VOLUME 44, NUMBER 11 · AUGUST 1985

Federation Proceedings

Official Publication of the
FEDERATION OF AMERICAN SOCIETIES
FOR EXPERIMENTAL BIOLOGY

FUTURE FASEB AND SOCIETY MEETINGS:

ASPET—AUGUST 18–22, 1985, Boston, MA
APS—OCTOBER 13–18, 1985, Niagara Falls, NY
ASCB—NOVEMBER 18–22, 1985, Atlanta, GA
FASEB—APRIL 13–18, 1986, St. Louis, MO
ASBC—JUNE 8–12, 1986, Washington, DC

Contents

Continued

FEDERATION PROCEEDINGS is published 15 times a year, monthly except 4 issues in March, by FASEB. All issues except *March* (nos. 3, 4, 5, and 6) contain papers presented at symposiums and articles especially written for FP. The *March* issues contain the abstracts of papers submitted for presentation at scientific sessions at the Annual Meeting of the Federation. The views expressed in articles and in meeting abstracts are those of the authors and not necessarily those of the Federation. Manuscripts that are considered to be of interest to readers of FP are welcome. All manuscripts submitted (including FASEB or constituent Society symposiums as well as any others submitted) are subject to review and approval by the Editors before publication in FP. Rejected manuscripts may be reconsidered if appealed through the Editorial Board. Second-class postage paid at Bethesda, Maryland, and at additional mailing offices. ISSN 0014-9446. Printed at Lancaster Press, Lancaster, Pennsylvania.

For comments on the Beaumont medal shown on the cover, see page 1 of the January 1985 issue.

Business Manager: All business matters should be directed to Mr. Austin H. Henry, Business Manager, FASEB, 9650 Rockville Pike, Bethesda, MD 20814 (301/530-7066).

Advertising Director: **William J. Wyatt,** TORQUE, INC., 2512 Maryland Avenue, Baltimore, MD 21218 (301/235-6519).

Subscription Price: Members of Constituent Societies $13.00 per year; Nonmembers (Personal) $65.00 per year; Institutional $90.00 per year; Students $35.00 per year with certification. **Postage Differentials:** Other Countries + $20.00. All subscriptions entered on a calendar year basis only and payable in advance. **Single Issues:** $12.00, except March 1, 5, 8, and 12, Nos. 3, 4, 5, and 6 (*Abstracts*), $40.00. Subscriptions and orders should be sent to: FEDERATION PROCEEDINGS, Subscription Department, 9650 Rockville Pike, Bethesda, Maryland 20814 (301/530-7027).

Evolution and expression of the transplantation antigen gene family[1]

JAMES J. DEVLIN,* GEORG WIDERA,* ANDREW L. MELLOR,† KAREN FAHRNER,* DAVID SHERMAN,* ELISABETH H. WEISS,‡ AND RICHARD A. FLAVELL*

*Biogen Research Corporation, Cambridge, Massachusetts 02142; †Clinical Research Center, Harrow, Middlesex HA1 3UJ, England; and ‡Institute for Immunology of the University of Munich, D-8000 Munich 2, Federal Republic of Germany

ABSTRACT

We have cloned 26 different class I genes that are located in the major histocompatibility complex of the C57BL/10 mouse. Two of the three class I genes found in the H-2 complex encode the H-2K^b and H-2D^b antigens; the other 23 class I genes map to the adjacent Tla complex. We have grouped the cosmids containing these genes into three clusters: one cluster links the *H-2K* and *I-A* regions, one cluster links the *H-2D* and *Qa-2* regions, and the final cluster maps to the *TL* region. The class I gene organizations in the *Qa-2* and *TL* regions of the C57BL/10 and BALB/c mice are generally similar, but there are several polymorphic segments. The *Qa-2* region of both mice seems to have evolved by the duplication of gene pairs; furthermore, the *H-2K* region may have been generated by the translocation of a gene pair from the *Qa-2* region. We have evidence that several of the genes in the *Qa-2* region are expressed.—**Devlin, J. J.; Widera, G.; Mellor, A. L.; Fahrner, K.; Sherman, D.; Weiss, E. H.; Flavell, R. A.** Evolution and expression of the transplantation antigen gene family. *Federation Proc.* 44: 2736–2740; 1985.

