Genet. Res., Camb. (1987), **50**, *pp.* 137–146 *With 4 text-figures Printed in Great Britain*

Nucleotide sequence analysis of class II genes borne by mouse *t* chromosomes

M. GOLUBIĆ, O. BUDIMIR, R. SCHOEPFER, M. KASAHARA, W. E. MAYER, F. FIGUEROA AND J. KLEIN†

Max-Planck-Institut für Biologie, Abteilung Immungenetik, Corrensstrasse 42, D-7400 Tübingen, F.R.G.

(*Received 5 January 1987 and in revised form 17 March 1987*)

Summary

Five class II *H-2* genes borne by *t* chromosomes were partially sequenced: $A_\beta^{w30.1}$ borne by the t^{Tuw10} chromosome of EDY589 ($H\text{-}2^{w2}$); $A_\beta^{w31.2}$ and E_β^{w31} borne by the t^{Tuw8} chromosome of CRO437 ($H\text{-}2^{w57}$), as well as $A_\beta^{w36.1}$ and $E_\beta^{w31.1}$ borne by the t^{Tuw7} chromosome of CRO435 ($H\text{-}2^{w37}$). These genes are representatives of the three major groups of alleles found associated with *t* chromosomes. The sequenced part consisted of almost the entire exon 2 and the entire exon 3 coding for the first and the second domain of the β polypeptide chains, respectively. The sequence was compared with the published sequences of A_β and E_β alleles borne, by non-*t* chromosomes. The comparison revealed that the *t*-associated alleles are no more similar to one another than they are to the corresponding genes present on non-*t* chromosomes in laboratory mice. This divergence in the nucleotide sequence among the class II genes is interpreted as evidence that the *t* complex is very old.

1. Introduction

Some 5–35% of all wild mice carry chromosome 17 with two inversions in the proximal region. The abnormal chromosomes, referred to as *t* haplotypes, are maintained in the population by a potent system of segregation distorters whose action is opposed by certain genes that cause male sterility and others that cause lethality of *t*/*t* embryos (for reviews see Klein & Hammerberg, 1977; Lyon, 1981; Silver 1985; Klein, 1986). The inversions span a number of loci, most of which apparently have no relation to the *t* complex genes themselves (Artzt, Shin & Bennett, 1982; Herrmann *et al.* 1986). The most prominent among these unrelated loci are the members of the *H-2* complex, the major histocompatibility complex (Mhc) of the mouse.

Different mouse populations carry different *t* haplotypes characterized by specific lethality genes (Dunn, 1955; Dunn, Beasley & Tinker, 1960; Klein, Sipos & Figueroa, 1984), alleles at the *H-2* complex loci (Hammerberg & Klein, 1975; Sturm, Figueroa & Klein, 1982; Nizetić, Figueroa & Klein, 1984; Figueroa *et al.* 1985), alleles at the enzyme-encoding loci (Nadeau, 1983, 1986; Ayane *et al.* 1987), and specific random DNA fragments (Röhme *et al.* 1984; Fox *et al.* 1985). Sixteen different complementation groups carrying specific lethality genes have been identified so far and there are indications that a few more may exist among wild mice (Klein, Sipos & Figueroa, 1984; F. Figueroa & J. Klein, unpublished data). The *H-2* typing of mice with *t* haplotypes has revealed the existence of at least three major groups (Nizetić, Figueroa & Klein, 1984; Figueroa *et al.* 1985).

Neither serological nor restriction enzyme analysis has revealed any geneological relationship among the three groups. These results suggest that the *H-2* haplotypes within each group are descended from a single ancestral haplotype but they do not tell us whether *all t*-associated *H-2* haplotypes are descended from a single ancestral haplotype. It is possible that the three branches of the genealogical tree separated from a common stem such a long time ago that the extent to which *H-2* loci are related in different branches is no longer obvious when studied serologically or by restriction fragment length polymorphism (RFLP). It is, however, also possible that the three branches reflect three independent origins of *t* chromosomes. To decide between these two possibilities, we sequenced representative *H-2* genes from the three branches of *t* haplotypes, hoping that the sequences might reveal relationships not revealed by the serological and RFLP analyses.

† Corresponding author.

2. Materials and Methods

The 3 strains used in this study were: CRO435 ($t^{Tuw\,7}$, $H\text{-}2^{w\,37}$), CRO437 ($t^{Tuw\,8}$, $H\text{-}2^{w\,57}$), and EDY589 ($t^{Tuw\,10}$, $H\text{-}2^{w\,2}$). The first two of these were derived from wild mice captured at Nahya, Giza, Governate, Egypt and the third originated from a mouse captured at Eday on the Orkney Islands. The alleles carried by the three *H-2* haplotypes are: $H\text{-}2^{w\,2}$: $K^{w\,30\cdot2}$ $A_\beta^{w30\cdot1}$ E_β^{w2} E_α^{w2} D^{w30}; $H\text{-}2^{w\,37}$: $K^{w\,30\cdot4}$ $A_\beta^{w36\cdot1}$ $E_\beta^{w31\cdot1}$ E_α^{w28} D^{w37}; $H\text{-}2^{w\,57}$: $K^{w\,57}$ $A_\beta^{w31\cdot2}$ E_β^{w31} $E_\alpha^{w29\cdot1}$ D^{w57}. All three of these wild-derived strains bear semi-lethal *t* haplotypes and hence produce a small fraction of viable *t*/*t* individuals. It was from such homozygotes that the DNA for sequencing was isolated. The detailed characterization of the *t* and *H-2* haplotypes carried by these strains has been previously reported (Nizetić, Figueroa & Klein, 1984; Klein, Sipos & Figueroa, 1984; Figueroa *et al.* 1985). The three *t* haplotypes represent different groups in terms of their *H-2* loci (Klein *et al.* 1985; Figueroa *et al.* 1985).

Cosmid genomic libraries prepared from kidney DNA of the three *t*-bearing strains were screened with mouse class II A_β and E_β probes. The A_β probe was a 5·6 kb *Eco* RI genomic fragment obtained from the 34·2 BALB/c cosmid clone provided by Steinmetz and co-workers (1982). It covers most of the A_β gene from the first exon to the central part of the 3′ untranslated region. The E_β probe was a 2 kb *Eco* RI fragment obtained from the 24·2 BALB/c genomic cosmid clone provided by Steinmetz and co-workers (1982). It corresponds to the second exon of the gene.

Cosmid clones hybridizing with the A_β or E_β probes were isolated and their restriction enzyme maps constructed. Four A_β clones and one E_β clone were isolated from the CRO435 library, one A_β clone and one E_β clone were obtained from the CRO437 library, and one A_β clone was isolated from the EDY589 library. The cosmid clones were digested with either *Eco* RI (A_β clones) or *Hin*dIII (E_β clones); the genomic DNA fragments containing, as judged by their restriction maps, the A_β and E_β sequences were isolated and subcloned in pUC8 (Viera & Messing, 1982). The EDY589 A_β fragments were additionally subcloned in the M13mp18 vector.

