Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 1782–1786, March 1986
Genetics

Conservation and diversity in the class I genes of the major histocompatibility complex: Sequence analysis of a *Tla*^{b} gene and comparison with a *Tla*^{c} gene

(multigene family/thymus leukemia antigen/DNA sequence)

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Communicated by Alex B. Novikoff, October 24, 1985

ABSTRACT **The thymus leukemia (TL) antigens, encoded by class I genes in the *Tla* subregion of the major histocompatibility complex (MHC), are cell surface molecules expressed on thymocytes of certain strains of mice and on certain T-cell leukemias. In order to study the fine structure and interrelationships of genes of the *Tla* subregion, a *Tla*-specific probe was isolated from the TL-encoding *T13*^{c} gene of BALB/c mice (*Tla*^{c} haplotype). The probe hybridized with two *Tla* genes in the *Tla*^{c} haplotype (*T13*^{c} and *T3*^{c}) and with only one in the *Tla*^{b} haplotype (*T3*^{b}). Examination of this subset of *Tla* genes (*T3*^{b}, *T3*^{c}, and *T13*^{c}) by restriction enzyme analysis and oligonucleotide hybridization studies confirmed that *T3*^{b} is the allele of *T3*^{c} and that *T3*^{c} and *T13*^{c} may have arisen by duplication. The *T3*^{b} gene, while not transcribed in the tissues of the TL$^-$ strain C57BL6, was shown to be transcriptionally active in the TL-expressing leukemic cell line ERLD derived from that strain. The *T3*^{b} gene was cloned and its complete DNA sequence was determined. These data permit complete comparison of two *Tla*-region genes, *T3*^{b} and its homologue *T13*^{c}, and allow us to conclude that these genes show extraordinarily high sequence conservation, in contrast to alleles of the *H-2K*- and *H-2D*-region genes. Comparison of *T3*^{b} with other class I sequences in the *H-2* and *Qa* subregions suggests that the *T3*-subset genes are the most divergent from other class I genes.**

The major histocompatibility complex (MHC) class I genes are a multigene group containing the *H-2* genes (*K*, *D*, and *L*), the *Qa* genes, and the *Tla* genes. The number of genes in each subregion varies according to the haplotype, from 2 to 4 for the *H-2* subregion, 8 to 10 for the *Qa* subregion, and 13 to 18 for the *Tla* subregion (1, 2). These genes have a number of features that distinguish them. The *H-2* gene system, in particular the *K* and *D* genes and to a smaller extent the *L* gene, is marked by an extreme polymorphism (>50 alleles per locus; ref. 3) and diversity (15–20% differences in alleles) possibly associated with the role of the products in guiding T lymphocytes to react to cells bearing foreign antigens. On the other hand, those products of the *Tla* and *Qa* subregions that have been studied are significantly less polymorphic [e.g., only 6 alleles of *Tla* have been described (*Tla*$^{a-f}$; ref. 4)]. In addition, unlike the *H-2* products which are expressed on all nucleated cells, the *TL* and *Qa* products have a more restricted tissue distribution. The functions of the products of the *Tla* and *Qa* genes are unknown.

To study the features of a specific gene (and its product) in the *Tla* subregion, we have cloned and determined the nucleotide sequence of the *T3*^{b} gene,¶ which encodes a putative TL protein from the *Tla*^{b} haplotype. The availability of this sequence allows the comparison of this gene to *Tla* genes of the *Tla*^{c} haplotype, as well as to other class I genes, and refines our understanding of the interrelationships of the class I gene group.

MATERIALS AND METHODS

Mice and Cell Lines. Mice were obtained from The Jackson Laboratory (C57BL, A, BALB/c, 129); from L. Flaherty (Albany, NY) (A.CA, B6.AC2-*Tla*^{d}, B6.AC1) or from Memorial Sloan-Kettering Cancer Center (B6.K1, B6.K2, C57BR-Gix(+), A-*Tla*^{b}). The *H-2*, *Qa*, and *Tla* haplotypes of each strain are shown in Fig. 1. The TL-expressing ERLD leukemia cell line, which was induced in the C57BL strain, was kindly provided by E. Stockert (New York, NY).