In 1937 Gorer reported that cell surface antigens mapping to what is now called the major histocompatibility complex (MHC) were responsible for allograft rejection (6). It has recently been shown that these class I MHC antigens are encoded by only a few members of a large gene family (14, 16) (Fig. 1*a*). Class I antigens consist of a ~45,000-dalton glycoprotein and the separately encoded, noncovalently associated, 12,000-dalton β_2-microglobulin; these heterodimers are usually expressed on the cell surface, but some class I molecules may be secreted (9). We have isolated 26 different class I heavy chain genes from C57BL/10 (B10) mouse (*b* haplotype); we have mapped all of these genes to the MHC, located on chromosome 17 in the mouse (Fig. 1*b*). Two of the three class I genes that we have mapped to the H-2 complex, *H-2K^b* and *H-2D^b*, encode the type of histocompatibility antigens reported by Gorer.

These H-2 antigens have an essential role in the detection of foreign antigens on the cell surface (3). For example, virally infected cells are recognized and lysed by cytotoxic T lymphocytes (CTL) only when viral glycoproteins on the surface of the infected cell are recognized in association with class I H-2 antigens. The recognition of foreign antigens on a wide variety of cell types is made possible by the expression class I H-2 antigens on virtually all nucleated cells. More than 50 different alleles of *H-2K* and *H-2D* have been identified; this extraordinary polymorphism may be necessary to allow the population to recognize many different foreign antigens (7). The less polymorphic class I antigens encoded in the Tla complex (e.g., Qa-2,TL) are expressed only on certain lymphocyte populations, and although the majority of class I genes lie in the Tla complex, the function of their gene products is not known.

To determine the organization of this gene family, we screened cosmid libraries by using class I cDNA clones (13) as probes and isolated over 100 cosmids, each containing an insert of ~40 kilobases (kb) of murine DNA. By restriction mapping and chromosomal walking experiments, we have arranged these clones into just three clusters of overlapping cosmids. One cluster links the *H-2K* and *I-A* regions; one cluster links the *H-2D* and *Qa-2* regions; and the final cluster maps to the *TL* region.

THE *H-2K* REGION

The two class I genes *K1* and *K^b* shown in Fig. 2 were mapped to the *H-2K* region by restriction fragment length polymorphisms (16); a similar gene pair has been mapped to the *H-2K* region of the BALB/c mouse (*d* haplotype) (14). L cells transformed with the *K^b* gene expressed the K^b antigen as determined by both CTL and antigen-binding assays (10). These class I genes have been linked to the newly identified $A_{\beta 3}$ class II sequence, which we isolated by using an $A_{\beta 1}$ probe. Previously identified

[1] From the Minisymposium *Molecular Biology and Chemistry of MHC- and Tla-Encoded Molecules* presented at the 68th Annual Meeting of The American Association of Immunologists, St. Louis, Missouri, June 4, 1984. Accepted for publication September 27, 1984.

0014-9446/85/0044-2736/$01.25.