All subclones, with the exception of the EDY589 A_β subclone, were sequenced by the chemical degradation method (Maxam & Gilbert, 1977). The EDY589 A_β subclone was sequenced by the primer extension method in the presence of 2′-3′ dideoxynucleotide triphosphates (Sanger, Nicklen & Coulson, 1977).

3. Results and Discussion

We have partially sequenced three A_β ($A_\beta^{30\cdot1}$, $A_\beta^{w31\cdot2}$, $A_\beta^{w36\cdot1}$) and two E_β (E_β^{w31}, $E_\beta^{w31\cdot1}$) genes. From each gene we sequenced most, if not all, of the exon coding for the $\beta1$ domain and the exon coding for the entire $\beta2$ domain. In some of the genes we also sequenced short stretches of intron 2 separating the two exons (data not shown). The sequencing strategy is shown in Fig. 1; the nucleotide sequence appears in Fig. 2. The amino acid sequence derived from the nucleotide sequence appears in Figs. 3 and 4, together with the sequence of further A_β and E_β polypeptides determined

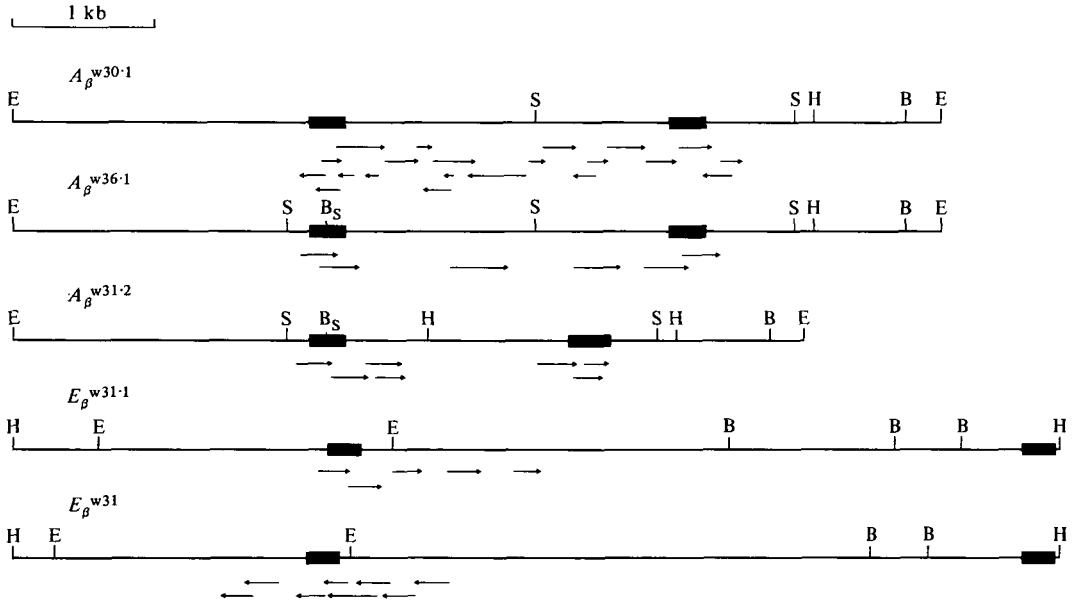

Fig. 1. Restriction enzyme maps of the A_β and E_β genes and the sequencing strategy. Solid rectangles in each gene correspond to the second and third exons. DNA fragments were subcloned in pUC8 and deletion subclones were prepared as described (Frischauf, Garoff & Lehrach, 1980). Arrows indicate the length of DNA regions that were sequenced. The 3′-labelled ends are the arrow tails. The $A_\beta^{w30\cdot1}$ was subcloned in M13mp18 and sequenced by the dideoxinucleotide chain termination method. Restriction enzymes used: B, *Bam* HI; E, *Eco* RI; H, *Hin*dIII,; S, *Sac* I.