DNA and RNA Preparation and Analyses. DNA was prepared from spleen cells as described (5). Restriction enzymes were used under the conditions specified by the supplier (Bethesda Research Laboratories). Digested DNA was electrophoresed in agarose gels and transferred to GeneScreen (New England Nuclear) (6). Whole-cell RNA was prepared from fresh tissues as described by Auffray and Rougeon (7). RNA was size-fractionated in agarose/formaldehyde gels and transferred to nitrocellulose. The filters were hybridized, washed, and subjected to autoradiography as described (5).

Isolation and ^{32}P-Labeling of Cloned TL Probe. A 0.1-kilobase (kb) *Pst* I–*Sac* I fragment was subcloned from λ-17.3 (8) (containing the *T13*^{c} gene) into pBR322. The recombinant plasmid was labeled by nick-translation and separated from unincorporated [α-^{32}P]dGTP as described by Maniatis *et al.* (9).

Preparation of TL-Specific Oligonucleotides. Oligonucleotides were chemically synthesized by either the phosphotriester (Bachem Fine Chemicals, City of Hope, CA) or the phosphoramidite (Applied Biosystems, Foster City, CA) solid-phase method.

Sequencing of *T3*^{b} Gene. Fragments of the H10 cosmid (2) were subcloned in both orientations, using M13 mp10, mp11, mp18, and mp19 vectors (Pharmacia). DNA sequencing was performed according to Sanger *et al.* (10), using either the specific M13 primers (Pharmacia) or *T3*-specific oligonucleotides.

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Abbreviation: kb, kilobase(s).

¶We use terminology (to be described elsewhere) in which the *Tla* genes are designated by the letter *T*, followed by a number referring to gene position along the chromosome, with a superscript referring to the *Tla* haplotype. Thus the previously described gene *17.3A* is called *T13*^{c} (see Fig. 2).

RESULTS AND DISCUSSION

Identification and Preparation of the TL-Specific Probe. To learn more about the interrelationships among *Tla* genes, we initiated studies to isolate and sequence a gene coding for a TL product in the Tla^b haplotype. Since peptide map analysis (11) had shown an extraordinary similarity between TL products of the Tla^b and Tla^c haplotypes, and a DNA fragment that expressed a Tla^c (*T13*) product was available (12), we subcloned from that DNA a 0.1-kb *Pst* I–*Sac* I fragment from the 3′ end of exon III (designated pTL). This probe was specific for the *Tla* subregion and hybridized to a subset of *Tla* genes when genomic DNA from a panel of inbred and recombinant mouse strains (Fig. 1) was examined. The pTL probe was shown to be a single-copy probe for the Tla^b haplotype, as it hybridized to a single *Bgl* II restriction enzyme fragment in C57BL DNA (Fig. 1). Similar results were obtained using *Bam*HI, *Eco*RI, *Hin*dIII, *Sma* I, and *Xba* I restriction endonucleases (data not shown).

The TL Probe Detects the $T3^b$ Gene. A panel of cosmids containing 26 class I genes spanning the *H-2*, *Qa*, and *Tla* subregions of C57BL mice (2) was screened with pTL (data not shown). Only the $T3^b$ gene from the H10 cosmid clone hybridizes with pTL, consistent with the finding that pTL hybridizes to a single gene in C57BL genomic DNA (Fig. 1).

Interrelationships of the Subset of Genes Resembling *T3*. The pattern of hybridization of the pTL probe with genomic DNA (Fig. 1) revealed different numbers of genes for different *Tla* haplotypes. For example, Tla^b had one band, both Tla^c and Tla^f had two bands, and both Tla^a and Tla^d had three bands. The finding of different numbers of genes resembling T3 in the various haplotypes is consistent with previous results (8) in which *Tla* subregions of the Tla^c and Tla^b haplotypes were aligned on the basis of restriction endonuclease maps (Fig. 2*A*). In that comparison, $T13^c$ was found to have no allele in Tla^b because it resided on a cluster of genes, $T11^c$–$T17^c$, that was not present in the C57BL mouse.

Since $T3^b$ and $T3^c$ share all *Kpn* I and *Eco*RI sites, and $T13^c$ lacks one *Eco*RI site and has additional *Xba* I and *Sma* I sites (Fig. 2*B*), our data indicate that $T3^b$ is the allele of $T3^c$ and not the allele of $T13^c$, thus confirming the alignment shown in Fig. 2*A*. Further support for the close relationship of $T3^b$ and $T3^c$ as compared to $T13^c$ comes from hybridization analyses with six $T3^b$-specific oligonucleotides (see Fig. 4) prepared from sequence differences between $T3^b$ and $T13^c$, which showed that $T3^b$ and $T3^c$ shared all of these sequences (data not shown). Although $T3^b$ and $T3^c$ seem most alike, $T13^c$ is clearly homologous to these genes. This finding is consistent with the idea that $T3^c$ and $T13^c$ arose by duplication.