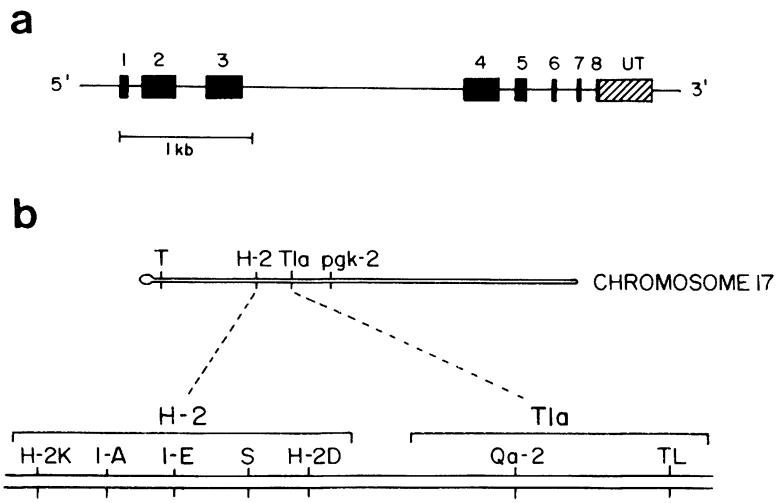


Figure 1. *a*) Intron-exon structure of the *H-2K*^{b} gene. The black boxes represent exons and the striped box represents 3′ untranslated sequences in the mRNA. Exon 1 encodes the leader sequence, which is removed from the mature protein. Exons 2, 3, and 4 encode the three external protein domains of the class I antigen; exon 5 encodes the transmembrane domain; and exons 6, 7, and 8 encode a cytoplasmic domain. We use the intron between exons 3 and 4 to divide class I genes into 5′ and 3′ regions in other figures. Other class I genes have a similar intron-exon structure. *b*) Murine chromosome 17. The centromere is at the left. Class I genes are found in the *K* and *D* regions of the H-2 complex; the *I-A* and *I-E* regions contain class II genes; the *S* region encodes components of the complement pathway. The Tla complex contains the majority of the murine class I genes, including those that encode the Qa-2 and TL antigens.

class II genes, which encode cell surface glycoproteins involved in the associative recognition of antigens by helper T cells, have been mapped to the *I-A* and *I-E* regions of the H-2 complex (Fig. 1*b*).

Although $A_{\beta 3}$ has not yet been linked to cosmids containing other class II genes in the *I-A* region, restriction fragment length polymorphisms suggest that $A_{\beta 3}$ is in fact located in the *I-A* region. The single-copy localization probe shown in Fig. 2 was hybridized to genomic blots of *Pvu*II-digested DNA from the strains of mice listed in Fig. 3. The probe detected different patterns of restriction fragments in the *k*, *q*, and *s* haplotypes. In the case of the recombinant strain A.TL, the probe hybridized to a *k* haplotype pattern. This strain is *s* haplotype in the *H-2K* region and *k* haplotype in the *I-A* region, thereby mapping the localization probe, and presumably also the $I\text{-}A_{\beta 3}$ sequence, to the *I-A* region in this strain. Restriction fragment length polymorphisms between the *q* and *k* haplotypes mapped the localization probe to the *H-2K* region in the B10.AQR recombinant strain; thus the recombinations that generated the A.TL and B10.AQR strains apparently occurred on different sides of the localization probe as shown in Fig. 3. This would establish a map order of: centromere, *K1*, K^b, *I-A* region. We have also confirmed the linkage of *H-2K* to $A_{\beta 3}$ in the *k* haplotype.

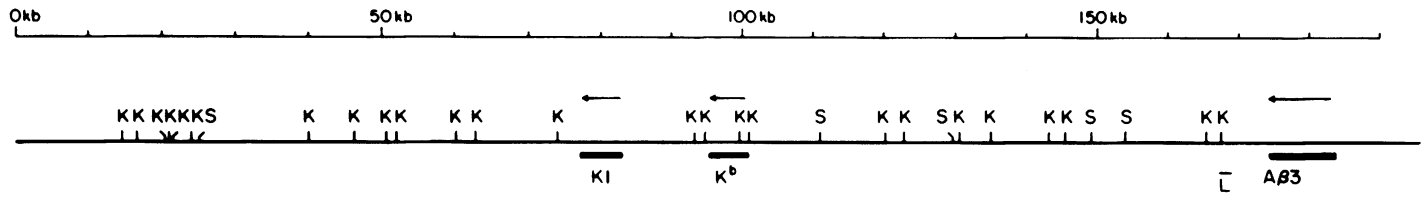
THE *H-2D* AND *Qa-2* REGIONS

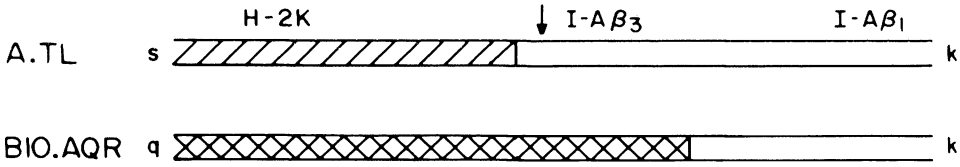
We have linked the *H-2D* and *Qa-2* regions with a 320-kb cosmid cluster (Fig. 4*a*). Restriction fragment length polymorphisms map the D^b gene to the *H-2D* region (16). L cells transformed with this gene express the D^b antigen on their surface as determined by CTL assays (15). Using restriction fragment length polymorphisms, we mapped probes isolated from immediately 5′ of the *Q1* and *Q9* genes to the *Qa-2* region, as defined by the B6.K1 and B6.K2 mice (4, 16). Thus, 10 of the genes in this cluster, all of which are in the same 3′ to 5′ orientation, map to the Tla complex.

