The EMBO Journal vol.4 no.6 pp.1431-1434, 1985

Gene duplications in the TL region of the mouse major histocompatibility complex

Ulf Hammerling, Hans Ronne[2], Eva Widmark, Bo Servenius, Maurizio Denaro[3], Lars Rask[1] and Per A. Peterson

Department of Cell Research, Uppsala University, and [1]Swedish University of Agricultural Sciences, The Wallenberg Laboratory, Box 562, S-751 22, Uppsala, Sweden

[2]Present address: Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, 701 W. 168th Street, New York, NY 10032, USA

[3]Present address: Divisione di Oncologia Sperimentale, Centro di Riferimento Oncologico, Via Pedemontana Occidentale, 33081 Aviano (PN), Italy

Communicated by P.A.Peterson

We have isolated a class I gene from the TL region of the A/J mouse. The gene, T2A, is a homologue of the C57BL/10 mouse gene T2. In the process of mapping this gene we screened a number of BALB/c class I cosmid clusters with a T2A flanking probe. Several of the hybridizing clusters were found to contain identical DNA segments and could therefore be linked together into one single BALB/c TL region which appears to be identical to the TL region of the C57BL/10 mouse. However, two of the hybridizing clusters do not overlap with the C57BL/10 TL region. It appears that these two clusters represent a partial duplication of the TL region in the BALB/c mouse.

Key words: gene duplications/TL region/Tla/class I antigens/ major histocompatibility complex

Introduction

The class I antigens of the major histocompatibility complex (MHC) play an important role in the vertebrate immune system. These cell surface molecules control self recognition (MHC restriction) in the immune response (Doherty and Zinkernagel, 1975). In the mouse, the H-2K, D and L loci of the MHC encode the classical class I antigens which are present on all nucleated cells (Klein *et al.*, 1983). However, the murine MHC contains many more class I genes, most of which reside in the Qa2,3 and TL clusters (Hood *et al.*, 1983; Weiss *et al.*, 1984). These regions comprise the telomeric part of the MHC and contain the Qa2,3 and Tla loci where lymphoid differentiation antigens are encoded (Boyse, 1984).

The Qa2,3 region contains at least 10 genes (Weiss *et al.*, 1984). The TL region contains even more class I genes. In the C57BL/10 mouse, overlapping cosmids have been isolated that cover the entire TL region, including at least 13 genes or gene fragments (Weiss *et al.*, 1984). In the BALB/c mouse, several cosmid clusters containing class I genes have been isolated (Steinmetz *et al.*, 1982), two of which were shown to be homologous to the C57BL/10 region (Weiss *et al.*, 1984), but the organization of the BALB/c TL region is still not clear. Five BALB/c cosmid clusters have been mapped to the Tla locus (clusters 3, 4, 7, 8 and 10) whereas two clusters (5 and 12) map to either Qa2,3 or Tla (Winoto *et al.*, 1983). These seven clusters contain a total of 21 genes or gene fragments.

We have isolated a phage λ clone containing class I sequences from the TL region of a third mouse strain, A/J. The λ clone contains one complete gene and two gene fragments. The complete gene, T2A, is a homologue of the C57BL/10 gene T2 (Weiss *et al.*, 1984). A probe from this clone was hybridized to class I cosmids from the BALB/c mouse (Steinmetz *et al.*, 1982). Our interpretation of the hybridization data is that several of the BALB/c Tla clusters overlap and define a TL region which is identical to that of the C57BL/10 mouse. Moreover, BALB/c clusters 5 and 12, which also hybrized to our probe, seem to represent a partial duplication of this TL region.

Results

Isolation and characterization of phage A/J λTL

The clone A/J λTL was isolated from an A/J genomic library using a probe from the class I cDNA clone pH-2d-3 (Bregegere *et al.*, 1981). A restriction map is shown in Figure 1. Within the clone, 3'-hybridizing sequences were mapped using the pH2-d-3 probe and 5' sequences using a probe from the human genomic clone HLA-12.4 (Malissen *et al.*, 1982). The clone, A/J λTL, contains one complete class I gene, T2A, and fragments of two more class I genes (Figure 1). One of the latter T1A, is orientated at the same direction as the T2A gene (Widmark *et al.*, in preparation). The orientation of the second fragment was not determined.

