germany; T4 DNA ligase was from New England Biolabs (Beverly, MA); reverse transcriptase of avian myeloblastosis virus was from Genofit (Geneva, Switzerland); SP6 polymerase, RNasin, and DNase RNase-free were from Promega Biotec (Madison, WI); T1, A RNases, and agarose were from Sigma Chemical Co. (Poole, United Kingdom). Nitrocellulose membranes (BA85) were obtained from Schleicher & Schüll (Dassel, Federal Republic of Germany); hybond-*N* was from Amersham Corp. (Amersham, United Kingdom); and nylon membrane (Biotrans) was from ICN Radiochemicals (Plainview, NY). Unlabeled nucleotides and the hexamers for random priming were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Labeled nucleotides (α -[³²P]UTP-SP6 grade, α -[³²P]dCTP, and γ -[³²P]dATP) were from Amersham Corp.

Mice and Cell Lines. All mice were bred at the Pasteur Institute. Cell lines EL4, F9 teratocarcinoma, and BW5147 were grown in RPMI 1640 medium supplemented with 10% FCS in 5% CO₂. BW1-J were grown as described (11). The 293 human cell line was grown in DME supplemented with 10% FCS in 8% CO₂.

Preparation of DNA and RNA. Plasmid and cosmid purifications were performed by the alkaline lysis procedure (12). Genomic DNA were isolated from mouse liver as described in reference 13. Total RNA from tissues was prepared by the LiCl procedure (14), and in the case of RNA from placenta, brain, and heart, by the method described in reference 15. Total RNA from cell lines was prepared by the hot phenol technique (16). Poly(A)⁺ mRNA was prepared by chromatography on oligo(dT) cellulose. Poly(A)⁺ mRNA from 173 myeloma (BALB/c) was a generous gift of Dr. P. Legrain (Institut Pasteur) and poly(A)⁺ mRNA from MBL2 (C57BL/6) and LSTRA (BALB/c) thymoma were generous gifts of Dr. P. Baldacci, Institut Pasteur (17). For each preparation, the quality of the RNA was tested by glyoxal agarose gel electrophoresis (18).

Isolation and Characterization of the 37 Genomic Clone. A genomic BALB/c sperm DNA library constructed in the vector Charon 4A (19) and containing 1.5×10^6 clones was screened with the 250 Pst I fragment of pH-2^d-37 (9) (37-specific probe), labeled by nick translation to a sp act of 4×10^8 cpm/ μ g. Positive clones were plaque purified and DNA prepared (12). The restriction enzyme map was done by using partial digests of the λ phage DNA hybridized to the end-labeled cosR oligonucleotides (20). The position of the genes on genomic DNA insert was determined by Southern blotting. After transfer, the digested DNA was hybridized either to the Eco RI-Kpn I (515 bp) or to the Sac I-Hind

III (306 bp) fragments of pH-2^d-33 (21). These fragments correspond respectively to 5' or 3' H-2 DNA probes.

Chromosome Walking. A B10.D2-H-2^{dml} liver DNA library constructed in the cosmid vector TL5 and containing ~360,000 clones (22) was screened with a 0.8 kb Eco RI–Bgl II fragment isolated from a subclone of phage λ .16 DNA (see Fig. 2A) and labeled to a sp act of 4×10^9 cpm/ μ g by the oligolabeling technique (23). Hybridization was performed as described in reference 24. Restriction map analyses were performed using the standard double-digestion method. Probes used for mapping included TL5 (vector), 37-specific third exon probe (9), the 0.8-kb Eco RI–Bgl II flanking sequence probe, and a 500-bp Kpn I–Eco RI fragment from a T9 cDNA clone (Hunt, S. W., III, K. Brorson, H. Cheroutre, and L. Hood, manuscript in preparation).

Preparation of Antisense RNA Probes and RNase Mapping. The 250-bp Pst I fragment which is contained in the second domain coding sequence of pH-2^d-37 (9) was prepared and subcloned at the Pst I site of pSp65 plasmid vector (25) by classical procedures. The Ribo-37.2 clone, which contained the insert in the proper orientation, was selected for the synthesis of an antisense RNA probe. The *T10^c* gene contained in cosmid 12.2 was a generous gift of Dr. M. Steinmetz (Basel Institute for Immunology, Basel, Switzerland). The *T11^b* gene, contained in a 9-kb Bam HI fragment subcloned in pUC13 vector, was a generous gift of R. A. Flavell (Biogen, Cambridge, MA). The 220-bp Pst I–Pvu II restriction fragments, which are located in homologous positions in the two genes and correspond to part of the third exon, were subcloned in pSp65 plasmid vector digested with Hinc II and Pst I restriction enzymes to generate the Ribo-T10 and the Ribo-T11 plasmids. Ribo-37.2, Ribo-T10, and Ribo-T11 DNAs were linearized at the Hind III site and used as templates for the synthesis of RNA probe (25), except that the labeled nucleotide was UTP instead of GTP.

RNase Mapping Analyses. RNase mapping analyses were performed according to Melton et al. (25), except that RNase A was used at 4 μ g/ml. Hybridization of the labeled probe (0.5 ng) with 10–20 μ g of total RNA or 0.5 μ g of poly(A)⁺ mRNA was performed overnight at 45°C. Protected fragments were analyzed by electrophoresis on 6 or 7% acrylamide sequencing gels. pBR322 digested by Hpa II and end labeled using Klenow fragment was used as size markers.

Southern Blot Analysis. The DNA was transferred onto membrane according to Southern (26) or Reed and Mann (24). DNA probe fragments were labeled either by nick translation (12) or by the oligolabeling technique (23). Hybridizations on total mouse genomic DNA were performed with a labeled RNA probe derived from Ribo-37.2 plasmid (see above).

Sequencing of the 37 Gene. Fragments of λ .16 DNA were subcloned in both orientations, using M13 tg130 or tg131 DNA (27); DNA sequencing was performed according to Sanger et al. (28) using either the specific M13 universal primer, or the 37-specific oligonucleotide.

Primer Extension. The oligonucleotide 5'GGGACTGTGGGGCTGACCAG3' complementary to positions 63–82 in the leader segment of the mRNA coding sequences was synthesized by the phosphoramidite method (29) and purified by polyacrylamide gel electrophoresis. ~0.3 ng of this oligonucleotide that had been end-labeled with T4 polynucleotide kinase (sp act 3.6×10^8 cpm/ μ g) (12) was coprecipitated with RNA. The pellet was resuspended in 10 μ l of buffer containing 50 mM Tris-HCl (pH 8.0), 50 mM KCl, 5 mM MgCl₂, and 15 mM DTT, heated for 3 min at 95°C, and allowed to hybridize for 2 h at 60°C. Elongation was initiated by the addition of the four deoxyribonucleotides (0.5 mM each) and reverse transcriptase (5 U) in the presence of RNasin (13 U). After 1 h of incubation at 42°C, the reaction mixture was extracted with phenol-chloroform (1:1), was ethanol precipitated, and was analyzed on a 10% polyacrylamide sequencing gel.