https://doi.org/10.1017/S0016672300023545 Published online by Cambridge University Press

```
                6                  10                  15                  20                  25
Aβw36·1  CAT TTC GTG TAC CAG TTC CAG CCC TTC TGC TAC TTC ACC AAC GGG ACG CAG CGC ATA CGG
Aβw31·2  --- --- --- C-- --- --- A-- GG- GAG --- --- -G- --- --- --- --- --- --- --G ---
Aβw30·1  --- --- --- --- --- --- A-- AG- GCG --- --- --- --- --- --- --- --- --- ---
H-2Aβᵇ   --- --- --- --- --- --- AT- GG- GAG --- --- --- --- --- --- --- --- --- --A
H-2Aβᵈ   --- --- --- GT- --- --- A-- GG- GAG --- --- -A- --- --- --- --- --- --- ---
H-2Aβᵏ   --- --- --- C-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβᵘ   --- --- T-- GT- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --A
H-2Aβˢ   --- --- --- -T- --- --- A-- GG- GAG --- --- --- --- --- --- --- --- --- --A
H-2Aβᵠ   --- --- --- GC- --- --G A-- GG- GAG --- --- --- --- --- --- --- --- --- --A
H-2Aβʲ   --- --- --- -C- --- --- A-- GG- GAG --- --- --- --- --- --- --- --- --- --A
Eβw31·1  T-G --T T-- G-A T-C -GT A-A T-T GAG --T C-T --- TA- --- --- --- --- --- G-G ---
Eβw31    TGG --T T-- G-A T-C -GT A-A T-T GAG --T C-T --- TA- --- A-- --- --- --- G-G ---
H-2Eβᵇ   TGG --T T-- G-A T-C -GT A-A TCT GAG --- C-T --- TA- --- --- --- --- --- G-G --C
H-2Eβᵈ   -GG --T T-- G-A T-C G-T ACA TCT GAG --T C-T --- TA- --- --- --- --- -A- G-G ---
H-2Eβᵏ   TGG --T T-- G-A T-C -G- A-A TCT GAG --T C-T --- TA- --- --- --- --- --- G-G ---
H-2Eβˢ   TGG --T T-- G-A T-T -CT ACA TCT GAG --T C-T --- TA- --- --- --- --- --- G-G ---
H-2Eβᵘ   -GG --T T-- GGA T-T -CT ACA TCT GAG --T C-T --- TA- --- --- --- --- --- G-G ---

                30                  35                  40                  45
Aβw36·1  CTT GTG ACC AGA TAC ATC TAC AAC CGG GAG GAG TTC ATG CGC TTC GAC AGC GAC GTG GGC
Aβw31·2  --C --- G-- --- --- --- --- --- --- --- --- -A- CC- --- --- --- --- --- --- ---
Aβw30·1  --C --- -G- --- A-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβᵇ   TA- --- --- --- --- --- --- --- --- --- --- -A- G-- --- -A- --- --- --- --- ---
H-2Aβᵈ   --C --- --- --- --- --- --- --- --- --- --- -A- G-- --- -A- --- --- --- --- ---
H-2Aβᵏ   --- --- -T- --- --- --- --- --- --- --- --- -A- G-- --- --- --- --- --- --- ---
H-2Aβᵘ   TA- --- --- --- --- --- --- --- --- --- --- -A- C-- --- --- --- --- --- --- ---
H-2Aβˢ   TC- --- GA- --- --- ------- --- --- --- --- -A- C-- --- --- --- --- --- --- ---
H-2Aβᵠ   TC- --- -A- --- --- --- --- --- --- --- --- -GG G-- --- --- --- --- --- --- ---
H-2Aβʲ   TC- --- GA- --- --- --- --- --- --- --- --- -A- C-- --- --- --- --- --- --- ---
Eβw31·1  T-- C-- AAA --- --- T-- --- --- -T- --- --- AA- C-- --- --- --- --- --- --- ---
Eβw31    --- C-- GAA --- --- --- --- --- -T- --- --- -AT T-A -A- --- --- --T --- C-- --G
H-2Eβᵇ   --- C-- GAA --- -T- --- --- --- -T- --- --- AA- C-- --- --- --- --- --- --- ---
H-2Eβᵈ   --- C-- GAG --- --- --- --- --- --- --- --- AA- C-- --- --- --- --- --- --- ---
H-2Eβᵏ   --- C-- GTA --- --- T-- --- --- -T- --- --- AA- C-- --- --- --- --- --- C-- ---
H-2Eβˢ   --- C-- GAA --- --- T-- --- --- -T- --- --- A-- C-- --- --- --- --- --- --- ---
H-2Eβᵘ   T-- C-- GAC --- --- T-- --- --- --- --- --- --- --- --- --- --- --- -- --- ---

                46                  50                  55                  60                  65
Aβw36·1  GAG TAC CGC GCG GTG ACC GAG CTG GGG CGG CCA GAC GTC GAG TAC TGG AAC AGC CAG ///
Aβw31·2  -G- --- --- --- --- --- --- -C- --- --- -AC TCA -C- --- --- -AC --T -AG --- ///
Aβw30·1  --- -T- --- --- --- --G --- --- --- --- --- --- --- --- --- --- --T -AG --- ///
H-2Aβᵇ   --- C-- --- --- --- --- --- --- -------- --- --- -C- --- --- --- --- --- --- CCG
H-2Aβᵈ   --- --- --- --- --- --- --- --- --- --- --- --- -C- --- --- --- --- --- --- CCG
H-2Aβᵏ   --- --- --- --- --- --- --- --- --- --- --- --- -C- --- --- --- --T -A- --- ///
H-2Aβᵘ   --- --- --- --- --- --- --- --- --- --- --- --- -C- --- --- -AC --T -A- --- ///
H-2Aβˢ   --- --- --- --- --- --- --- --- --- --- --- --- -C- --- --- -AC --T -AG --- ///
H-2Aβᵠ   --- --- --- --- --- --- -------- --- --- --- --- -C- --- --- --- --- --- --- CCG
H-2Aβʲ   --- --- --- --- --- --- --- --- --- --- T-- --- -C- --- --- -AC --T -AG --- ///
Eβw31·1  --- -T- --- --- --- --- --- --- --- --- --- --- -C- --- A-- --- --- --- --- CCG
Eβw31    --- -T- --- --- --- --- --- --- --- --- --- --- -C- --- A-- --- --- --- --- CCG
H-2Eβᵇ   --- -T- --- --- --- --- --- --- --- G-- --- --- -C- --- A-- --- --- --- --- CCG
H-2Eβᵈ   --- --- --- --- --- --A --- --- --- --- --- --- -C- --- A-- --- --- --- --- CCG
H-2Eβᵏ   --- -T- --- --- --- --- --- --- --- --- --- --- -C- --- A-- --- --- --- --- CCG
H-2Eβˢ   --- -T- --- --- --- --- -------- --- --- --- --- -C- --- A-- --- --- --- --- CCG
H-2Eβᵘ   --- --- --- --- --- --- --- -------- --- --- --A -C- --- A-- --- --- --- --- CCG

                70                  75                  80                  85
Aβw36·1  TAC /// CTG GAG CGA ACG CGG GCC GAG CTG GAC ACG GTG TGC AGA CAC AAC TAC GAG AAG
Aβw31·2  --- /// --- --- --- --- --- --- --- --- --- --- -C- --- --- --- --- --- --- G--
Aw30·1   --- /// --- --- -A- -A- --- --- -C- G-- --- --- --- --- --- --- --- --- --- GG-
H-2Aβᵇ   G-G ATC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GG-
H-2Aβᵈ   G-G ATC --- --- --- --- --- --- --- G-- --- --- -C- --- --- --- --- --- --- GG-
H-2Aβᵏ   --G     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβᵘ   --- /// --- --- --- --- --- --- --- --- --- --- --- --- --- T-- --- --- --- G--
H-2Aβˢ   --- /// --- --- -A- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GG-
H-2Aβᵠ   G-G ATC --- --- --- --- A-- --- --- G-- --- --- --- --- --- --- --- --- --- GG-
H-2Aβʲ   --- /// --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- GG-
Eβw31·1  G-G TTC --- --- -A- -A- --- --- -C- G-- --- --- TAC --- --- --- --- --T --- -TC
Eβw31    G-G TTC --- --- -A- -A- --- --- --- G-- --- --- --- --- --- --- --- --T --- -TC
H-2Eβᵇ   G-G TTC --- --- -A- -A- --- --- --- G-- --- --- --- --- --- --- --- --T --- -TC
H-2Eβᵈ   G-G ATC --- --- GAT G-- --- --- TC- G-- --- --- TAC --- --- --- --- --T --- -TC
H-2Eβᵏ   G-G TTC --- --- CA- -A- --- --- --- --- --- --- --- --- --- --- --- --T --- -TC
H-2Eβˢ   G-G TTC --- --- -A- -G- --- --- -C- --- --- --- TAC --- --- --- --- --T --- -TC
H-2Eβᵘ   G-G ATC --- --- -A- --- --- --- -C- G-- --- --- TAC --- --- --- --- --T --- -TC
```