Screening of BALB/c class I cosmids

Our aim was to determine the part of the MHC from which the A/J λTL clone was derived. Accordingly, a 600-bp intergenic *Pvu*II fragment (Figure 1) was hybridized to the BALB/c class I cosmids of Steinmetz *et al.* (1982). The results are shown in Table I. The probe did not hybridize to any cosmids from the H-2 or Qa2,3 regions. However, it hybridized to cosmids in clusters 4, 7 and 8 from the Tla locus as well as to cosmids in clusters 5 and 12. Curiously, the probe hybridized to identical fragments in clusters 4, 7 and 8 when tested with three different enzymes (not shown). This suggested that the clusters possibly contain overlapping and thus identical pieces of DNA. Accordingly, we searched the cluster maps (Steinmetz *et al.*, 1982) for regions of identity.

Overlap of BALB/c clusters 3, 4, 7 and 8

As shown in Figure 2 the BALB/c cosmid clusters 3, 4, 7 and 8 do form a continuous overlapping segment of DNA, provided that one protruding end is eliminated from each cluster. Since these protruding ends lack class I hybridizing sequences and are derived from single cosmids, it is likely that they represent cases where two unrelated DNA fragments have been ligated to one another. Indeed, it has been shown that the protruding end of cluster 7 does not map to the MHC, the expected result for an unrelated piece of DNA (Winoto *et al.*, 1983). These authors also noted the similarity of clusters 7 and 8 to each other and suggested that the protruding end of cluster 8 could be such an unrelated DNA fragment.

Strong independent support for the proposed overlap is pro-

© IRL Press Limited, Oxford, England.

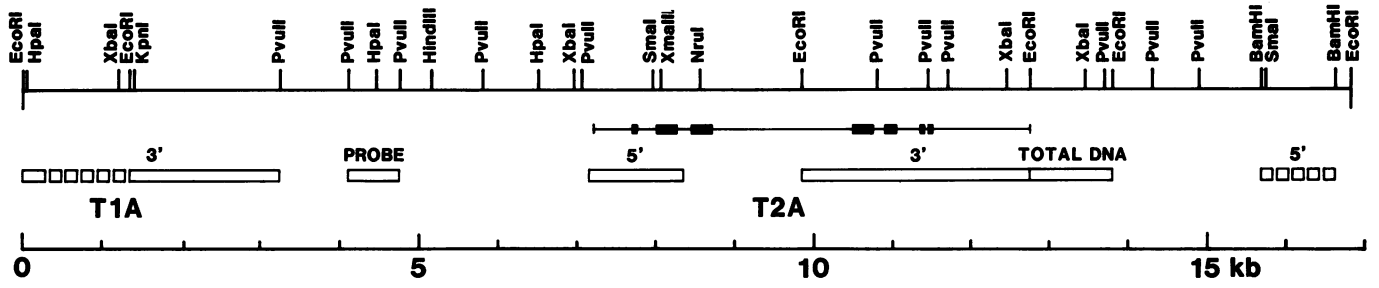

Fig. 1. Restriction map of the A/J λTL insert. The fragments that hybridized to 5′ and 3′ class I probes and to total mouse DNA (repetitive sequences) are shown below the map as open boxes. Also shown is the location of the 600-bp intergenic *PvuII* probe. The exon/intron organization of the T2A gene as determined from the sequence (Widmark *et al.*, in preparation) is outlined as black boxes.