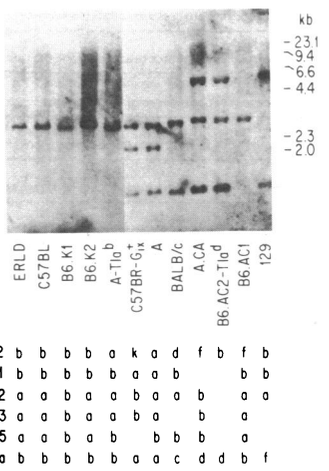

FIG. 1. Hybridization analyses using the pTL probe. Characterization of *Bgl* II genomic restriction fragments by hybridization to the pTL probe shows that the sequences map to the TL region and identifies a single band for Tla^b strains. Each lane contains 20 μg of digested DNA. λ phage DNA digested with *Hin*dIII endonuclease was included as size marker; the position and size (in kb) of the fragments are shown at right. The *H-2*, *Qa*, and *Tla* haplotypes are indicated below each lane.

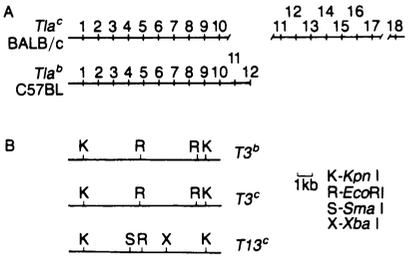

FIG. 2. Interrelationships of *Tla* genes. (A) Diagram of the organization of the *Tla* subregion in BALB/c and C57BL mice, adapted from ref. 13. Numbered vertical bars indicate each locus (e.g., 3 for T3). Genes in the Tla^b subregion corresponding to $T11^c$–$T18^c$ are absent. (*B*) Restriction enzyme map comparison of members of the *T3* subset. Digestions and fragment separations were performed according to Maniatis *et al.* (9). $T3^b$ is more similar to $T3^c$ than to $T13^c$. See text for discussion.

$T3^b$ Is Transcribed in ERLD. Unlike the H-2 antigens, which are expressed on virtually all nucleated cells, the TL products have a more restricted tissue distribution; i.e., thymocytes of certain strains of mice (designated TL^+ strains), certain leukemias of both TL^+ and TL^- strains, and activated T lymphocytes (4, 13, 14). The pTL probe detected an mRNA species from the polyadenylylated fraction of RNA from the TL-expressing ERLD leukemia cell line, but not from thymocytes of the TL^- strain C57BL (Fig. 3). The pTL probe also hybridized to polyadenylylated RNA from thymocytes of TL^+ strains (A, BALB/c, 129) but not to RNA from other tissues (brain, liver, kidney, spleen) of these strains (data not shown). This pattern correlated with the reported expression of TL antigens in different tissues (4). The several different mRNA species that are transcribed in the TL-expressing leukemia ERLD must all be from $T3^b$ (Fig. 3) because this is the only gene recognized by pTL in the genomic DNA of C57BL tissues and ERLD (Fig. 1). These bands were not detected in the poly(A)$^-$ fraction from ERLD (Fig. 3, lane D).

Structure of the $T3^b$ Gene. The $T3^b$ gene from the C57BL cosmid identified by the pTL probe was subcloned into M13 for DNA sequencing. The sequence of the entire gene included in a 5.2-kb stretch of DNA is shown in Fig. 4, which also presents a comparison to the $T13^c$ gene, the only other completely sequenced *Tla* gene.