A comparison of the B10 *Qa-2* region restriction map to the maps of the BALB/c cosmid clusters reported by Steinmetz et al. (14) is shown in Fig. 4*b*. For the most part, the B10 and BALB/c restriction maps are in good agreement; however, in some areas (e.g., *Q2* and *Q3*) the maps are substantially different. This comparison also suggests that the 5′ end of the BALB/c gene 7 was derived from a *Q8*-like gene whereas the 3′ end of gene 7 was derived from a *Q9*-like gene. Thus, the BALB/c gene 7 may be a fusion gene formed by the deletion shown in Fig. 4*b*.

The B10 restriction map also shows that the *Q6* and *Q7* gene pair is very closely related to the *Q8* and *Q9* gene pair. The *Q4* and *Q5* gene pair is also similar; however, the map of *Q4* diverges somewhat from *Q6* and *Q8*. The similarity of these gene pairs suggests that this region has evolved by the duplication of gene pairs. In fact, probes that hybridize to the 5′ and 3′ flanking regions of these genes show that genes *Q4–Q10* are all closely related (16). These flanking probes do not hybridize near the *TL* or *Q1*, *Q2*, and *Q3* genes, and only the 3′ flanking probe hybridizes to the $H\text{-}2D^b$ gene. In fact, other 5′ flanking probes indicate that the *Q1*, *Q2*, and *Q3* genes are closely related to the *TL* genes (16).

Figure 2. The *H-2K* region. The genes are indicated by thick black bars. The arrows above them point in the direction of transcription or to the 3′ end of the gene. The L indicates the location of the probe used to map the $A_{\beta 3}$ gene. The enzymes used for the restriction map were *Kpn*I (K) and *Sal*I (S).





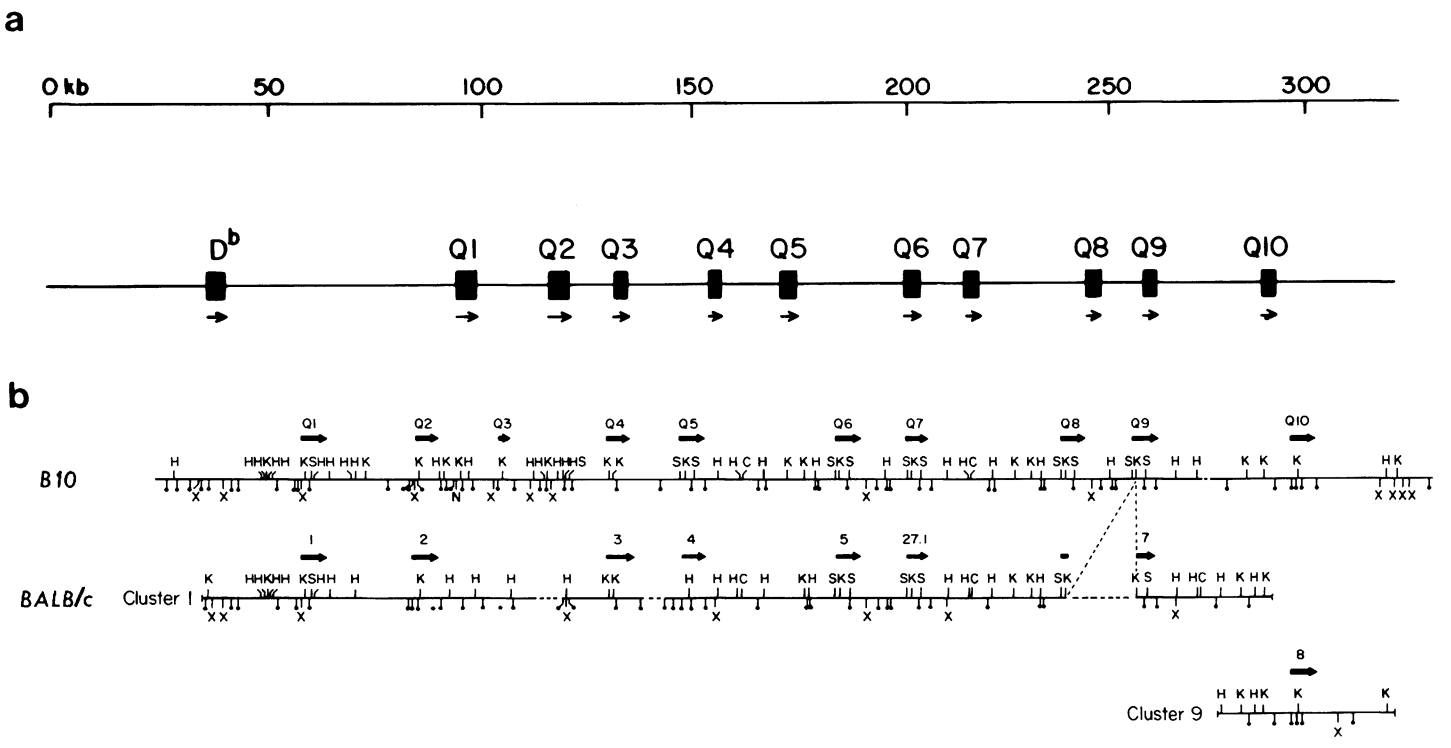
	H-2K	I-Aβ1
A.SW	s	s
A.TL	s	k
A	k	k
BIO.AQR	q	k
BIO.G	q	q