Transient Expression Assays. About 5×10^5 cells of the human cell line 293 were spread on 5-cm plates. The next day, 5 μ g of the 37 gene contained in p37EH12 plasmid DNA (Hind III–Eco RI fragment of λ .16 subcloned in pGEM4) were added per plate using the calcium phosphate precipitation technique (30). 48 h after transfection, total

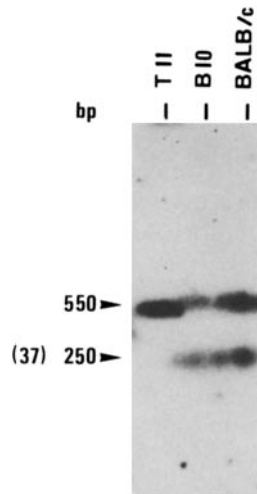


FIGURE 1. Hybridization patterns of *T11^b* gene DNA and genomic DNAs from B10 (H-2^b) and BALB/c (H-2^d) mice with the 37 probe. The DNA origin is indicated above each lane. Size markers used were *Hinf* I-digested pBR322 plasmid and *Hind* III-digested λ phage DNA. Southern blots and hybridization with Ribo-37.2 RNA probe were performed as described in Material and Methods. (37) indicates the fragment specific for the 37 gene. Sizes of hybridizing fragments are indicated in base pairs on the left side. The restriction enzyme used was *Pst* I.

RNA was extracted and assayed for the presence of the 37 mRNA by RNase mapping analyses.

Results

Isolation and Mapping of the Gene Corresponding to pH-2^d-37. Cosmid cloning has allowed the isolation and mapping of most class I genes in BALB/c and B10 mice. When the 37 probe (i.e., the *Pst* I 250-bp fragment encompassing the presumptive second domain coding region) (9) was used to screen the B10 class I cosmid clones of Weiss et al. (5), only one gene, named *T11^b*, was found positive (Flavell, R. A., personal communication). Upon Southern blot analysis of *Pst* I-digested B10 and BALB/c genomic DNA, the 37 probe revealed two fragments of 250 and 550 bp (Fig. 1), the former being specific for the 37 gene (9). Both the B10 *T11^b* and BALB/c *T10^c* genes gave rise to 550-bp *Pst* I fragments that hybridized to the 37 probe (Fig. 1 and reference 9). Therefore, the *T11^b* gene is probably the allele of the *T10^c* gene as already suggested (5) and neither of them encodes the transcript cloned as pH-2^d-37.

Since the corresponding gene was absent from two well-characterized cosmid libraries, we used the 37 probe to screen λ clones from a genomic library of BALB/c sperm DNA (19). Four positive clones were isolated and their DNAs were analyzed by Southern blotting with the 37 probe. One clone (λ .16) carried a 16.1-kb insert, which gave rise to the 250-bp *Pst* I and 1,600-bp *Pvu* II-positive fragments that are characteristic for the 37 gene (see above and reference 9), while the other three contained the related *T10^c* gene.

The λ .16 insert was further characterized by restriction enzyme mapping and hybridization with H-2 probes derived from the first of third domain coding sequences of an H-2K^d cDNA clone (21) (data not shown). These analyses revealed the presence of two class I genes spaced by \sim 7 kb and in the same 5' to 3' orientation, one of which specifically reacted with the 37 probe (Fig. 2A).

Since the *T10^c* gene also reacts with the 37 probe and because duplications of large regions of DNA in the *Tla* region of the BALB/c mouse have been described (5, 10), the restriction maps of the λ .16 insert and that of the *T10^c* gene region were compared. Indeed, several restriction sites (e.g., *Bam* HI, *Kpn*

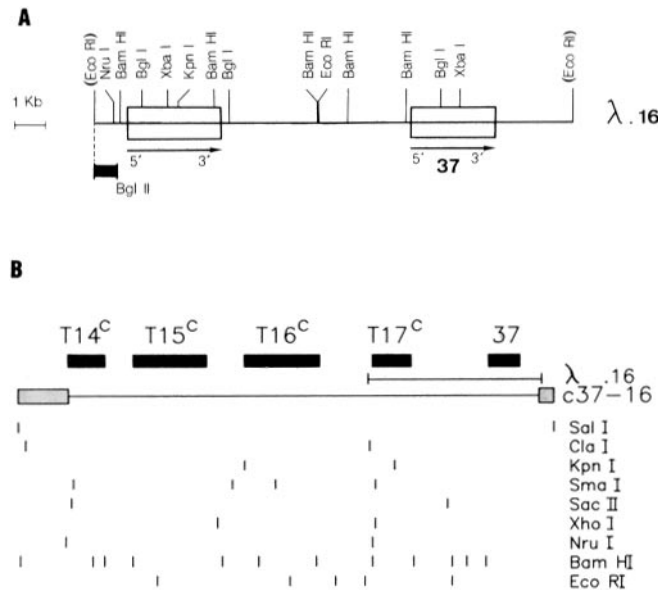


FIGURE 2. Localization of the *37* gene in λ .16 phage DNA and in c37-16 cosmid clone DNA. (A) Restriction map of λ .16 clone insert. Open boxes correspond to DNA fragment hybridizing with the H-2-specific probes (see Materials and Methods and the text). The 5' to 3' arrows correspond to direction of transcription. Position of the *37* gene is indicated. The *Eco* RI sites in brackets refer to the sites generated by the cloning technique (18). The *Eco* RI-*Bgl* II fragment used for chromosome walking is indicated. (B) Restriction map of the c37-16 cosmid clone. The cosmid DNA has been linearized at the *Sal* I site in the vector. The arms of the cosmid vector are shaded. The position of the λ .16 clone is indicated above the cosmid map. The black boxes indicate the positions of the different class I genes in the cosmid.

I, and *Nru* I) were in similar relative positions, suggesting that the *37* and the *T10^c* genes may also have arisen via gene duplication. Since the four genes, *T6^c*, *T7^c*, *T8^c*, and *T9^c* located 5' to the *T10^c* gene have been duplicated to yield *T14^c*, *T15^c*, *T16^c*, and *T17^c*, it seemed possible that the class I gene linked 5' to the *37* gene in the λ .16 insert was the *T17^c* gene. This was confirmed in two ways.

First, a T9 cDNA probe that reacts only with the *T9^c* and *T17^c* genes (Hunt, S. W., III, K. Brorson, H. Cheroute, and L. Hood, manuscript in preparation) strongly hybridized with the λ .16 insert. Second, using the same probe and a low copy probe that maps 9 kb 5' to the *37* gene in the λ .16 insert (Fig. 2A), we screened cosmid clones from a B10.D2-H-2^{dml} genomic library (22) and identified a cosmid clone (c37-16) that contained the *37* gene. As shown in Fig. 2B, the c37-16 cosmid clone contains the *37* gene and three other class I genes. Its restriction map in the region 5' to the *37* gene is identical to the restriction map of the region containing the *T15^c* to *T17^c* genes in the BALB/c mouse (10).

Altogether these results place the *37* gene 7 kb 3' to the *T17^c* gene in the Tla gene cluster shown previously to contain the *T11^c* to the *T17^c* genes.

These data confirm our previous assignment of the *37* gene to the Tla complex using recombinant mouse strains between A mice and B10 mice that contain, respectively, a single and two *37*-related genes (31).