The $T3^b$ gene contains six exons homologous to the first six exons of other class I genes (Fig. 4). The 5′ flanking region of $T3^b$ contains regions corresponding to the TATAAA and CCAAT sequences of the RNA polymerase II promoters (15). The first ATG encountered 3′ of the "TATAAA box" serves as the initiation codon. The sequences neighboring

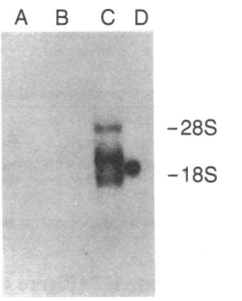

FIG. 3. Hybridization analysis of RNA blots shows that the TL probe hybridizes to RNA from TL^+ leukemia ERLD but not to RNA from C57BL (TL^-) thymocytes. Lanes: A, poly(A)$^+$ RNA of C57BL thymus; B, poly(A)$^-$ RNA of C57BL thymus; C, poly(A)$^+$ RNA of ERLD; D, poly(A)$^-$ RNA of ERLD. Five micrograms of RNA was used per lane. 18S and 28S rRNA positions are indicated.

this region agree with the consensus sequences for translational initiation sites, namely an A in position −3 and C in positions −1 and −5 (16). A change in the position of the initiation codon to 15 nucleotides 5′ of the conventional class I initiation site creates a signal peptide that is five amino acids longer than that of other class I molecules.

Exons II–V correspond both in sequence and in the position of intron–exon borders to other class I sequences. In contrast, whereas murine class I cytoplasmic domains are encoded by three exons, VI–VIII, the putative cytoplasmic regions of both $T3^b$ and $T13^c$ may be entirely encoded by the sixth exon. However, the cytoplasmic portion of $T3^b$ appears to be 23 amino acids longer than that of $T13^c$ because of the insertion of two nucleotides prior to the translational termination (UGA) codon identified for $T13^c$. There is a region 3′ of the termination codon in exon 6 for both genes that shares homology with exon VII of other class I genes. The function of this region is not understood.

Another unique feature of the $T3^b$ and $T13^c$ genes is a 1.1-kb insertional sequence in the third intron, flanked by a 10-base-pair inverted inexact repeat (Fig. 4). This insert begins with a repetitive T-rich tract and ends with a B2 *Alu*-family repeated sequence (17). This stretch is highly conserved in both the $T3^b$ and $T13^c$ genes. To our knowledge, $T3^b$ and $T13^c$ are the only class I genes known so far to contain this 1.1-kb insert.