Figure 3. Localization of the $A_{\beta 3}$ gene. The approximate points of recombination relative to the *H-2K*, $I\text{-}A_{\beta 3}$, and $I\text{-}A_{\beta 1}$ genes are shown for the A.TL and B10.AQR mice. The open bars indicate *k* haplotype DNA; the striped bar indicates *s* haplotype DNA; the hatched bar indicates *q* haplotype DNA. The arrow indicates the relative position of the localization probe. The table gives the haplotypes of the *H-2K* and $K\text{-}A_{\beta 1}$ genes in the strains of mice used in these experiments. Data from ref 8.

The flanking probes that hybridize near the paired genes in the *Qa-2* region show similar patterns of hybridization in the *H-2K* region (Fig. 5). For example, one of the 5′ flanking region probes hybridizes to a 640 base pair *Bam*HI fragment flanking the 5′-most gene of the *Qa-2* and *H-2K* region gene pairs while it hybridizes to a 680 base pair fragment flanking the 3′-most gene in each pair. These hybridization patterns indicate that the *H-2K* region gene pair is more closely related to the *Qa-2* region gene pairs than to other class I genes, which suggests that the *H-2K* region was generated by the translocation of a pair of genes from the *Qa-2* region. In another example of possible information transfer from the *Qa-2* region to the *H-2K* region, the *Q10* gene has been identified as a potential donor of a 13–51 base pair segment of DNA that generated the $H\text{-}2K^{bm1}$ mutation by gene conversion (12).

We have preliminary evidence that several of the genes in the *Qa-2* region are expressed. The exons of the *Q10* gene are more than 99% homologous to a gene from the *q* haplotype that encodes a secreted class I antigen (11), and this gene is apparently transcribed in the *b* haplotype (2). We have also synthesized a 19 base pair oligonucleotide complementary to a region in the third exon of the *Q8* gene. This oligomer also hybridizes to the *Q6* gene, which is not surprising because *Q6* and *Q8* occupy analogous positions in the *Qa-2* region gene pairs. This probe detects a class I-sized mRNA in Northern blots of RNA from the cloned CTL line 2C. We have constructed a cDNA library from this same CTL line and isolated

Figure 4. *a*) Class I genes in the *H-2D* and *Qa-2* regions. The arrows point to the 3′ end of the genes. *b*) Comparison of the *Qa-2* regions of the B10 and BALB/c mice. The arrows point to the 3′ end of the genes. The dashed lines indicate what seem to be deletions in the BALB/c chromosome with respect to the B10 chromosome. The enzymes used for the restriction map were *Kpn*I (K), *Hpa*I (H), *Sal*I (S), *Cla*I (C), *Xho*I (X), *Nru*I (N), and *Bam*HI (line with dot on end).