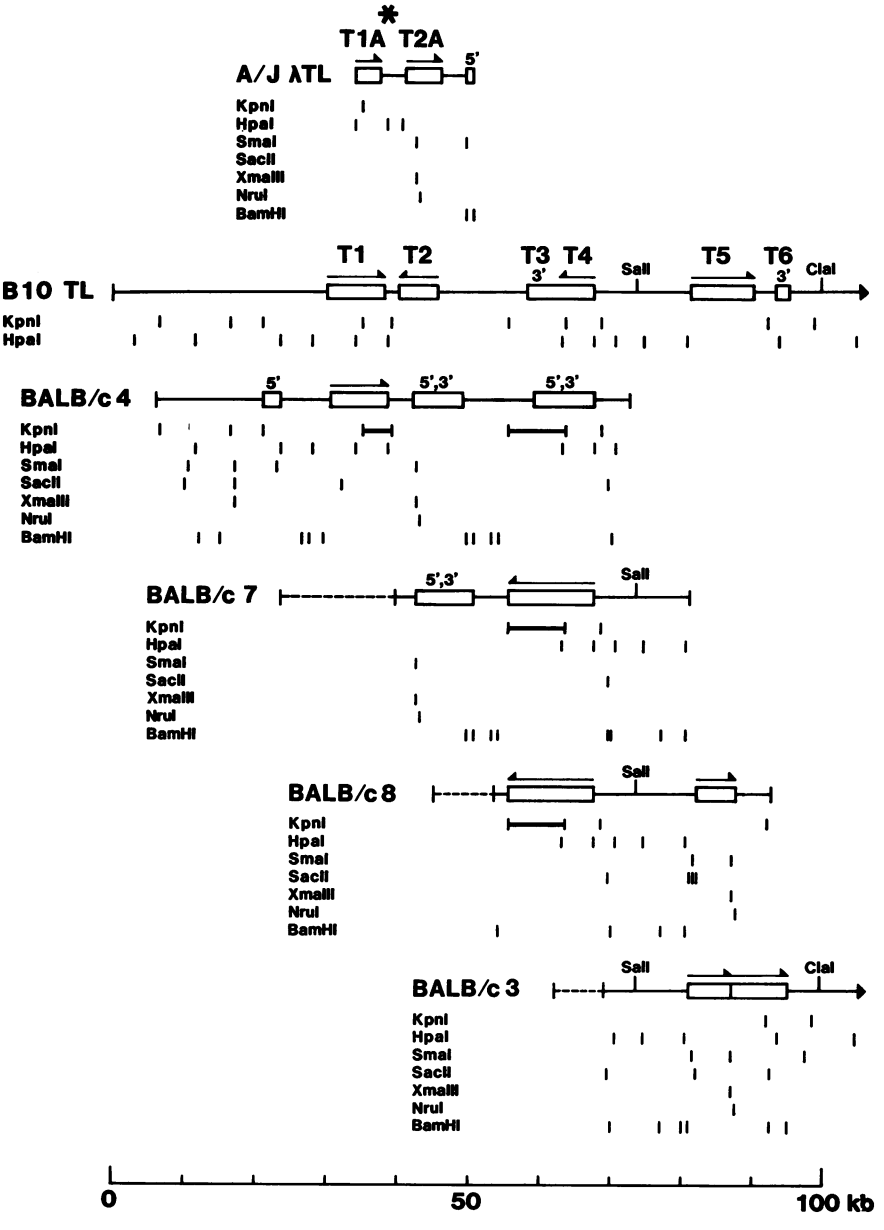

Fig. 2. Alignment of A/J and BALB/c TL clones to the C57BL/10 (B10) TL region. Open boxes mark the approximate locations of class I genes (as determined from hybridization data). Arrows show assigned gene orientations. The location of the T1A/T2A intergenic probe is marked by an asterisk. *KpnI* fragments that hybridize to the probe are drawn as thick lines. The C57BL/10 data are from Weiss *et al.* (1984) and the BALB/c data from Steinmetz *et al.* (1982). In the BALB/c cosmid clusters 7, 8 and 3 some segments of the cluster maps have been eliminated. These segments correspond to protruding single cosmids and are indicated by dashed lines. At positions 70 and 80 kb there are three minor differences between the maps of cluster 7, 8 and 3. However, these differences all involve the question whether a given enzyme has one or several sites at virtually the same position, which is difficult to establish by restriction mapping. The homology between the B10 TL region and BALB/c cluster 3 (Weiss *et al.*, 1984) continues outside the area shown in the figure.

vided by a comparison with the TL region of the C57BL/10 mouse (Weiss *et al.*, 1984). Thus, the BALB/c overlap is co-linear with the C57BL/10 TL region and all restriction sites which have been mapped in the two strains are identical (Figure 2). For two of the clusters (3 and 4) the alignment thus obtained is identical to the one proposed by Weiss *et al.* (1984). Since most of the remaining part of the C57BL/10 TL region (genes T7 – T11) is homologous to the BALB/c cluster 3, it appears that the BALB/c and C57BL/10 TL regions are organized in the same way. The only difference is at the end of the region, at genes T12 and T13. Since this is another place where the BALB/c data are derived from a single protruding cosmid, it is also possible that this difference could be eliminated.