Fig. 2. For caption see page 141

https://doi.org/10.1017/S0016672300023545 Published online by Cambridge University Press

```
                 86                  90                  95
Aβw36·1     ACG GAG ACC CAC ACC TCC CTG CGG CGG TTT G
Aβw31·2     --- --- --- -C- --- --- --- --- --- C-- -
Aw30·1      C-- --- --- --- --- --- --- --- --- --- -
H-2Aβ^b     C-- --- --- --- --- --- --- --- --- C-- -
H-2Aβ^d     C-- --- --- AG- --- --- --- --- --- --- -
H-2Aβ^k     --- --- --- -C- --- --- --- --- -G- --- -
H-2Aβ^u     --- --- GT- -C- --- --- --- --- --- C-- -
H-2Aβ^s     GT- --- --- --- --- --- --- --- --- C-- -
H-2Aβ^q     GT- --- --- --- --- --- --- --- --- C-- -
H-2Aβ^f     GT- --- --- -C- --- --- --- --- --- C-- -
Eβw31·1     TT- --T -AA TT- CTT /// G-- -C- --- AGA -
Eβw31       T-- --T -AA TT- CTT /// G-- --- --- AGA -
H-2Eβ^b     T-- --T -AA TT- CTT /// G-- --- --- AGA -
H-2Eβ^d     T-- --T -AA TT- CTT /// G-- --- --- AGA -
H-2Eβ^k     TTC --T --- TT- CTT /// G-- --- --- AGA -
H-2Eβ^s     TT- --T -AA TT- CTT /// G-T --- --- AGA -
H-2Eβ^u     TC- --T AAA TT- CTT /// G-- --- --- AGA -

                 96                 100                 105                 110                 115
Aβw36·1     AA CAG CCC AAT GTC GTC ATC TCC CTG TCC AGG ACA GAG GCC CTC AAC CAC CAC AAC ACT
Aβw31·2     -- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Aβw30·1     -- --- --- --- A-- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^b     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^d     -- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^k     -- --- --- -G- --- --- --- --- --- --- --- --- --- --- --- --- ------- --- --G
H-2Aβ^u     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^s     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^q     -- --- --- --- --- -C- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^f     -- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Eβ^b     TT G-- --T -CG --G ACT G-G GA- -CC A-A -A- --G C-- C-- --G G-A --- --- --- CTC
H-2Eβ^d     TT G-- --T -CG --G ACT G-G -A- -CC A-A -A- --G C-- C-- --G G-A --- --- --- CTG
H-2Eβ^k     TT G-- --T -CG --G ACT G-G GA- -CC A-A -A- --G C-- C-- --G G-A --- --- --- CTC
H-2Eβ^s     TT G-- --T -CG --G ACT G-G -A- -CC A-A -A- --G C-- C-- --G G-A --- --- --- CTC
H-2Eβ^u     TT G-- --T -CG --G ACT G-G -A- -CC A-A -A- --G D-- C-- --G G-A --- --- --- CTC

                                120                 125                 130                 135
Aβw36·1     CTG GTC TGC TCA GTG ACA GAT TTC TAC CCA GCC AAG ATC AAA GTG CGC TGG TTC CGG AAT
Aβw31·2     --- --- --T --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Aw30·1      --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^b     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^d     --- --- --T --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-- ---
H-2Aβ^k     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^u     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^s     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^q     --- --- --- --G --- --- --- --- --- --- --- --- --- --- --- --- --- --- A-- ---
H-2Aβ^f     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Eβ^b     --- --- --- --T --- AGT --C --- --- --T -G- --C --T G-- --C A-A --- --- --- ---
H-2Eβ^d     --- --- --- --T --- AGT --C --- --- --T -G- --C --T G-- --C A-A --- --- --- ---
H-2Eβ^k     --- --- --- --T --- AGT --C --- --- --T -G- --C --T G-- --C A-A --- --- --- ---
H-2Eβ^s     --- ------- --T --- AGT --C --- --- --T -G- --C --T G-- --C A-A --- --- --- ---
H-2Eβ^u     --- --- ------T --- AGT --C --- --- --T -G- --C --T G-- --C A-A --- --- --- ---

                136                 140                 145                 150                 155
Aβw36·1     GGC CAG GAG GAG ACG GTG GGG GTC TCA TCC ACA CAG CTT ATT AGG AAT GGG GAC TGG ACC
Aβw31·2     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
Aβw30·1     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^b     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^d     --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^k     --- --- --- --- --A --- --- --- --G --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^u     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^s     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --T --- --- --- ---
H-2Aβ^q     --- --- --- --- --A --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Aβ^f     --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
H-2Eβ^b     --- A-- --- --- -AA ACA --A A-T GTG --- --G GGC --G G-C CCA --- --A --- --- ---
H-2Eβ^d     --- A-- --- --- GAA ACA --A A-T GTG --- --G GGC --G G-C C-A --- --A --- --- ---
H-2Eβ^k     --- A-- --- --- -AA ACA --A A-T GTG --- --G GGC --G G-C C-A --- --A --- --- ---
H-2Eβ^s     --- A-- --- --- -AA ACA --A A-T GTG --- --G GGC --G G-C C-A --- --A --- --- ---
H-2Eβ^u     --- A-- --- --- -AA ACA --A A-T GTG --- --G GGC --G G-C C-A --- --A --- --- ---
```

Fig. 2. For caption see opposite

https://doi.org/10.1017/S0016672300023545 Published online by Cambridge University Press