Structural Analysis of the *37* Gene. The cloned *37* gene is 2.73 kb long. Its restriction map is shown in Fig. 3A and its entire nucleotide sequence is given in

Fig. 3*B*. When compared with the pH-2^d-37 cDNA sequence (isolated from DBA/2 mouse) (9), the gene sequence differs at three nucleotide positions (see Fig. 3*B*), none of which leads to an amino acid change. At all three positions, a G in the genomic sequence is replaced by an A in the cDNA sequence. This may be due to either polymerase errors during the synthesis of the cDNA, or to polymorphism between the BALB/c and DBA/2 mouse strains.

Structurally, the gene can be divided into seven exons. All intron-exon junctions obey the GT/AG rule (32). Exons 1–5 are distributed as in all other murine class I genes characterized to date (1, 9). In contrast, the putative cytoplasmic domain of the 37 gene product is encoded by only two exons (exons 6 and 7), whereas three exons (exons 6–8) are used in the classical class I genes and a single exon (exon 6) is used in the genes that encode the TL antigens (10, 33, 34).

The translation termination codon occurs in exon 7 and is followed by a 3' noncoding sequence. The latter consists of a B2 (Alu like) element (35) that contains several overlapping polyadenylation signals AATAAA.

Another distinctive feature of the 37 gene structure is the relatively small size of the third intron. This intron is 539 bp long, and does not contain the 1.1 kb insertional sequence that is present in two *Tla* genes coding for TL antigens (10, 34), nor does it contain insertions characteristic of H-2 class I genes (36).

Table I shows the percent sequence homology between exons 1 to 5 of gene 37 and the corresponding exons for other mouse class I genes located in the K (*K^d*) (37), the D (*D^d*) (38), Qa (*Q7^d*) (39) and the *Tla* (*T13^c*) (10) regions. The overall homology of the 37 gene with the H-2 or Qa class I genes is greater than with the *Tla* gene. Moreover, the third exon, which is used as a 37 gene-specific probe in this study and was previously shown to be the most divergent exon when compared with H-2 and Qa region genes, is even more divergent when compared with the third exon of the *Tla* region gene, *T13^c* (9).

In the vicinity of the putative first exon, the nucleotide sequence contains a CAAT box at the same position as for the H-2 and Qa region class I genes, but does not show a conventional TATA box. It was therefore conceivable that the

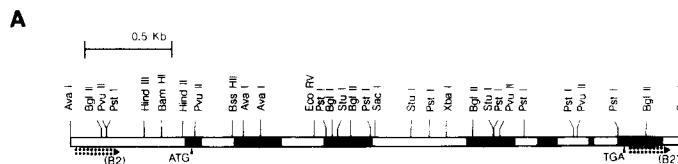


FIGURE 3. Restriction map and sequence of the 37 gene. (A) Fine restriction map of 37 gene. Solid boxes indicate the coding region deduced from comparison with the cDNA (9). The position of the B2 repeated sequence (35) is underlined by dotted horizontal arrows. Position of initiation and termination codons are indicated. The scale is in kilobases. (B) Sequence of the 37 gene and the 5' flanking region. Coding regions are represented as triplets. Position +1 correspond to the mRNA start site. In the promoter region the CAAT box is boxed and the TATA-like boxes are underlined. The three G nucleotides, which are As in the cDNA sequence, are represented by a black triangle. In the 3' untranslated region that is a complete B2 sequence, only the first one of the polyadenylation sites is boxed. The perfect direct repeats that border the B2 sequence are indicated by horizontal arrows.¹

¹ These sequence data have been submitted to the EMBL/GenBank Data Libraries under the accession number Y00629.

TABLE I
*Percent Nucleotide Homology between the 37 Gene and Other Class I
 Genes from H-2^d Haplotype*

Sequence	<i>K</i> ^d	<i>D</i> ^d	<i>Q7</i> ^d	<i>T13</i> ^c
Leader	44	48	43	48
Exon 2 (α 1)	83	84	84	68
Exon 3 (α 2)	72	70	71	64
Exon 4 (α 3)	93	95	94	91
Exon 5 (TM)	88	87	87	66

α 1- α 3, corresponding domains; TM, transmembrane region.

actual promoter sequence lay upstream of an unsuspected more 5' exon. To clarify this problem, we determined the location of the transcription start site, using primer extension experiments. To maximize the specificity of the extension reaction the primer was chosen to be complementary to a specific sequence in the first exon. As shown in Fig. 4, two bands corresponding to 82 and 83 nucleotides fragments are observed with poly(A)⁺ (lane 1) or total RNA (lane 2) from DBA/2 mouse liver. These fragments were not detected with total RNA from an H-2^r teratocarcinoma cell line (lane 3). The major transcription start point inferred from these results is indicated as +1 in the gene sequence (Fig. 3B). The corresponding A is the second nucleotide of the published pH-2^d-37 cDNA sequence (9), which is, therefore, a full-length cDNA.

Furthermore, in transient transfection assays, we have observed that the 37 gene, when flanked by only 240 bp of sequence upstream from its cap site, is transcribed in human cells (not shown), suggesting that an active promoter lies in this region.

Tissue Distribution of the 37 mRNA. We could not reliably investigate the tissue distribution of the 37 mRNA using Northern blots because the 37 probe hybridizes to both the *T10*^c (or *T11*^b) and 37 genes. Therefore, we undertook RNase mapping analyses, using a labeled antisense RNA probe derived from pH-2^d-37. The 250-bp Pst I-Pst I fragment, which contains most of the second domain coding sequence (9), was subcloned in the appropriate orientation at the Pst I site of the pSp65 vector (25), yielding the Ribo-37.2 clone. Linearization of Ribo-37.2 DNA by Hind III allows the synthesis of a 305-nucleotide probe that includes 54 nucleotides of vector sequence in addition to the 37 exon 3 sequence. Thus, the undigested probe should be easily distinguished from the fragment protected by the 37 mRNA (250 nucleotides). This was verified in reconstruction experiments (Fig. 5). The pH-2^d-37 DNA (lane *e*) protects a riboprobe fragment the size of which corresponds to the elimination of the vector sequence. In contrast, DNA containing the *T10*^c (lane *i*) and *T11*^b (lane *g*) genes protects shorter fragments; the largest one, which has the same size for both DNAs, is about 122 nucleotides long. These results suggest that the heteroduplexes formed between *T10*^c or *T11*^b gene DNAs and the 37 riboprobe contain several mismatches which are effectively recognized by the mixture of A and T1 RNases. Both enzymes are required since upon digestion with T1 alone, larger fragments are protected (lanes *d*, *h*, and *f*) and incomplete digestion of the probe occurs (lane *c*).