Alignment of BALB/c clusters 5 and 12 to the TL region

The two remaining BALB/c clusters which hybridized to the A/J λTL probe were clusters 5 and 12. These clusters are located in the MHC telomeric to H-2D, but they have not been mapped with respect to the Qa2,3 and Tla loci (Winoto *et al.*, 1983). Interestingly, two of the hybridizing *Kpn*I fragments from these clusters are identical in size to hybridizing fragments from clusters 4, 5 and 8 in the TL region (Table I). This suggests that clusters 5 and 12 are related to this part of the TL region. Indeed, when the two clusters are aligned to the BALB/c TL region in such a way that the hybridizing *Kpn*I fragments of identical size match, extensive similarities are seen between the restriction maps (Figure 3). Thus, for the seven enzymes shown in Figure 3, 10 of the 16 sites that have been mapped in cluster 12 are also found in the BALB/c TL region. For cluster 5, 13 out of 25 mapped sites are shared with the TL region. The alignment to the TL region also suggests that clusters 5 and 12 may overlap in the genome, since the aligned clusters coincide for about 8 kb, within which their restriction maps are identical (Figure 3). If this segment is counted only once, 21 out of 38 sites in clusters 5 and 12 are shared with the TL region.

This degree of restriction map similarity is much too high to be coincidental, particularly since the alignment was derived from hybridization data. Accordingly we suggest that clusters 5 and 12 represent a duplication of part of the TL region (genes T1 – T6). This duplication apparently is present in BALB/c, but absent in the C57BL/10 mouse, since no region corresponding to clusters 5 and 12 was found in the latter strain (Weiss *et al.*, 1984).

Table I. BALB/c *Kpn*I fragments that hybridize to the A/J λTL probe

Cluster	Map position	Size of fragments (kb)		
4	Tla	3.6	8.0	
7	Tla		8.0	
8	Tla		8.0	
5	Telomeric to H-2D		8.0	12.5
12	Telomeric to H-2D	3.6		

The A/J T2A gene

The A/J λTL probe hybridized to several distinct locations within the duplication T1 – T6 region (Figure 3). A comparison of the restriction maps (Figures 2 and 3) shows that the T1A/T2A gene pair is particularly closely related to the T1/T2 gene pair and also to the first two genes in BALB/c cluster 12, which represent a duplication of this gene pair. We therefore conclude that the A/J T2A gene is a homologue of the C57BL/10 gene T2.