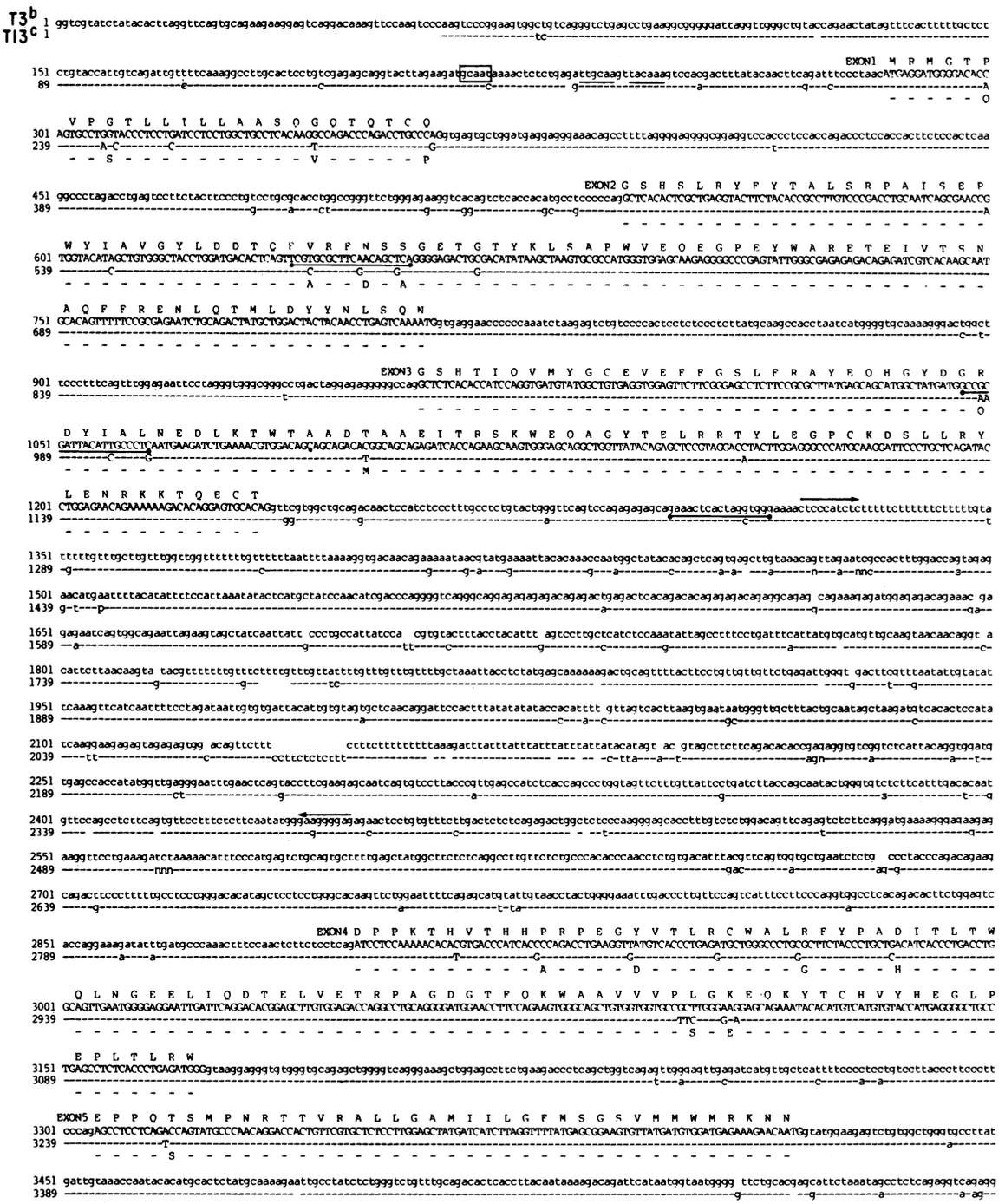

FIG. 4. (Figure continues on the next page)

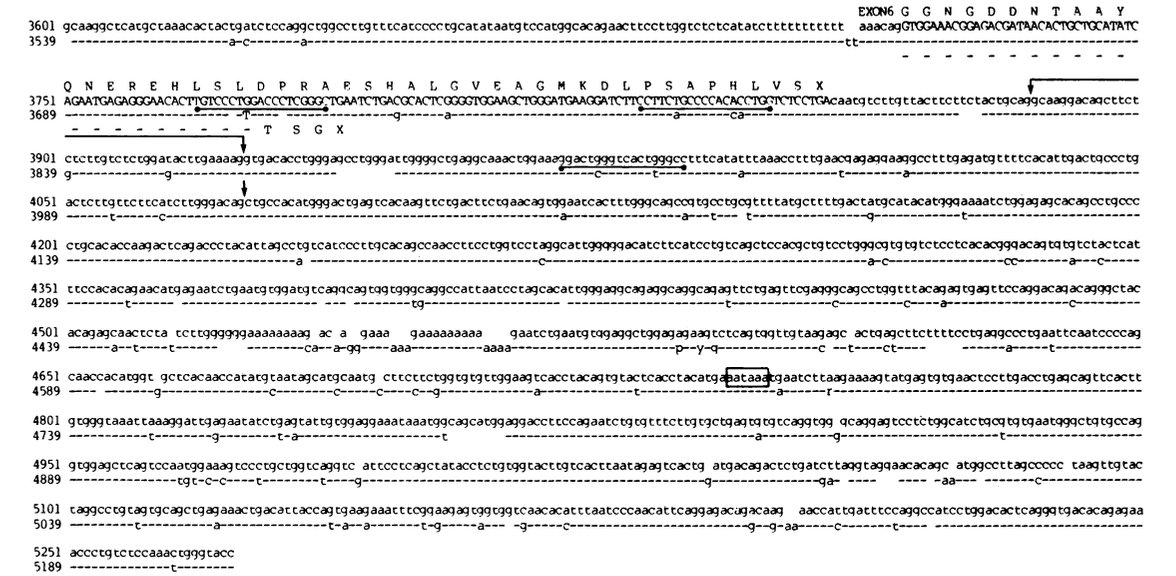

FIG. 4. Sequence of *T3*^{b} and comparison with *T13*^{c}. Coding regions for *T3*^{b} are shown as capital letters with the translated amino acids (single-letter code) above the DNA sequence. Amino acid changes in T13^c are shown under its sequence. The homologous nucleotides for *T13*^{c} are represented by dashes, and spaces are inserted to maximize homology. The two possible TATAAA sequences are underlined and the CCAAT sequence is boxed. In the third intron, the two inexact inverted repeats are indicated by horizontal arrows. Near the 3′ end of the gene, vertical arrows indicate possible splice junctions bordering exon 7 and beginning exon 8. The first AATAAA polyadenylylation signal is boxed. The six *T3*^{b} oligonucleotide probes specific for differences between the two sequences (see text) are designated ●—●.