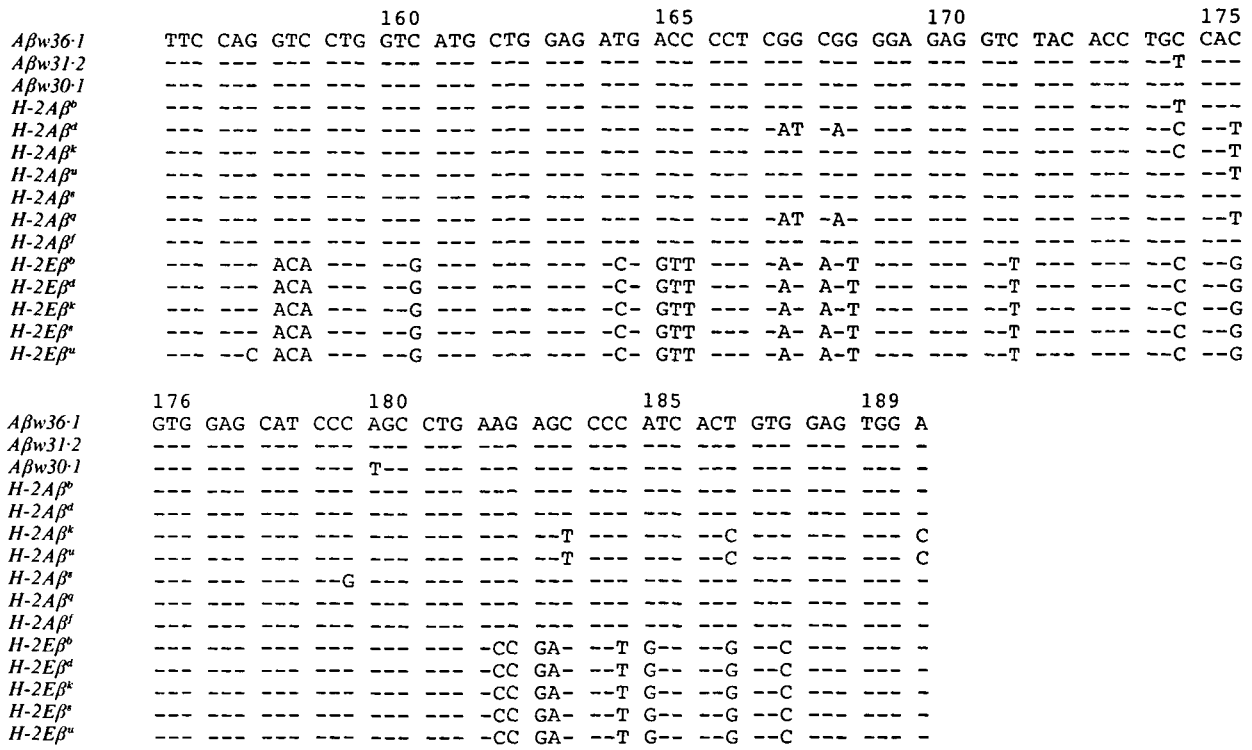

Fig. 2. Nucleotide sequence of *t*-associated class II genes. Dash ('–') indicates identity with the nucleotide in the top line; slash (/) indicates absence (deletion) of a nucleotide; codons are numbered to correspond with the amino acid residues for which they code in the polypeptide chain. For comparison, the sequences of A_β and E_β genes borne by non-*t* laboratory mice are also shown. The A_β sequences are taken from Larhammer *et al.* (1983) and Estess *et al.* (1986). The E_β sequences are taken from Widera & Flavell (1984), Saito *et al.* (1983) and Mengle-Gaw & McDevitt (1983, 1985).

by other investigators. The percentages of similarity between the various genes and polypeptide chains are given in Tables 1–3.

The ten A_β first-domain sequences can be divided into two groups: in the first group the codons specifying amino acid residues at positions 65 and 67 are deleted; in the second group these two codons are present. The former group (A_β^Δ) contains the A_β alleles *f*, *k*, *s*, *u*, *w30·1*, *w31·2* and *w36·1*; the latter contains A_β alleles *b*, *d* and *q*. Hence all three A_β genes carried by the *t* chromosomes have the A_β^Δ form of the gene. Since the three genes represent the three main groups of *t* haplotypes, it is likely that most if not all *t* chromosomes bear the A_β^Δ gene. The three genes not carrying the deletions (A_β^b, A_β^d, A_β^q) show from 93% to 95% similarity to one another in their nucleotide sequence and only 84–91% similarity to the A_β^Δ genes. Among the A_β^Δ genes, the A_β^s and A_β^f alleles show 98% similarity. One further group of genes showing from 92% to 96% similarity consists of A_β^u, A_β^k and $A_\beta^{w36\cdot1}$ alleles. The $A_\beta^{w30\cdot1}$ and $A_\beta^{w31\cdot2}$ alleles show only an average of 90% similarity to these two groups.

The similarities among the ten A_β genes in the third exon (i.e. that coding for the second domain) are significantly higher than those among the second exons. Since the differences range from only 1% to 4% it becomes difficult, if not impossible, to draw any firm conclusions from the comparisons of these genes. Bearing this point in mind, it is nevertheless apparent, that there are two groups of very similar sequences, A_β^d and A_β^q, as well as A_β^b, A_β^f, and $A_\beta^{w36\cdot1}$ (within-group similarity of 99%). The high similarity of A_β^d and A_β^q could be expected from the foregoing discussion. The relative dissimilarity of A_β^b from either A_β^d or A_β^q is surprising because all three of these genes are members of the same group, as determined by comparisons of the second exons. The result could indicate either that the comparisons in the third domain are highly unreliable or that in the A_β^b gene, crossing-over occurred some time in the past between the second and the third exon which obscured the relationship among the A_β genes. Such an event is by no means improbable; it appears that most of the crossing-overs within the class II region lead to exchanges of exons within a gene (Steinmetz *et al.* 1982). Be that as it may, the third exons again reveal no closer similarity among the *t*-associated A_β genes than among the genes not associated with *t* chromosomes or between *t* and non-*t* genes.

The comparisons of the E_β sequences reveal that the E_β^q allele is most dissimilar from all other alleles (from 90% to 92% similarity in the nucleotide sequence and from 78% to 84% similarity in the amino acid sequence). The E_β^q allele is therefore the most distant and at the same time approximately equidistant from all other alleles. This observation suggests the existence

https://doi.org/10.1017/S0016672300023545 Published online by Cambridge University Press

```
                1         10        20        30        40        50        60        70        80        90
Aβb             GDSERHFVYQFMGECYFTNGTQRIRYVTRYIYNREEYVRYDSDVGEHRAVTELGRPDAEYWNSQPEILERTRAELDTVCRHNYEGPETHTSLRRLE
Aβd             -N------V--K----Y--------L--------------------Y--------------------------V--A----------S-------
Aβk             .....---H--QPF-----------L-I----------F----E-Y----------------K--Y-----------------KT--P-------
Aβf             .....---S--KSA-----------S-D----------L-F------Y---------S----Y-K-/Y/-----------------V--P-------
Aβq             .....---A-LQSA-----------S-N--------C--F------Y--------------------------V----------V----------
Aβs             .....---F--QSA-----------S-D----------L-F------Y-------------Y-K-/Y/--Q---------------V----------
Aβu             .....--LV--QPF-----------------------L-F------Y-------------Y-N-/Y/-------------Y---ET-VP-------
Aβw30·1         .....------KSA---------M-L-S-N------FM-F------F----------V----K-/Y/--QK--AV-------------------F-
Aβw36·1         .....-------QPF---------M-L----------FM-F------Y----------V------/Y/-----------------KT--------F-
Aβw31·2         .....---H--K----C------M-L-A---------P-F-----GY-----P--HS---Y-K-/Y/----------A------ET--P-------

                  100       110       120       130       140       150       160       170       180
Aβb             QPNVVISLSRTEALNHHNTLVCSVTDFYPAKIKVRWFRNGQEETVGVSSTQLIRNGDWTFQVLVMLEMTPRRGEVYTCHVEHPSLKSPITVEW
Aβd             ----A-------------------------------------------------------------------HQ--------------------
Aβk             --S-T-------------------------------------------------------------------------------------------
Aβf             ------------------------------------------------------------A--------------------------------
Aβq             ----A-------------------------------------------------------------------HQ--------------------
Aβs             ------------------------------------------S--------------------------------------------------
Aβu             -------------------------------------------------------------------------------------------
Aβ30·1          ---I---------------------------------------------------------------------------C-------------
Aβ36·1          ----A-------------------------------------------------------------------------------------------
Aβ31·2          -------------------------------------------------------------------------------------------
```