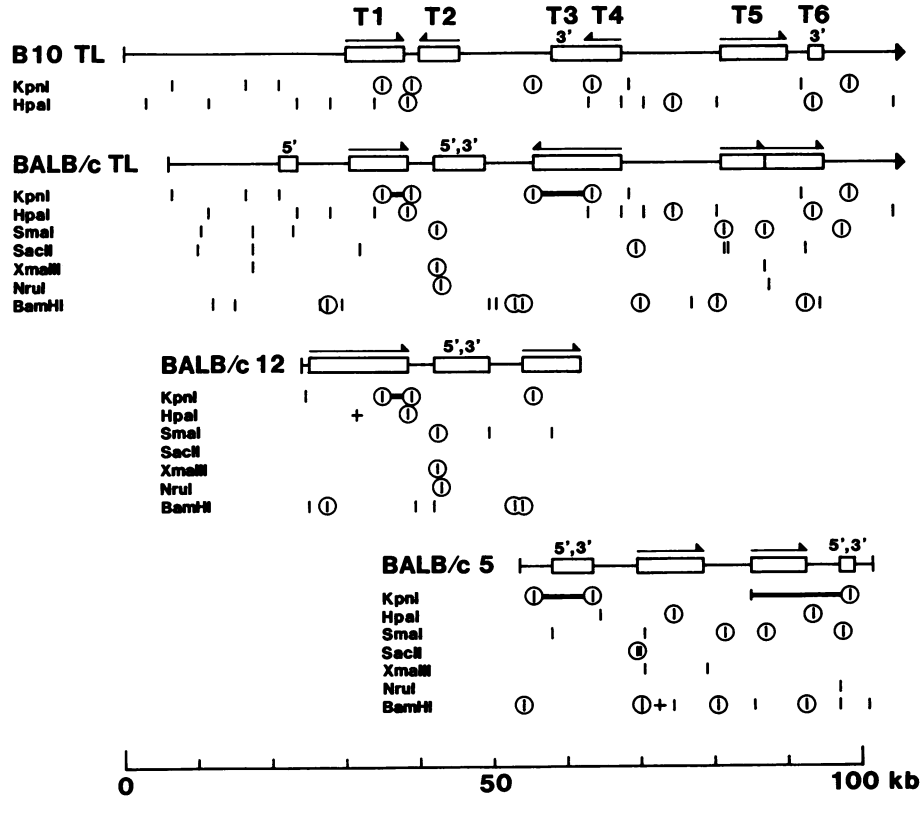

Fig. 3. Alignment of BALB/c clusters 5 and 12 to the TL region. Restriction sites that are found in both the TL region and in clusters 5 and 12 are circled. The BALB/c TL map was derived from the overlaps in Figure 2. The B10 TL map is from Weiss *et al.* (1984) and the maps of clusters 5 and 12 from Steinmetz *et al.* (1982). Open boxes mark the approximate locations of class I genes. Arrows show assigned gene orientations, *Kpn*I fragments that hybridize to the T1A/T2A intergenic probe are drawn as thick lines.

Since we do not know the number of class I genes in the A/J mouse we cannot rule out the possibility that there are several such T2 homologues, similar to the two duplicated genes in BALB/c. In any case, the similarity of the maps suggests that the T2 and T2A genes should have the same orientation. It is therefore surprising that the assigned direction of the C57BL/10 T2 gene differs from that of the T2A gene (Figure 2). The latter has been confirmed by sequencing (Widmark *et al.*, in preparation) whereas the T2 direction was mapped by blotting (Weiss *et al.*, 1984). Since we observed a weak 5′ signal close to the 3′ end of the T2A gene (Figure 1) it seems possible that such a signal could have interfered with the mapping of the T2 gene.

Discussion

The number of class I genes in the mouse

There have been several different estimates of the number of class I genes in different mouse strains. Thus, it was suggested that the BALB/c genome contains at least 36 class I genes (Steinmetz *et al.*, 1982; Hood *et al.*, 1983) whereas the C57BL/10 mouse has ~26 genes (Weiss *et al.*, 1984). If our interpretation of the BALB/c data is correct, then the number of the genes in that strain would be reduced to 32. However, the BALB/c mouse would still have more class I genes than the C57BL/10 mouse because clusters 5 and 12 appear to be absent in the latter strain. There are also two more BALB/c clusters which have not been accounted for in the C57BL/10 mouse (clusters 6 and 10). It remains to be determined whether these clusters are due to aberrant cosmids or whether they represent additional genes in the BALB/c mouse.

Duplications in the TL region

The hybridization data and restriction map similarities suggest that the BALB/c clusters 5 and 12 were created by a duplication of TL genes T1 – T6. Clusters 5 and 12 have not been mapped to any specific locus (Winoto *et al.*, 1983) and there is no evidence that they are immediately continuous with the TL region. However, the observation that a gene product from cluster 5 is precipitated by an antiserum against TL determinants (Goodenow *et al.*, 1982) also suggests that this cluster is closely related to the TL region.

The duplication apparently occurred a long time ago, since a number of restriction site differences also are seen (Figure 3). It is therefore likely that clusters 5 and 12 have been deleted in the C57BL/10 mouse rather than having been generated by a recent duplication in the BALB/c strain. This conclusion is further supported by the observation that the BALB/c T1 – T6 region is more similar to the C57BL/10 T1 – T6 region than to the BALB/c clusters 5 and 12 (Figure 3), which suggests that the duplication occurred before the haplotype separation. The fact that the T1A/T2A probe hybridizes to several distinct locations within the duplication segment (Figure 3) suggests that the T1 – T6 region itself may have been generated by a series of duplication events, prior to the duplication of the whole region.