The 3′ untranslated region, starting at the end of the sixth exon, includes two tandemly arranged *Alu*-family repeated sequences, B1 and B2 (18, 17). At the end of the B2 sequence is the first polyadenylylation site (Fig. 4). Since this polyadenylylation signal is 800 nucleotides 3′ from the translational termination codon, we expect the transcribed product of this gene to be about 2.2 kb long. This is consistent with the detection by hybridization of an 18S band in ERLD mRNA (Fig. 3). The identification of RNA of larger molecular weight might be accounted for by alternative polyadenylylation, as in the case of *H-2L*^{d}, in which two effective polyadenylylation sites also provided by *Alu*-family sequences were found (19). Alternatively, these messages may represent unprocessed or alternatively spliced products of this gene. Additional studies are required to distinguish between these and other possibilities.

The expression of TL in leukemias of TL$^-$ strains [e.g., ERLD (13)] has suggested that all strains have the structural sequences for TL but differ with regard to regulatory sequences controlling its expression. Our data on the sequence and organization of *T3*^{b} suggest that it is a functional gene. The finding of a *T3*^{b} transcript in ERLD and the absence of such a transcript in C57BL thymocytes demonstrate its ability for differential expression. However, the elements that control this trait are still unidentified.

Properties of the T3^b Protein Inferred from the DNA Sequence. The *T13*^{c} gene encodes a TL product of TL.1 and TL.2 specificity upon DNA-mediated transfer into mouse fibroblasts (12). The high conservation of amino acid sequence between *T3*^{b} and *T13*^{c} products (≈98% identity in the first two domains; Table 1) suggested to us that *T3*^{b} encodes a TL product in ERLD cells of serological specificity similar to that of the product encoded by *T13*^{c} in BALB/c thymocytes. Indeed, TL antigens isolated from BALB/c thymocytes and ERLD cells share the TL.1, TL.2, and TL.4 specificities (20).

The T3^b protein, inferred from the DNA sequence, contains general features that classify it as a class I antigen. Thus, compared to other class I antigens, the α1, α2, and α3 domains are of the same length; the cysteines required for the formation of disulfide bridges are conserved in the α2 and α3 domains; and the α3 domain, thought to bind β_2-microglobulin, shows ≈90% homology with other class I products (Table 3). On the other hand, the high dissimilarity for regions other than α3 between *T3*^{b} and *H-2* products may account for some of their biological differences. Thus the *T3*^{b} product differs from the *K*^{b} product by 43% in the first domain and 35% in the second domain (Table 3). Whereas D^b and K^b differ from each other in the transmembrane and cytoplasmic regions to the extent of 25% and 20%, respectively, the transmembrane and cytoplasmic regions of K^b differ from those of the T3^b product by 53% and 80%. In addition, there are differences in the leader peptide and glycosylation sites between T3^b and K^b. For example, the change in the initiation site leads to a T3^b signal peptide that is five amino acids longer than that of K^b and other class I molecules. The leader peptide also differs from that of K^b by ≈45% (Table 3). The only two potential N-linked glycosylation sites are in the α1 domain of T3^b. This is in contrast to the K^b protein, which is glycosylated in both the α1 and α2 domains, and the D^b protein, which is glycosylated in three sites, one in each

Table 1. Percent nucleotide and predicted amino acid divergence between *T3*^{b} and *T13*^{b} and alleles of *H-2K*

	% divergence			
	T3^b vs. *T13*^{c}		*K*^{b} vs. *K*^{d}	
	Gene	Protein	Gene	Protein
5′ Intron	4		6	
Exon I (leader)	7	15	7	5
Intron 1	5		9	
Exon II (domain α1)	2	3	9	20
Intron 2	2		6	
Exon III (domain α2)	2	2	11	19
Intron 3	5		ND	
Exon IV (domain α3)	4	7	7	13
Intron 4	5		8	
Exon V (TM)	1	3	7	13
Intron 5	3		3	
Exons VI–VIII (Cyto)	6		3	9

TM, transmembrane domain; Cyto, cytoplasmic domain; ND, not determined.