Fig. 3. Amino acid sequence of the polypeptides encoded in the A_β genes. For comparison, the sequence of A_β chains present in non-*t* laboratory mice is also shown. For references see Fig. 2. Dash ('–') indicates identity with the residue in the top line; dot (.) stands for 'not tested', and slash (/) for the absence of a residue (deletion).

```
                1         10        20        30        40        50        60        70        80        90
Eβk             .ASFRPWFLEYCKSECHFYNGTQRVRLLVRYFYNLEENLRFDSDVGEFRAVTELGRPDAENWNSQPEFLEQKRAEVDTVCRHNYEIFDNFLVPRRV
Eβu             RGDS--R--G-ST-------------F-D-----R--WV--------Y---------E---------I---T--A---Y-------S-K---R---
Eβb             VRDS-----G-----------------E-------------------------------------------------------S-K---R---
Eβd             VRDT--R----VT----------H--F-E-FI--R------------Y-----------------I--DA--S---Y-------S-K---R---
Eβs             ......-----ST---------------E-------------------------------------R--A---Y-------L-K-------
Eβw17           VRDS--R-------------------F-K-----------------------------------------I------A----------------R---
Eβw31           ......--------------R-------E-------------------------------------------------S-K---R--.
Eβw31·1         ......*-------------------F-K-----------------------------------------A---Y-------L-K------.
```

Fig. 4. Amino acid sequence of the polypeptides encoded in the E_β genes. For comparison, the sequence of E_β chains present in non-*t* laboratory mice is also shown. For references see Fig. 2. Dash ('–') indicates identity with the residue in the top line; dot (.) stands for 'not tested'.

Table 1. *Percent nucleotide and amino acid similarity between the β1 domains of different A_β alleles (allomorphs)*[a]

Amino acid similarity, allomorph	Nucleotide similarity, allele									
	b	*d*	*q*	*s*	*f*	*w31·2*	*k*	*u*	*w36·1*	*w30·1*
b	—	95·2	94·8	91·1	90·4	86·3	89·3	88·5	89·6	88·1
d	87·8	—	93·7	88·9	88·1	87·8	88·9	87·0	88·5	88·1
q	86·7	88·9	—	91·5	91·1	84·4	87·8	87·0	87·8	87·0
s	82·2	82·2	86·7	—	98·1	91·5	91·9	92·6	90·0	91·5
f	81·1	82·2	86·7	95·6	—	91·5	91·9	92·2	89·3	90·0
w31·2	75·6	76·7	73·3	81·1	83·3	—	91·1	89·3	88·1	88·1
k	83·3	82·2	82·2	86·7	87·8	83·3	—	94·4	95·9	91·1
u	80·0	76·7	76·7	84·4	85·6	80·0	90·0	—	92·6	87·4
w36·1	83·3	78·9	81·1	83·3	82·2	77·8	91·1	85·6	—	91·9
w30·1	77·8	80·0	77·8	80·0	81·1	72·2	80·0	73·3	85·6	—

[a] Similarity was calculated by dividing the number of identities between any two alleles (allomorphs) by the total number of nucleotides (amino acid residues) compared. References for sequences of *b*, *d*, *q*, *s*, *f*, *k*, and *u* are given in the legend to Figs. 3 and 4.

Table 2. *Percent nucleotide and amino acid similarity between the β2 domains of different A_β alleles (allomorphs)*[a]

Amino acid similarity, allomorph	Nucleotide similarity, allele									
	d	*q*	*w31·2*	*f*	*s*	*b*	*w36·1*	*w30·1*	*k*	*u*
d	—	99·6	97·5	97·5	96·1	96·5	96·8	96·1	95·0	96·1
q	100	—	97·2	96·8	96·5	96·8	97·2	96·5	95·4	96·5
w31·2	96·8	96·8	—	98·2	97·9	98·9	98·2	97·9	96·1	97·2
f	95·7	95·7	98·9	—	98·9	99·3	99·3	98·9	97·2	98·2
s	95·7	95·7	98·9	97·8	—	98·9	98·9	98·6	96·8	97·9
b	96·8	96·8	100	98·9	98·9	—	99·3	98·9	97·2	98·2
w36·1	97·8	97·8	98·9	97·8	97·8	98·9	—	98·9	97·5	98·6
w30·1	94·6	94·6	97·8	96·8	96·8	97·8	96·8	—	96·8	97·9
k	95·7	95·7	98·9	97·8	97·8	98·9	97·8	96·8	—	98·9
u	96·8	96·8	100	98·9	98·9	100	98·9	97·8	98·9	—

[a] See footnote to Table 1.

Table 3. *Percent nucleotide and amino acid similarity between the β1 domains of different E_β alleles (allomorphs)*[a]

Amino acid similarity, allomorph	Nucleotide similarity, allele						
	w31	*b*	*k*	*w17*	*s*	*w31·1*	*d*
w31	—	97·8	97·8	97·0	96·0	96·3	91·5
b	98·1	—	98·1	97·4	96·3	96·7	91·9
k	94·4	91·7	—	96·3	95·9	96·3	90·0
w17	93·3	91·0	93·3	—	93·7	95·6	91·5
s	91·0	92·1	91·0	86·5	—	97·4	92·6
w31·1	91·0	92·1	92·1	92·1	93·3	—	92·2
d	82·0	83·1	78·7	84·3	83·1	82·0	—

[a] See footnote to Table 1.

of a linkage disequilibrium between the A^d_β and E^d_β genes. (The sequence of the E^q_β gene is not available and the E^b_β gene is relatively dissimilar from E^d_β, as one would expect if an intragenic crossing-over occurred in the A^b_β gene.) The E^{w31}_β gene is almost identical with the E^b_β gene (98 % similarity in both the nucleotide and amino acid sequences). The $E^{w31\cdot1}_\beta$ gene is relatively similar to E^s_β (97 % similarity in nucleotide sequence). The *t*-associated E_β genes, again, are no more similar to one another than they are to other alleles (excluding E^d_β which is, as already pointed out, very dissimilar from other alleles). In the $E^{w31\cdot1}_\beta$ gene, a mutation

https://doi.org/10.1017/S0016672300023545 Published online by Cambridge University Press

produced a stop codon right at the beginning of the second exon, so that the gene cannot be expressed on the cell surface.

How do these data fit into the present knowledge about the origin of the *t* chromosomes obtained from other studies? The analysis of the RFLP of class I Mhc loci has revealed a remarkable uniformity of restriction enzyme patterns among the various *t* chromosomes, in contrast to a considerable variability of the same loci among inbred strains carrying non-*t* chromosomes (Silver, 1982; Shin *et al.* 1982). Random DNA probes obtained by microdissection of chromosome 17 have failed to indicate any RFLP among the known *t* chromosomes analysed by several restriction endonucleases (Röhme *et al.* 1984; Silver *et al.* 1986). High resolution, two-dimensional gel electrophoresis analysis of several testes-specific proteins encoded in *t* complex genes disclosed no polymorphic variation among *t* haplotypes (Silver, Artzt & Bennett, 1979; Silver *et al.* 1983). These observations lead to the conclusion that all the *t* haplotypes originated from a single ancestral *t* chromosome (Klein *et al.* 1985). Yet the serological analysis of class I and class II Mhc loci (Nizetić, Figueroa & Klein, 1984; Artzt *et al.* 1985), RFLP analysis of class II and C4 loci (Figueroa *et al.* 1984; Golubić *et al.* 1985; Artzt *et al.* 1985), and the nucleotide sequence analysis of the class II loci (this communication), all seem to contradict this conclusion. These analyses indicate that the class II (class I, C4) genes fall into at least three groups. Genes within each group are closely related to one another but genes in different groups are no more similar to one another than they are to genes borne by non-*t* chromosomes.