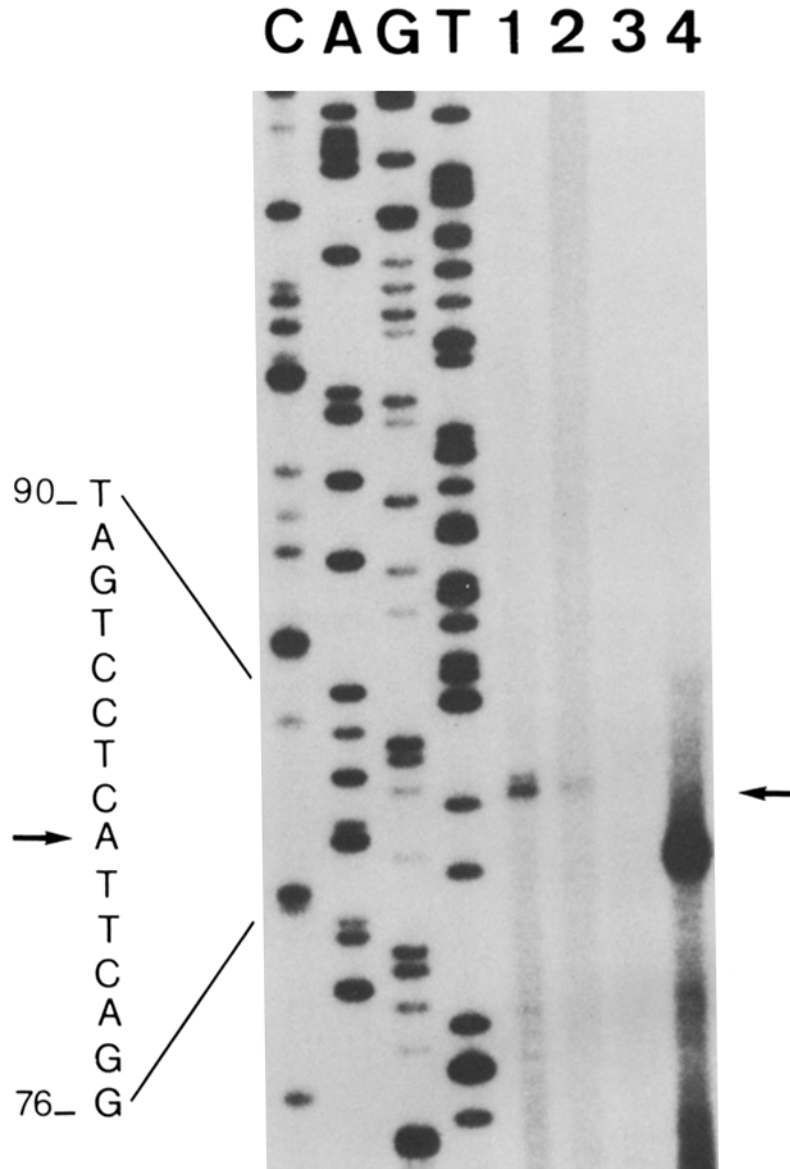
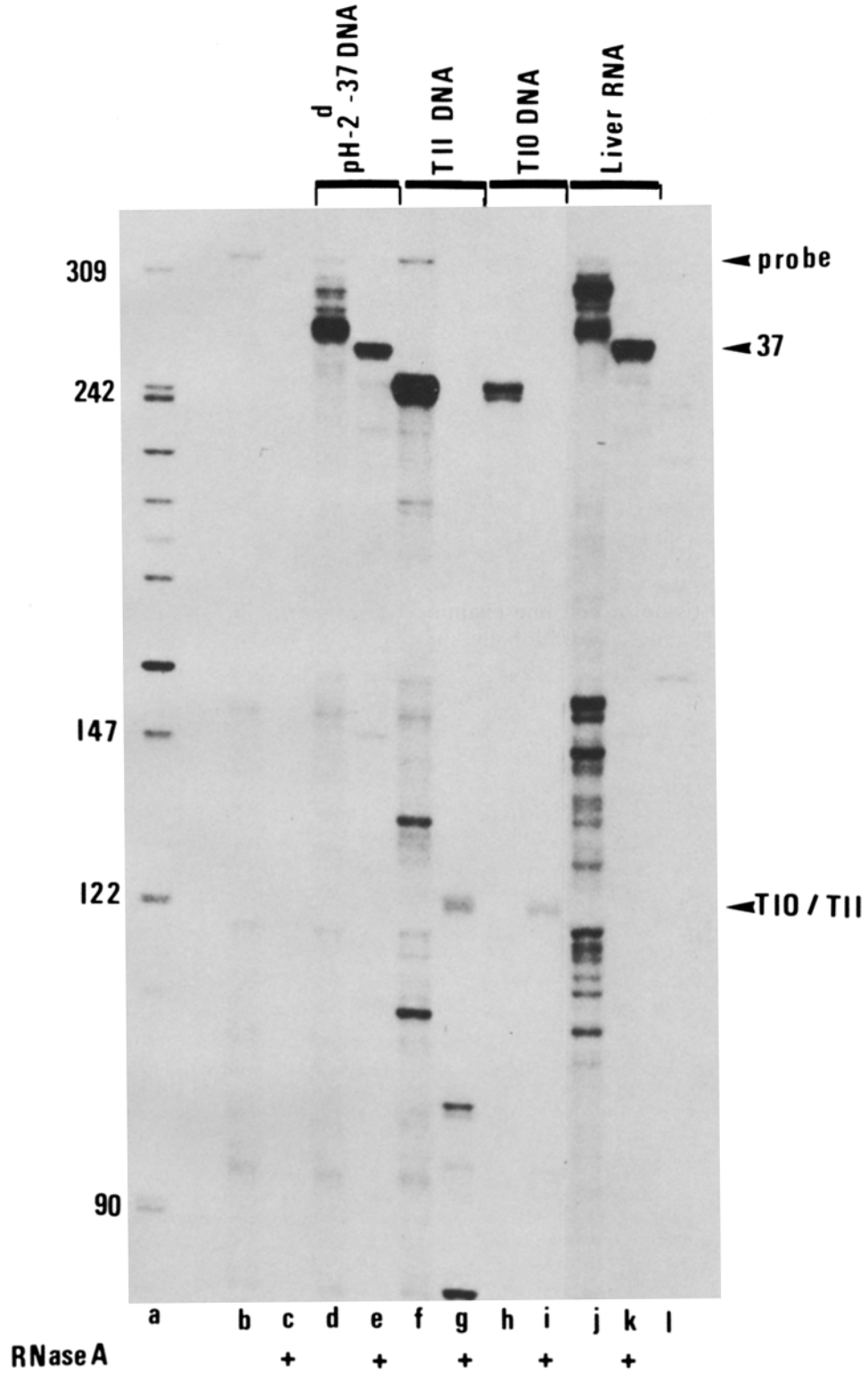


FIGURE 4. Primer extension analysis of the 5' end of the 37 mRNA. The end-labeled oligonucleotide complementary to 20 nucleotides of exon 1 (see Materials and Methods) was hybridized either to 4 μg of poly(A)⁺ (lane 1) or 35 μg of total RNA (lane 2) from DBA/2 mouse liver, and to 35 μg of total RNA from undifferentiated F9 cells (lane 3) or 20 μg of yeast tRNA (lane 4). The hybrids were processed as described in Materials and Methods. DNA sequence of the 5' region of the 37 gene using the M13 sequencing technique (28) with the end-labeled oligonucleotide as primer was carried out to determine the exact transcription start, which is indicated by arrows. On the left side of the figure the enlarged sequence corresponds to the mRNA strand.

As pH-2^d-37 was isolated from a DBA/2 liver cDNA library, DBA/2 liver mRNA was included as a control. In lane 4, a major band is observed at the same position as for pH-2^d-37 DNA. We did not observe a fragment that would



correspond to the protection of the probe by $T10^c$ mRNA. Therefore, if the $T10^c$ gene is transcribed in this tissue, its corresponding mRNA is at least 10–20 times less abundant than 37 mRNA.