Conclusion

Previous investigators have described a number of class I cosmid clusters from the BALB/c mouse, several of which map to the Tla locus (Steinmetz *et al.*, 1982; Winoto *et al.*, 1983). Two of these clusters were aligned to the TL region of the C57BL/10 mouse (Weiss *et al.*, 1984), but several BALB/c clusters remained unaccounted for, and the overall organization of the BALB/c TL region was not clear.

On the basis of hybridization data and a re-interpretation of the BALB/c cluster maps we suggest that BALB/c clusters 3, 4, 7 and 8, all of which map to the Tla locus, can be joined together into one continuous overlap which is identical to the C57BL/10 TL region. We also conclude that the two BALB/c clusters 5 and 12, whose map positions are unknown, are homologous to this TL region as revealed by hybridization data and extensive restriction map similarities. Thus, these two clusters represent a duplication of TL genes T1 – T6, which are present in BALB/c, but apparently absent in C57BL/10.

Materials and methods

The λ-phage genomic library of the A/J mouse strain (Young *et al.*, 1981) was propagated, plated and screened using standard procedures (Maniatis *et al.*, 1982). A 600-bp *Pst*I fragment from the class I cDNA clone pH2-d-3 (Bregegere *et al.*, 1981) was used to screen the library and to identify 3′ class I sequences in the A/J λTL insert. As a 5′ probe we used an 1850-bp *Eco*RI/*Bam*HI fragment from the human genomic clone HLA-12.4 (Malissen *et al.*, 1982). The restriction map was established by single and double digestions. The blotting procedures have been described (Larhammar *et al.*, 1983). All work involving recombinant plasmids was carried out as outlined in the National Institutes of Health guidelines for recombinant DNA research.

Acknowledgement

We thank Dr M. Steinmetz for doing the hybridizations of the BALB/c cosmids, Dr U. Schibler for the A/J library and Dr B. Jordan for the HLA-12.4 clone. This work was supported by grants from the Swedish Cancer Society.

References

Boyse,E.A. (1984) *Cell*, **38**, 1-2.

Bregegere,F., Abastado,J.P., Kvist,S., Rask,L., Lalanne,J.L., Garoff,H., Cami,B., Wiman,K., Larhammar,D., Peterson,P.A., Gachelin,G., Kourilsky,P. and Dobberstein,B. (1981) *Nature*, **292**, 78-81.

Doherty,P.C. and Zinkernagel,R.M. (1975) *J. Exp. Med.*, **141**, 502-507.

Goodenow,R.S., McMillan,M., Nicholson,M., Sher,B.T., Eakle,K., Davidson, N. and Hood,L. (1982) *Nature*, **300**, 231-237.

Hood,L., Steinmetz,M. and Malissen,B. (1983) *Annu. Rev. Immunol.*, **1**, 529-568.

Klein,J., Figueroa,F. and Nagy,Z.A. (1983) *Annu. Rev. Immunol.*, **1**, 119-142.

Larhammar,D., Hammerling,U., Denaro,M., Lund,T., Flavell,R.A., Rask,L. and Peterson,P.A. (1983) *Cell*, **34**, 179-188.

Malissen,M., Malissen,B. and Jordan,B.R. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 893-897.

Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning: A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.

Steinmetz,M., Winoto,A., Minard,K. and Hood,L. (1982) *Cell*, **28**, 489-498.'

Weiss,E.H., Golden,L., Fahrner,K., Mellor,A.L., Devlin,J.J., Bullman,H., Tiddens,H., Bud,H. and Flavell,R.A. (1984), *Nature*, **310**, 650-655.

Winoto,A., Steinmetz,M. and Hood,L. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3425-3429.

Young,R.A., Hagenbuchle,O. and Schibler,U. (1981) *Cell*, **23**, 451-458.

Received on 22 January 1985; revised on 25 March 1985