These seemingly contradictory observations can, theoretically, be reconciled in one of two ways. One possibility is that the *t* chromosomes recombine with non-*t* chromosomes and the *t* complex genes thereby become associated with new *H-2* genes (Artzt *et al.* 1985). Although recombination within the *t* complex is known to occur (Lyon & Phillips, 1959), the recombination frequency is very low ($< 0{\cdot}5\%$) and the event almost always changes complete haplotypes into partial ones (Lyon & Phillips, 1959). It is, therefore, difficult to envisage how the complete *t* haplotypes could have acquired by recombination the different *H-2* haplotypes now associated with them.

The second possibility is that the groups of *H-2* genes on *t* chromosomes are as old as the groups of *H-2* genes borne by non-*t* chromosomes. The grouping would then be a reflection of gene differentiation from a single ancestral form.

The strongest argument in favour of the common origin of all *t*-associated class II alleles is the presence of the two deletions in the $A\beta$ exon 2. It is extremely unlikely that these deletions arose repeatedly and independently and hence the fact that probably all *t* chromosomes carry the A^{Δ}_{β} genes supports the notion that they all arose from a single ancestral chromosome. The observation that many non-*t* chromosomes also carry the A^{Δ}_{β} genes could then be explained in one of two ways. Either the A^{Δ}_{β} genes are continuously being 'released' from *t* chromosomes during recombination with non-*t* chromosomes, or the two deletions occurred before the generation of the ancestral *t* chromosome. That all non-*t* A^{Δ}_{β} genes are derived from *t* chromosomes seems rather unlikely to us since their number is already large and is likely to increase as additional genes are analysed. The release of A^{Δ}_{β} genes from *t* chromosomes could be expected to be a rare event so that not many alleles could be introduced into mouse populations in this way. (It is, however, possible that the release occurred a long time ago and that the extant non-*t* A^{Δ}_{β} alleles are all derived from one originally-released A^{Δ}_{β} gene.) On the other hand, the very great similarity of A^{k}_{β} and $A^{w36{\cdot}1}_{\beta}$ suggests a derivation of the former from a *t* chromosome. Similarly, as we pointed out earlier (Klein *et al.* 1985), some of the E°_{α} mutations that are not associated with *t* haplotypes are also likely to have originated from *t* chromosomes.

We propose therefore the following explanation for the generation of *t*-associated class II gene polymorphisms: the major alleles present in contemporary mouse populations were founded long before the extant mouse species appeared on the evolutionary scene (Klein, 1980). Some of these alleles carried the two deletions in the $A\beta$ exon 2, and it was one such gene that was included in the ancestral *t* chromosome when the *t* complex arose. The A^{Δ}_{β} alleles, both *t* and non-*t*, then differentiated to generate the present major groups. Some of the A^{Δ}_{β} genes have been released from the *t* chromosomes rather recently and these show a high similarity with *t*-associated alleles. Other non-*t* A^{Δ}_{β} genes have persisted since the time before the generation of *t* chromosomes and these are much less similar to *t*-associated alleles. The deletion (mutation) in the E°_{α} gene (Jones, Murphy & McDevitt, 1978; Dembić, Singer & Klein, 1984) occurred probably after the generation of the *t* chromosome but this gene, too, has subsequently been released from the *t* complex by recombination (Klein *et al.* 1985). All the E°_{α} genes now found in laboratory and wild mice on non-*t* chromosomes are derived, according to this explanation, from *t* chromosomes by double crossing-over within the inverted region (Klein *et al.* 1985) or by gene conversion.

We thank Ms. Lynne Yakes for editorial assistance and Dr Zlatko Dembić and Dr Michael Steinmetz, Basel Institute for Immunology, who were involved in the preparatory steps of the present work. This work was supported by grant no. 8 R01 AI14736 from the National Institutes of Health, Bethesda, Maryland, U.S.A., and grant no. Fi 337/1-1 from the Deutsche Forschungsgemeinschaft, Bonn.

References

Artzt, K., Shin, H.-S. & Bennett, D. (1982). Gene mapping within the T/t complex of the mouse. II. Anomalous position of the *H-2* complex. *Cell* **28**, 471–476.

Artzt, K., Shin, H.-S., Bennett, D. & Dimeo-Talento, A. (1985). Analysis of major histocompatibility complex haplotypes of *t*-chromosomes reveals that the majority of diversity is generated by recombination. *Journal of Experimental Medicine* **162**, 93–104.

Ayane, M., Klein, D., Margetić, E., Figueroa, F. & Klein, J. (1987). *Neuraminidase-1* variation among mice carrying *t* chromosomes. Immunogenetics (in press).

Choi, E., McIntyre, K., Germain, R. N. & Seidman, J. G. (1983). Murine $I\text{–}A_\beta$ chain polymorphism: Nucleotide sequences of three allelic $I\text{–}A_\beta$ genes. *Science* **221**, 283–286.

Dembić, Z., Singer, P. A. & Klein, J. (1984). E°: a history of mutation. *The EMBO Journal* **3**, 1647–1654.

Dunn, L. C. (1955). Widespread distribution of mutant alleles (*t*-alleles) in populations of wild house mice. *Nature* **176**, 1275–1276.

Dunn, L. C., Beasley & Tinker, H. (1960). Polymorphism in populations of wild house mice. *Journal of Mammalogy* **41**, 220–229.

Estess, P., Begovich, A. B., Koo, M., Jones, P. P. & McDevitt, H. O. (1986). Sequence analysis and structure-function correlations of murine *q*, *k*, *u*, *s*, and *f* haplotype $I\text{–}A_\beta$ cDNA clones. *Proceedings of the National Academy of Sciences USA* **83**, 3594–3598.

Figueroa, F., Golubić, M., Nizetić, D. & Klein, J. (1985). Evolution of mouse major histocompatibility complex genes borne by *t* chromosomes. *Proceedings of the National Academy of Sciences USA* **82**, 2819–2823.

Fox, M., Martin, G., Lyon, M. F., Herrmann, B., Frischauf, A.-M., Lehrach, H. & Silver, L. M. (1985). Molecular probes define different regions of the mouse *t* complex. *Cell* **40**, 63–69.