In conclusion, our assay unambiguously detects 37 mRNA, as well as $T10^c$ and $T11^b$ mRNA expressed alone or when coexpressed with the 37 mRNA to a comparable extent.

We then assayed RNAs originating from various tissues and from mice of various H-2 haplotypes, and included the DNA controls mentioned above as markers. As shown in Fig. 6, 37 mRNA is detected in all DBA/2 (H-2^d) tissues analyzed, i.e., kidney, spleen, thymus (lanes 4–6), placenta, brain, and heart (lanes 9–11), as well as in C3H (H-2^k) liver (lane 3).

Likewise, results in Fig. 7 indicate that a 37 mRNA species is detected in established cell lines from various origins, i.e., in the two fibroblastic cell lines, 3T3 (H-2^d) and L (H-2^k) (lanes 2 and 4), in the hepatoma cell line BW1-J (H-2^b) (lane 3), in the three thymoma cell lines, EL4 (H-2^b), LSTRA (H-2^d), and MBL2 (H-2^b) (lanes 6, 9, and 10), and in the myeloma cell line, M173 (H-2^d) (lane 8). In contrast, RNA from F9 cells did not protect any fragment (lane 7), in agreement with the previous finding that H-2 class I mRNAs do not accumulate in this cell line nor in other embryonic carcinoma cell lines (40).

These results have two major implications. First, they indicate that the 37 mRNA has a very broad tissue distribution since it was detected in every type of differentiated tissue or cell line examined, apart from the BW5147 thymoma cell line (Fig. 7, lane 5). Additionally, they confirm that the polymorphism of the 37 gene in the region covered by the probe is very limited since the protected fragment has the same size in H-2^k and H-2^b haplotypes as in the H-2^d haplotype. By comparison, the K^k and K^b genes show 23 mismatches in the same region (41). On the other hand, liver RNA from A mice gave rise to smaller protected fragments (Fig. 6, lane 2). In fact, the pattern observed is actually the same as that seen for $T10^c$ DNA, suggesting that in the H-2^a haplotype the single gene that hybridizes with the 37 probe is more related to the $T10^c$ gene than to the 37 gene. In addition, no signal corresponding to protection by $T10^c$ or $T11^b$ mRNAs is detectable except in the case of the three thymoma cell lines, BW5147, EL4, and MBL2 (Fig. 7, lanes 5, 6, and 10, respectively).

Expression of the BALB/c $T10^c$ and the B10 $T11^b$ mRNA. To clarify the question of the expression of the $T10^c$ and $T11^b$ genes we decided to undertake reciprocal experiments using an RNase mapping assay with antisense RNA probes derived from these two genes. A 220-bp Pst I–Pvu II restriction fragment that is contained in the third exon of both the $T10^c$ and $T11^b$ genes was prepared and

FIGURE 5. Use of a pH-2^d-37-derived antisense RNA probe in an RNase mapping assay for the discrimination between the respective second domain coding sequences of the 37, $T10^c$, and $T11^b$ genes. The procedures used for preparation of the Ribo-37.2 probe, hybridization with DNA or RNA, digestion with RNases, and gel electrophoresis are described in Materials and Methods. (Lanes *a* and *l*) Hpa II-digested pBR322 plasmid DNA. The origin of the DNA or RNA hybridized with the probe is indicated above the corresponding lanes. Liver RNA was prepared from DBA/2 (H-2^d) mice. In *b* and *c* the probe was hybridized with yeast tRNA alone. The plus sign below a lane indicates that the digestion was performed with RNase A in addition to RNase T1. Arrows indicate the positions of specific fragments: P for nondigested probe, 37 for the fragment protected by 37 DNA, T10 for the fragments protected by $T10^c$ DNA, and T11 for the fragments protected by $T11^b$ DNA.

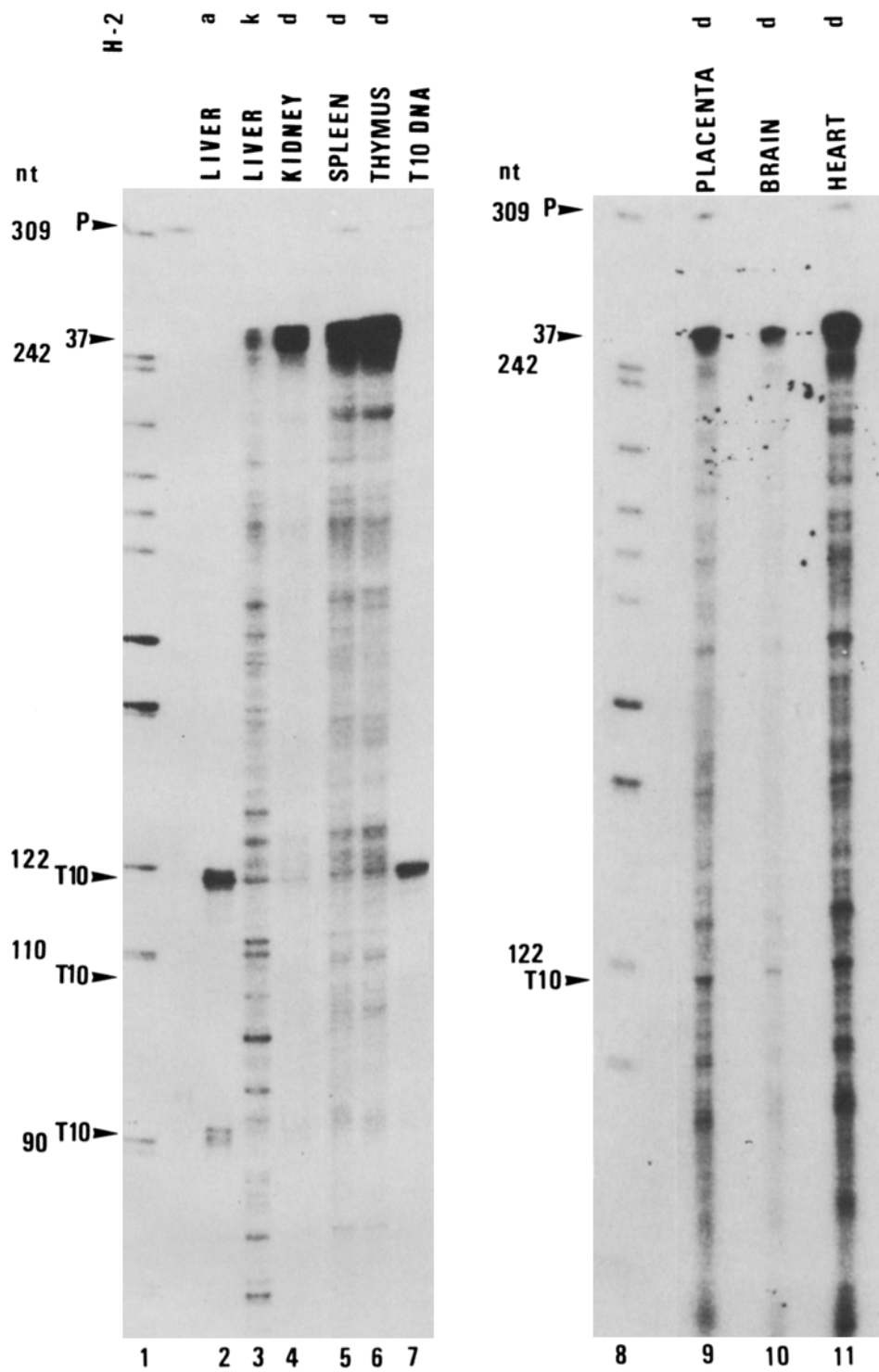


FIGURE 6. Expression of the 37 mRNA in different tissues. The Ribo-37.2 probe and the RNase mapping assay used are described in Materials and Methods. The origin of the RNA or the DNA used for the hybridization with the probe is indicated above each lane, as well as the corresponding H-2 haplotype. H-2^d tissues were recovered from DBA/2 mice, H-2^k tissues from C3H mice, and H-2^a tissues from A mice. Arrows indicate the position of specific fragments (see legend to Fig. 5). Lane 1 corresponds to Hpa II-digested pBR322 DNA.