Table 2. Percent nucleotide differences (for exons I–VI) between class I genes from C57BL mice

	K^b						$Q10^b$						$T3^b$					
	I	II	III	IV	V	VI	I	II	III	IV	V	VI	I	II	III	IV	V	VI
D^b		8	12	4	13	0		14	11	3	17	12		30	25	8	35	33
K^b							15	12	13	5	24	12	40	26	25	8	36	34
$Q10^b$													40	30	25	8	40	36

Table 3. Percent amino acid differences between class I gene products from C57BL mice

	K^b						$Q10^b$						$T3^b$					
	L	α1	α2	α3	TM	Cyt.	L	α1	α2	α3	TM	Cyt.	L	α1	α2	α3	TM	Cyt.
D^b		16	21	10	25	20		21	23	9				44	41	10	53	85
K^b							14	18	19	13			45	43	35	11	53	80
$Q10^b$													50	45	36	11		

L, leader; α1–α3, domains; TM, transmembrane region; Cyt., cytoplasmic region.

domain. Such differences may reflect biological differences between the H-2 and TL molecules.

Allelic Members of the *T3* Subset Are Highly Conserved. The degree of nucleotide sequence conservation between $T3^b$ and $T13^c$, although they are not alleles, is ≈96% overall. This represents an extremely high level of homology for class I genes. When the sequence of $T3^c$ is available, we expect the conservation to be even greater between $T3^b$ and $T3^c$, since restriction maps and oligonucleotide hybridizations suggest a greater similarity between these two genes (Fig. 2*B*). On the other hand, comparison of the *K* alleles K^b and K^d (21, 22) shows that these sequences are much less conserved (Table 1).

The contrast in conservation and diversity is even more striking at the protein level. For example, the α1 domains of $T3^b$ and $T13^c$ show about 3% divergence, compared to 20% between α1 domains of K^b and K^d, and α2 domains show about 2% divergence between $T3^b$ and $T13^c$, compared to 19% between products of *K* alleles. We would expect such conservation of protein sequence to relate to the function of the *T3/T13* products, in contrast to the suggested importance of diversity for the H-2 system, for which the function is antigen recognition.

Interrelationships of *H-2*, *Qa*, and *Tla* Genes. When examined for all coding regions, K^b, D^b, and $Q10^b$ are more homologous to each other than they are to $T3^b$ (Table 2). For example, the nucleotide divergence of exon III is about 12% between K^b, D^b, and $Q10^b$ genes (23) but about 25% between $T3^b$ and either $Q10^b$ or K^b. Although only partial sequences have been published for a number of other class I gene sequences, comparison of the available data permits further conclusions about the interrelationships of class I gene sequences. Thus, comparison of exon III of the *Qa* genes *Q4* of C57BL (24) and *27.1* of BALB/c (25), the *Tla* genes *T5* and *T8* (26) of BALB/c and pH37 (27) of DBA/2 reveal again the similarity between *H-2* and *Qa* genes, with sequence divergence ranging from 10 to 15% in exon III. However, comparison among the partial sequences of *Tla* genes shows that the range of divergence among these *Tla* loci (except for the homologues *T3* and *T13*) is 30–40% in exon III. Comparison of exon III of *Tla* genes to exon III of *Qa* and *H-2* genes also shows a range of divergence of 25–35%. Thus, even though the complete sequences are not available, we can conclude that the few *Tla* loci examined differ from each other at least as much as they do from *H-2* and *Qa* genes.

Note Added in Proof. Obata *et al.* (28) have published the sequence of a TL gene (isolated from ERLD leukemia) that appears to be the $T3^b$ gene.

We acknowledge the excellent assistance of Ms. D. McGovern, J. Trojnacki, E. Palmieri, R. Raval, and C. Whelan. This work was supported in part by Public Health Service Grants AI07289 and AI10702, National Cancer Institute Grant P30-CA-13330, and a grant from the American Cancer Society (IM-236). S.G.N. is a member of The Irvington House Institute for Medical Research. R.A.Z. is anEstablished Fellow of the New York Heart Association. H.M. was supported in part by National Institutes of Health Training Grant T32 GM7288. P.A.P. was supported by the Ministere de la Recherche et de l'Industrie.

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