Frischauf, A. M., Garroff, H., Lehrach, H. (1980). A subcloning strategy for DNA sequence analysis. *Nucleic Acids Research* **8**, 5541–5549.

Golubić, M., Figueroa, F., Tosi, M. & Klein, J. (1985). Restriction fragment length polymorphism of C4 genes in mice with *t*-chromosomes. *Immunogenetics* **21**, 247–256.

Hammerberg, C. & Klein, J. (1975). Linkage disequilibrium between *H-2* and *t* complexes in chromosome 17 of the mouse. *Nature* **258**, 296–299.

Herrmann, B., Bućan, M., Mains, P. E., Frischauf, A.-M., Silver, L. M. & Lehrach, H. (1986). Genetic analysis of the proximal portion of the mouse *t* complex: evidence for a second inversion within *t* haplotypes. *Cell* **44**, 469–476.

Jones, P. P., Murphy, D. B. & McDevitt, H. O. (1978). Two-gene control of the expression of a murine Ia antigen. *Journal of Experimental Medicine* **148**, 925–939.

Klein, J. (1980). Generation of diversity at MHC loci: Implications for T cell receptor repertoires. In *Immunology 80* (ed. M. Fougereau and J. Dausset), pp. 239–253. London: Academic Press.

Klein, J. (1986). *Natural History of the Major Histocompatibility Complex*, pp. 683–795. New York: Wiley.

Klein, J. & Figueroa, F. (1986). *Evolution of the Major Histocompatibility Complex*. CRC Immunology. **6**, 295–386.

Klein, J. & Hammerberg, C. (1977). The control of differentiation by the T-complex. *Immunological Reviews* **33**, 70–104.

Klein, J., Sipos, P. & Figueroa, F. (1984). Polymorphism of *t*-complex genes in European wild mice. *Genetical Research* **44**, 39–46.

Klein, J., Nizetić, D., Golubić, M., Dembić, Z. & Figueroa, F. (1985). Evolution of *H-2* genes on *t* chromosomes. In *Cell Biology of the Major Histocompatibility Complex* (ed. B. Pernis and H. J. Vogel), pp. 97–106. New York: Academic Press.

Larhammar, D., Hämmerling, U., Denaro, M., Lund, T., Flavell, R. A., Rask, L. & Peterson, P. A. (1983). Structure of the murine immune response $I\text{–}A_\beta$ locus: Sequence of the $I\text{–}A_\beta$ gene and an adjacent β-chain second domain exon. *Cell* **34**, 179–188.

Lyon, M. F. (1981). The T-complex and the genetical control of development. *Symposium of the Zoological Society, London* **47**, 71–104.

Lyon, M. F. & Phillips, R. J. S. (1959). Crossing-over in mice heterozygous for *t*-alleles. *Heredity* **13**, 23–32.

Malissen, M., Hunkapiller, T. & Hood, L. (1983). Nucleotide sequence of a light chain gene of the mouse I–A subregion: A^d_β. *Science* **221**, 750–754.

Maxam, A. M. & Gilbert, W. (1977). A new method for sequencing DNA. *Proceedings of the National Academy of Sciences USA* **74**, 560–564.

Mengle-Gaw, L. & McDevitt, H. O. (1983). Isolation and characterization of a cDNA clone from the murine $I\text{–}E_\beta$ polypeptide chain. *Proceedings of the National Academy of Sciences USA* **80**, 7621–7625.

Mengle-Gaw, L. & McDevitt, H. O. (1985). Predicted protein sequence of the murine $I\text{–}E^s_\beta$ polypeptide chain from cDNA and genomic clones. *Proceedings of the National Academy of Sciences USA* **82**, 2910–2914.

Nadeau, J. (1983). Absence of detectable gametic disequilibrium between the *t*-complex and linked allozyme-encoding loci in house mice. *Genetical Research* **42**, 323–333.

Nadeau, J. H. (1986). A glyoxalase-1 variant associated with the *T*-complex in house mice. *Genetics* **113**, 91–99.

Nizetić, D., Figueroa, F. & Klein, J. (1984). Evolutionary relationships between the *t* and *H-2* haplotypes in the house mouse. *Immunogenetics* **19**, 311–320.

Röhme, D., Fox, H., Herrmann, B., Frischauf, A.-M., Edstrom, J. E., Mains, P., Silver, L. M. & Lehrach, H. (1984). Molecular clones of the mouse *t* complex derived from microdissected metaphase chromosomes. *Cell* **36**, 783–788.

Saito, H., Maki, R. A., Clayton, L. J. & Tonegawa, S. (1983). Complete primary structures of the E_β chain and gene of the mouse major histocompatibility complex. *Proceedings of the National Academy of Sciences USA* **80**, 5520–5524.

Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences USA* **74**, 5463–5467.

Shin, H.-S., Starnezer, J., Artzt, K. & Bennett, D. (1982). The genetic structure and origin of *t*-haplotypes of mice, analysed with *H-2* cDNA probes. *Cell* **29**, 969–976.

Silver, L. M. (1982). Genomic analysis of the *H-2* complex region associated with mouse *t* haplotypes. *Cell* **29**, 961–968.

Silver, L. M. (1985). Mouse *t* haplotypes. *Annual Reviews of Genetics* **19**, 179–208.

Silver, L. M., Artzt, K. & Bennett, D. (1979). A major testicular cell protein specified by a mouse T/t complex gene. *Cell* **17**, 275–284.

Silver, L. M., Uman, J., Danska, J. & Garrels, J. (1983). A diversified set of testicular cell proteins specified by genes within the mouse *t* complex. *Cell* **35**, 35–45.

Silver, L. M., Hammer, M., Fox, H., Garrels, J., Bucan, M., Herrmann, B., Frischauf, A.-M., Lehrach, H., Winking, H., Figueroa, F. & Klein, J. (1986). Molecular evidence that all mouse *t* haplotypes are descended from a single

ancestral chromosome. *Molecular Biology and Evolution*. (In the Press.)

Steinmetz, M., Minard, K., Horvath, S., McNicholas, J., Frelinger, J., Wake, C., Long, E., Mach, B. & Hood, L. (1982). A molecular map of the immune region from the major histocompatibility complex of the mouse. *Nature* **300**, 35–42.

Sturm, S., Figueroa, F. & Klein, J. (1982). The relationship between *t* and *H-2* complexes in wild mice. I. The *H-2* haplotypes of 20 *t*-bearing strains. *Genetical Research* **40**, 73–88.

Viera, J. & Messing, J. (1982). The pUC plasmids and M13 mpt derived system for insertion mutagenesis and sequencing with synthetic universal primers. *Gene* **19**, 259–268.

Widera, G. & Flavell, R. A. (1984). The nucleotide sequence of the murine I–E^{b}_{β} immune response gene: evidence for gene conversion events in class II genes of the major histocompatibility complex. *The EMBO Journal* **3**, 1221–1225.