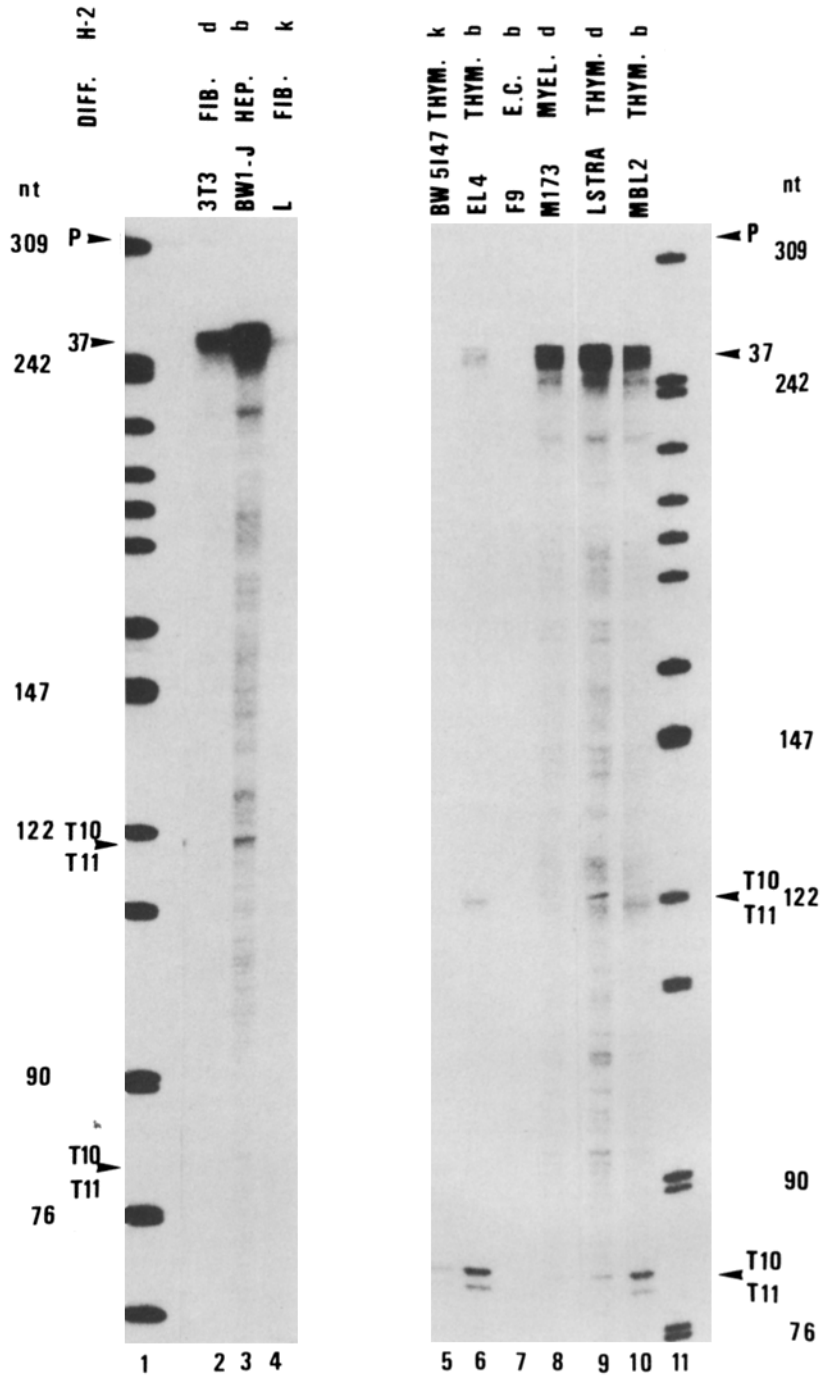


FIGURE 7. Expression of the 37 mRNA in different cell lines. The Ribo-37.2 probe and the RNase mapping assay used are described in Materials and Methods. The origin of the RNA and the H-2 haplotype of the corresponding cell line is indicated above each lane. The differentiation type (*Diff*) is also indicated. *FIB*, fibroblastic; *HEP*, hepatoma; *THYM*, thymoma; *E.C.*, embryonal carcinoma; *MYEL*, myeloma.

subcloned into the pSp65 vector (25). The resulting subclones Ribo-T10 and Ribo-T11 were then used to prepare the corresponding antisense RNA probes. Fig. 8 shows the pattern of protection observed with pH-2^d-37, *T11*^b, and *T10*^c DNAs and with A mice liver RNA. The Ribo-T10 probe gives rise to patterns nearly symmetrical to those obtained with Ribo-37 probe (compare Fig. 8, lanes 11–14 to Fig. 5). In contrast, fragments of Ribo-T11 probe that are protected by either pH-2^d-37 or *T10*^c DNAs are small (~67 and 76 nucleotides, respectively), suggesting that certain mismatches are more efficiently recognized in this configuration (Fig. 8, lanes 4–7). With each probe, A mouse liver RNA gives a pattern similar to *T10*^c DNA, which confirms the structural relationship between the *Tla* gene of the A mouse and the *T10*^c gene of the BALB/c mouse. As shown in Fig. 9, low levels of *T10*^c or *T11*^b mRNA are detected in most RNAs analyzed; however, the 37 mRNA species is at least 10 times more abundant than its *T10*^c or *T11*^b counterpart (Fig. 9, lanes 5–8).

Discussion

Considerable information about class I genes and their organization has been brought about by gene cloning. However, little is known about the expression and function of many of the identified genes. cDNA cloning allows one to study functionally active genes (in the transcriptional sense). It may even lead to the identification of new genes, as illustrated by the unexpected isolation of pH-2^d-37; the corresponding gene had not been previously identified among existing BALB/c nor B10 class I cosmid clones (reference 9 and this paper).

The pH-2^d-37 Transcript Originates from a Gene of the Tla Region. In the present study, we have isolated the BALB/c gene that produces the pH-2^d-37 transcript and have established its physical linkage to *Tla* genes cloned previously by Steinmetz et al. (6) and characterized by Fisher et al. (10). From our results and those obtained by Fisher et al. (10), we conclude that in the *Tla* region of the BALB/c mouse, two groups of linked genes, *T6^c-T10^c* and *T14^c-37* have arisen via DNA duplication. Accordingly, the total number of class I H-2 genes in BALB/c mouse now reaches 36. In the B10 mouse, only the cluster equivalent to the BALB/c mouse *T6^c-T10^c* group, called *T7^b-T11^b*, has been cloned (5). Since a gene producing the pH-2^d-37 transcript also exists in B10 mice, we conclude that it has been duplicated individually, or that there is another cluster of *Tla* region genes in the B10 mouse that has not yet been isolated. On the other hand, this duplication might be a quite recent event in the evolution of the mouse species since some mouse strains, such as the A strain, contain a single gene related to the 37 gene.