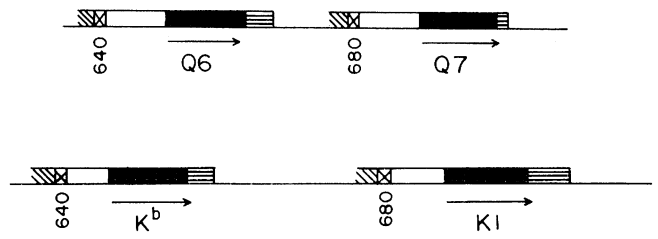


Figure 5. Comparison of *H-2K* and *Qa-2* region gene pairs. The genes are indicated by black boxes; the arrows point to the 3′ end of the genes. The other boxes indicate the regions that hybridize to three different 5′ flanking probes and one 3′ flanking probe. The numbers indicate the size in nucleotides of the *Bam*HI fragment to which the middle 5′ flanking probe hybridizes.

clones containing class I sequences. An oligonucleotide complementary to the 3′ untranslated region of one of these cDNA clones hybridized to only genes *Q5*, *Q7*, and *Q9*. We are currently working on precisely identifying the class I genes in the *Qa-2* as well as the *TL* region that are expressed.

THE *TL* REGION

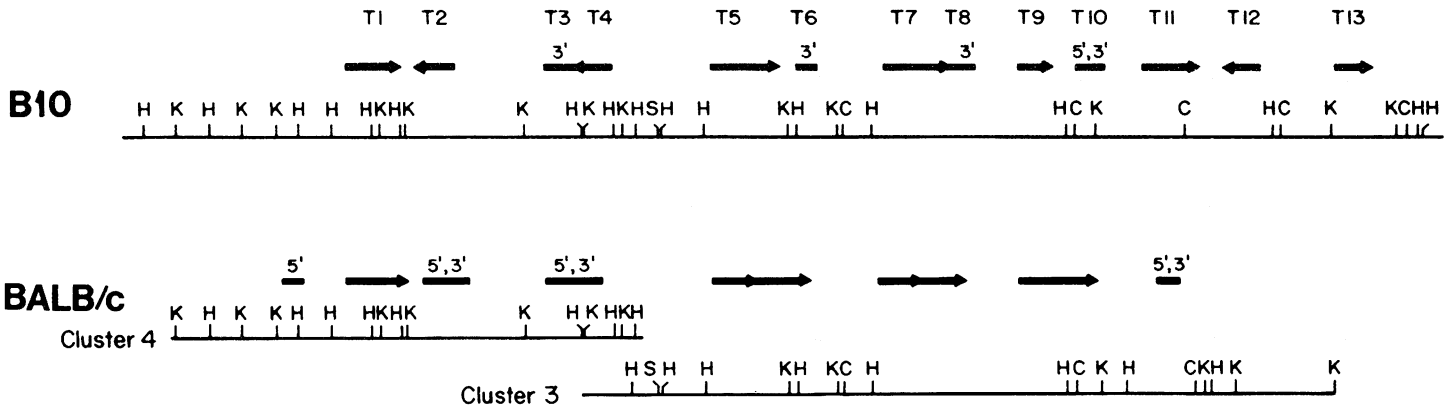
We have cloned 13 class I genes that map to the *TL* region. These genes are in a 180-kb cosmid cluster; unlike the *Qa-2* region genes, these genes are not all in the same 3′ to 5′ orientation. Restriction fragment length polymorphisms allowed us to map a probe isolated from just 5′ of the *T1* gene to the *TL* region, as defined by the B6.K2 mouse (4, 16). The orientation of this cluster with respect to the centromere is not known.

Comparison of restriction maps shows that the BALB/c (14) clusters 3 and 4 can be aligned with the B10 *TL* cluster (Fig. 6). Although the restriction site maps differ in the area between the *T11* to *T13* genes, all 27 restriction sites are identical in the *T1* to *T10* region. This is the most conserved class I gene region we have identified in comparing the BALB/c and B10 mice. We have not isolated a gene that corresponds to the gene reported to express the TL antigen in the BALB/c mouse (5).

THE ROLE OF Tla COMPLEX CLASS I GENES

We are currently examining the transcription of the 23 Tla complex class I genes in different tissues and stages of development. This information should be helpful in determining the biological function of the class I genes in the *Qa-2* and *TL* regions. However, one function for these genes has been suggested that does not require their expression: they may serve as a reservoir of sequence information that can be transferred to H-2 complex class I genes by gene conversion (1). Thus these genes may be an important part of the mechanism for generating allelic polymorphism in the H-2 complex.