Structure of the BALB/c 37 Gene. DNA sequence analysis of the 37 gene and of its 5' upstream region has revealed several features that may have biological implications. First, comparison of coding sequences indicates that the 37 gene has diverged much further from genes coding for TL antigens than from the known H-2 and Qa region genes (Table I). This was previously noted by Pontarotti et al. (34) in their study of two *Tla* genes.

Second, the promoter region of the 37 gene has no obvious similarity with those of other known H-2 and Qa class I genes sequenced to date, except that it displays a CAAT box at the same position relative to the cap site. Promotor

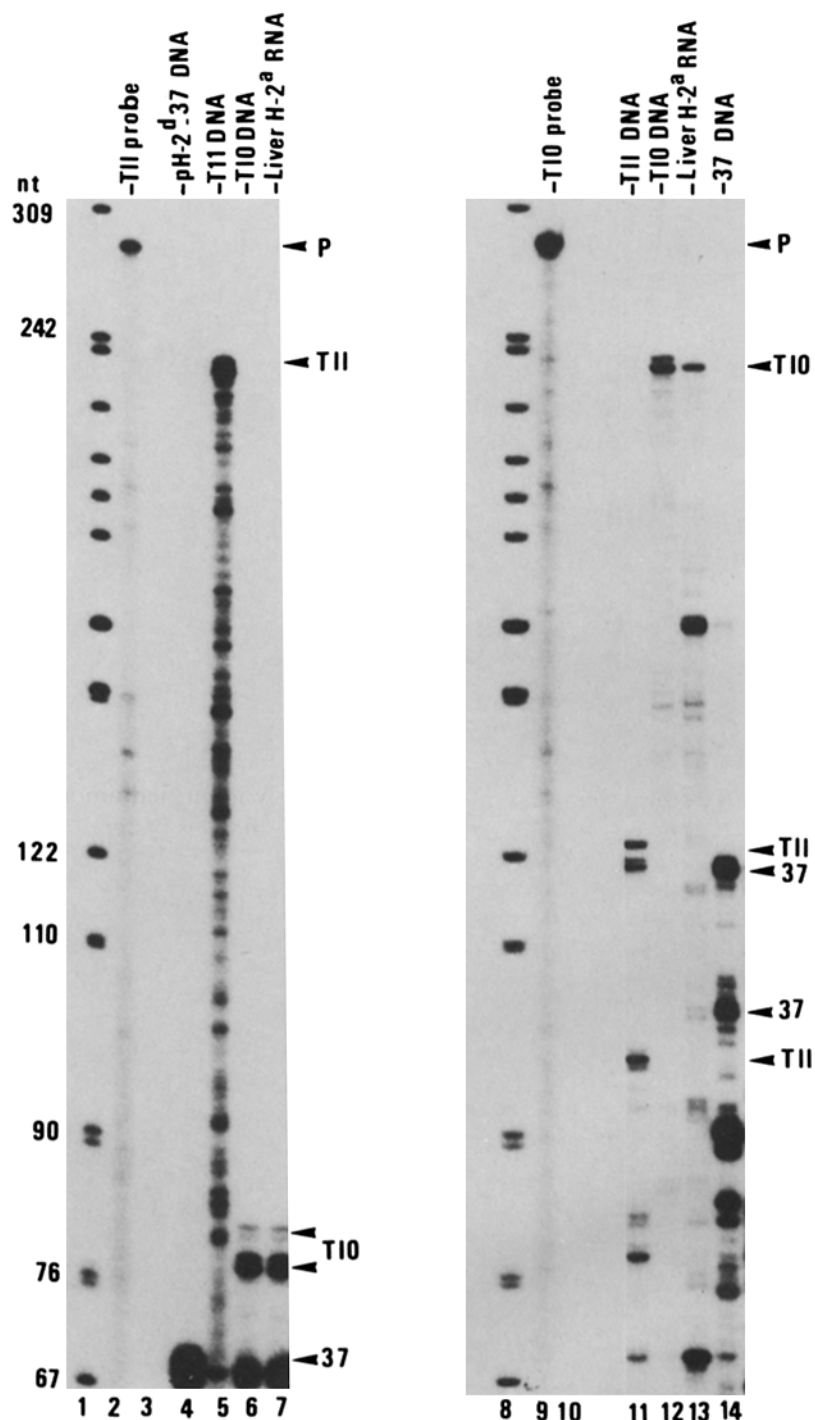


FIGURE 8. Use of *T10^c* gene- and *T11^b* gene-derived antisense RNA probes in RNase mapping assays for discrimination between the respective second domain coding sequences of the *37*, *T10^c*, and *T11^b* genes. Preparation of Ribo-T10 and Ribo-T11 probes and the RNase mapping assays are described in Materials and Methods. The experiment performed with the Ribo-T11 probe is shown on the left panel (lanes 2-7) and those with the Ribo-T10 probe on the right panel (lanes 9-14). (2) Nondigested Ribo-T11 probe; (3) Ribo-T11 probe hybridized with yeast tRNA alone; (4-7) the origin of the DNA or the RNA hybridized with the Ribo-T11 probe is indicated above; (9) nondigested Ribo-T10 probe; (10) Ribo-T10 probe hybridized with yeast tRNA alone; (11-14) the origin of the DNA or the RNA hybridized with the Ribo-T10 probe is indicated above each lane; (1-8) Hpa II-digested pBR322 DNA as size markers. Arrows indicate the positions of specific fragments (see legend to Fig. 5).