Figure 6. Comparison of the C57BL/10 and BALB/c *TL* regions. The genes are indicated by black bars with arrows pointing to the 3′ end of the genes. The symbols 5′,3′ above genes indicate that the 5′ to 3′ orientation has not been established. Those bars with only 5′ or 3′ above them indicate that only the 5′ or 3′ section of a class I gene has been detected. The symbols for restriction enzymes are the same as those used in Fig. 4.



REFERENCES

1. **Brégégère, F.** A directional process of gene conversion is expected to yield dynamic polymorphism associated with stability of alternative alleles in class I histocompatibility antigens gene family. *Biochemie* 65: 229–237; 1983.
2. **Cosman, D.; Kress, M.; Khoury, G.; Jay, G.** Tissue-specific expression of an unusual H-2 (class I)-related gene. *Proc. Natl. Acad. Sci. USA* 79: 4947–4951; 1982.
3. **Dorf, M.,** ed. *The role of the major histocompatibility complex in immunobiology.* New York: Garland; 1981.
4. **Flaherty, L.** The Tla region of the mouse: identification of new serologically defined locus, Qa2. *Immunogenetics* 3: 533–539; 1976.
5. **Goodenow, R. S.; McMillan, M.; Nicolson, M.; Sher, B. T.; Eakle, K.; Davidson, N.; Hood, L.** Identification of the class I genes of the mouse major histocompatibility complex by DNA-mediated gene transfer. *Nature (London)* 300: 231–237; 1982.
6. **Gorer, P. A.** The antigenic basis of tumor transplantation. *J. Pathol. Bacteriol.* 47: 231–252; 1938.
7. **Klein, J.** The major histocompatibility complex of the mouse. *Science* 203: 51b; 1979.
8. **Klein, J.; Figueroa, F.; David, C.** H-2 haplotypes, genes and antigens:

second listing II. The H-2 complex. *Immunogenetics* 17: 553–596; 1983.

9. **Kress, M.; Cosman, D.; Khoury, G.; Jay, G.** Secretion of a transplantation-related antigen. *Cell* 34: 189–196; 1983.
10. **Mellor, A. L.; Golden, L.; Weiss, E.; Bullman, H.; Hurst, J.; Simpson, E.; James, R. F. L.; Townsend, A. R. M.; Taylor, P. M.; Schmidt, W.; Ferluga, J.; Leben, L.; Santamaria, M.; Atfield, G.; Festenstein, H.; Flavell, R. A.** Expression of murine H-2K^b histocompatibility antigen in cells transformed with cloned H-2 genes. *Nature (London)* 298: 529–534; 1982.
11. **Mellor, A. L.; Weiss, E. H.; Kress, M.; Jay, G.; Flavell, R. A.** A nonpolymorphic class I gene in the murine major histocompatibility complex. *Cell* 36: 139–144; 1984.
12. **Mellor, A. L.; Weiss, E. H.; Ramachandran, K.; Flavell, R. A.** A potential donor gene for the bm1 gene conversion event in the C57BL mouse. *Nature (London)* 306: 792–795; 1984.
13. **Steinmetz, M.; Moore, K. W.; Frelinger, J. G.; Sher, B. T.; Shen, F-W.; Boyse, E. A.; Hood, L.** A pseudogene homologous to mouse transplantation antigens: transplantation antigens are encoded by eight exons that correlate with protein domains. *Cell* 25: 683–692; 1981.
14. **Steinmetz, M.; Winoto, A.; Minard, K.; Hood, L.** Clusters of genes encoding mouse transplantation antigens. *Cell* 28: 489–498; 1983.
15. **Townsend, A. R. M.; Taylor, P. M.; Mellor, A. L.; Askonas, B. A.** Recognition of D^b and K^b gene products by influenza-specific cytotoxic T cells. *Immunogenetics* 17: 283–294; 1983.
16. **Weiss, E. H.; Golden, L.; Fahrner, K.; Mellor, A.; Devlin, J. J.; Bullman, H.; Tiddens, H.; Bud, H.; Flavell, R. A.** Organization of class I genes in the major histocompatibility complex of the C57Bl/10 mouse. *Nature (London)* 310: 650–655; 1984.