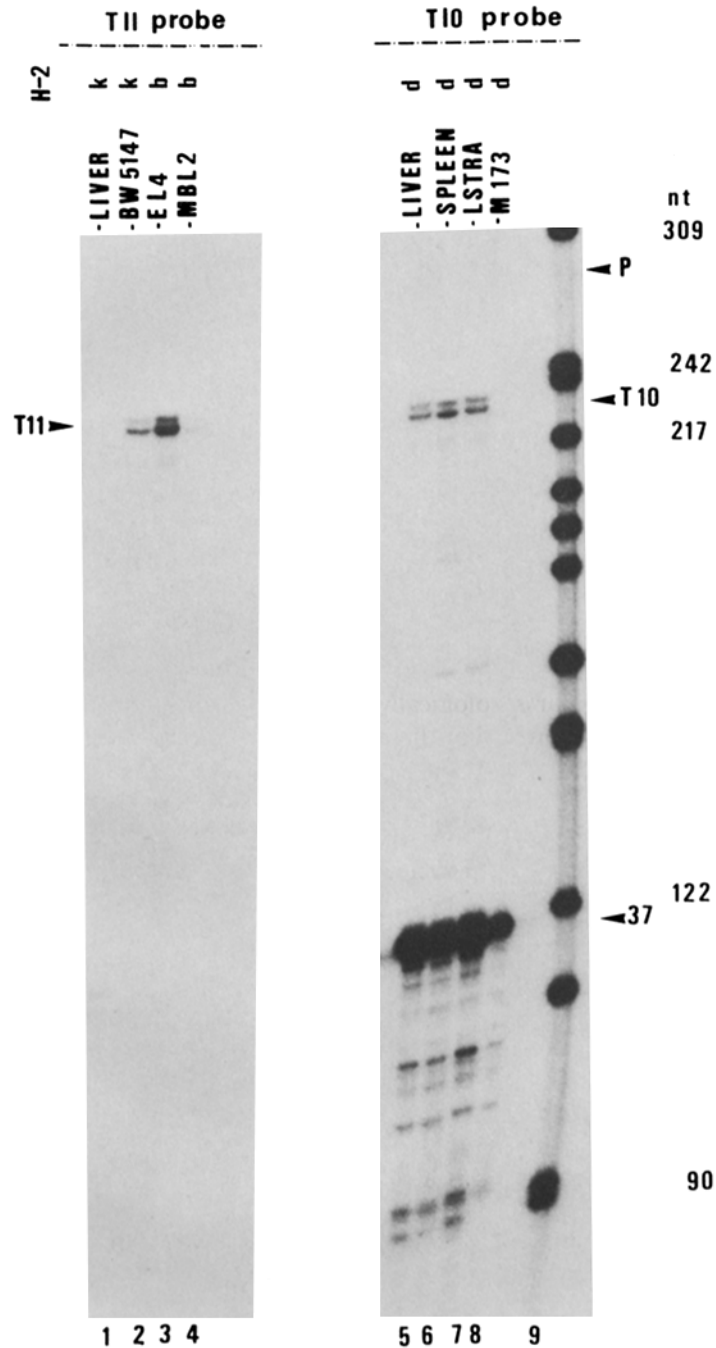


FIGURE 9. Expression of the T10^c and T11^b mRNAs in different tissues and cell lines. The Ribo-T10 and Ribo-T11 probes and the RNase mapping assays are described in Materials and Methods. The origin of the RNA hybridized with the probe as well as the H-2 haplotype of the corresponding tissue or cell line is indicated above each lane. Arrows indicate the position of the specific fragments (see legend to Fig. 5). Lane 9, Hpa II-digested pBR322 DNA as size markers.

regions of H-2 and Qa region genes contain TATA and CAAT boxes and show high sequence homology. In particular, one region containing an enhancer element and the regulatory interferon response sequence (IRS) is highly conserved (42). In contrast, it appears that two genes coding for TL antigens do not contain a typical TATA, nor a typical CAAT box (34), and their putative promoter regions, although very similar to each other, are quite divergent from those of the H-2 and Qa class I genes. The 37 gene promoter region does not show significant homology with the corresponding regions of these *Tla* genes, but like the *Tla* genes it is devoid of a conventional TATA box. Instead two TATA-like sequences are observed. One sequence (TCTCAAA) is very close to the consensus sequence seen in human class I genes (43). The other sequence (TACGAA) is found in the same position as the TATAA boxes of the H-2 class I genes (42).

Tissue Distribution of 37 mRNA. To study the tissue distribution of the 37 mRNA we have used RNase mapping assays that allowed us to analyze, in parallel, the expression of the 37 gene and its related gene. The 37 mRNA was detected in cell lines from various tissue origins and in particular in fibroblastic cell lines. It thus appears unlikely that the signal observed with RNAs extracted from total organs is due to a minority of expressing cells. Instead, the 37 gene is probably expressed in a large variety of cell types. In this respect, it behaves more like the genes coding for the K, D, and L transplantation antigens than like the genes coding for serologically defined TL antigens, the expression of which, both at the protein and at the mRNA levels, is restricted to immature T cells (i.e., mostly thymocytes) of certain mouse strains (44). We have not, however, compared the relative amounts of the 37 and classical H-2 class I gene mRNAs. The 37 gene might show quantitative variations in its level of expression according to cell types.

We have not studied *T10^c* and *T11^b* gene expression nearly as extensively, but it too appears that this expression is not restricted to cells in the early steps of the T cell differentiation pathways. The level of transcription of the *T10^c* and *T11^b* genes is usually far lower than that of the 37 gene, suggesting that the two genes are controlled in different ways although they are the result of a gene duplication event.

Polymorphism. Our RNase protection experiments confirm that the 37 gene is poorly polymorphic in its second domain coding region. RNases detect no mismatches between the H-2^d haplotype probe and RNAs from the H-2^k and H-2^b haplotypes. However, the single 37-related gene present in A mice displays intermediate properties; like the 37 gene, it is easily detectable in liver but it is structurally closer to the *T10^c* gene as judged by the pattern of protection observed with the different probes used.

Possible Functions of the 37 Gene Product. Assuming that 37 mRNA is translated into a protein, which remains to be identified, what could be the function of a class I molecule that is both widely distributed and poorly polymorphic? Several hypotheses can be put forward.

The 37 antigen might be a recognition element for cells known to interact with other cells regardless of their tissue origin. Among the immune cells, most of the CTLs display this property. CTL function is often class I MHC-restricted

in the sense that it generally requires H-2K, H-2D, or H-2L identity between the target and the T effector cells (reviewed in reference 45). However, in mouse and man, several exceptions have been reported. Cells that could kill both syngeneic and allogeneic murine tumor targets have been described for tumors induced by different viruses (e.g., the murine sarcoma virus [46], the Friend leukemia virus [47], and the mouse mammary tumor virus [48]). Seeley et al. (49) have reported that in man, specific CTLs elicited during the primary response to Epstein Barr virus infection are not HLA restricted. Thus, it is conceivable that, when the antigen recognition by CTLs appears not to be MHC restricted, the antigen is presented by a nonpolymorphic class I antigen such as that encoded by the *37* gene.

A second possibility stems from the well-documented data that imply that the Lyt-2 cell surface antigen plays an accessory role in target recognition by both anti-class I allogeneic and class I-restricted CTLs. This antigen is found on CTLs that recognize class I antigens, whereas L3T4 is expressed on anti-class II or class II-restricted cells (reviewed in references 50 and 51). In many cases, anti-Lyt-2 antibodies are able to inhibit target lysis by the Lyt-2⁺ CTLs (52, 53). Since Lyt-2 is poorly polymorphic and does not show clonal variability, it has been proposed that this antigen interacts with a nonpolymorphic determinant shared by the class I transplantation antigens and thus stabilizes low-affinity interactions between the T cell antigen receptor and its ligand (54, 55). Thus, the *37* gene product could provide this nonpolymorphic determinant, which then does not be present on the polymorphic K, D, and L molecules.

Experiments are in progress to isolate the *37* gene product and test whether and how it plays the role of a constant element in cellular interactions modulated by the polymorphic H-2K, D, and L antigens.

Summary

We have previously described (9) the isolation of pH-2^d-37, a cDNA clone that encodes a so far unknown, poorly polymorphic, class I surface molecule. We report here the isolation of the corresponding gene, its nucleotide sequence, and its localization in the *Tla* region of the murine MHC. Using a RNase mapping assay, we have confirmed that the second domain coding region of the *37* gene displays very limited polymorphism, and that the gene is transcribed in a broad variety of cell types, in contrast to the genes encoding the known Qa and TL antigens. Possible functions are discussed.

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