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E^o: a history of a mutation

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Eighteen mouse *t* haplotype-carrying strains were found not to express cell-surface E molecules controlled by class II genes of the *H-2* complex (= E^o strains). Northern and Southern blot analysis of these and other, non-*t* strains that also fail to express the E molecule, has revealed two kinds of defect. Three strains (CRO437, t^{w2}, and presumably t^o) were found to transcribe the E_α gene, but they were not able to convert the message into a functional protein. All other E^o strains fail to transcribe the E_α gene because of a deletion encompassing the promoter region, the RNA initiation site, and the first exon. The length of the deletion is ~650 ± 50 bp. These two defects closely resemble those found previously in standard inbred strains carrying the *H-2*^{f}, *H-2*^{q} (failure of E mRNA to be expressed functionally), *H-2*^{b}, and *H-2*^{s} (deletion of a part of the E_α gene) haplotypes. In particular, the location and length of the E_α deletion appear to be the same in the strains carrying this mutation. The E_α deletion is in linkage disequilibrium with certain alleles at other *H-2* loci in some of the strains. These observations, combined with the growing evidence that *H-2* haplotypes associated with *t* chromosomes derive from a single ancestral haplotype, suggest that the E_α deletion is an old mutation and that it has been disseminated in mouse populations by the *t* chromosomes.

Key words: major histocompatibility complex/*t* complex/evolution

Introduction

The major histocompatibility complex (MHC) of the mouse contains at least four functional class II genes: A_α and A_β which code for the two chains of the A molecule, and E_α and E_β which code for the α and β chains of the E molecule (Klein *et al.*, 1983b). Some of the inbred strains carry mutations that cause the failure of the expression of the E molecules on the cell surface (Jones *et al.*, 1978, 1981). These mutations are of three kinds (Mathis *et al.*, 1983; Hyldig-Nielsen *et al.*, 1983). In the *H-2*^{b} and *H-2*^{s} strains, the E_α gene contains a deletion that prevents the transcription of this gene, and since no E_α protein is synthesized, the E_β chain alone cannot be inserted into the plasma membrane. Mice of the *H-2*^{f} haplotype synthesize predominantly an E_α mRNA of aberrant size, and mice of the *H-2*^{q} haplotype seem to have a defect in RNA processing or stability.

Recently, we found E^o mutations (i.e., mutations that cause the absence of E molecules on the cell surface) in more than one half of the *t*-bearing strains (Nižetić *et al.*, 1984). The *t* haplotypes are abnormal regions of chromosome 17, the same chromosome that carries the *H-2* complex, the MHC of the mouse. The abnormality manifests itself in several developmental and genetic disturbances: shortening of the tail, arrest of embryogenesis in some *t*/*t* homozygotes, distorted transmission of the *t* chromosome through the sperm, sterility of males, and suppression of crossing-over in *t*/+ heterozygotes (Klein and Hammerberg, 1977; Lyon, 1981). The genes responsible for embryonic lethality fall into 16 complementation groups, eight of which have been identified by us recently (Klein *et al.*, 1984). The *t* haplotypes have been found with a frequency of up to 40% in most of the sampled wild mouse populations. The *H-2* haplotypes of the different *t* chromosomes are all related and probably arose from a single ancestral haplotype (Nižetić *et al.* 1984). The finding of E^o mutations in *t* chromosomes raises questions about the origin of these mutations and their relationship to similar mutations found in inbred strains. As a first step in an attempt to answer these questions, we have tested all the *t* haplotypes available to us for the presence of the E_α mRNA and analyzed their E_α genes by digestion with restriction endonucleases. For comparison, we included in this study the *H-2* haplotypes *b, s, f* and *q*, and six other strains which also fail to express cell-surface E molecules: NMRI/Navy, STS/A, NFS/N, B10.LG, GPC151, and B10.CAS2.

Results

Analysis of poly(A)$^+$ RNA

To determine whether the E^o strains transcribe the E_α gene, we isolated poly(A)$^+$ RNA from them and analyzed it by the dot blot technique. Because strains carrying lethal *t* haplotypes could not be made homozygous for their *H-2* haplotypes, we used F_1 hybrids of *t* lines with strains carrying the *H-2*^{b} haplotype (C57BL/10Sn, B6.K1, or B6.K2) or the *H-2*^{w17} haplotype (B10.CAS2). Strains carrying semilethal or viable *t* haplotypes were used as *H-2* homozygotes. The poly(A)$^+$ RNA was spotted onto nitrocellulose filters and hybridized with a probe derived from the E_α^d gene and encompassing the segment from the first to the fourth intron (Steinmetz *et al.*, 1982). Of all the tested E^o strains, the probe hybridized only with the mRNA of t^{w2} and CRO437 (t^{Tuw7}), carrying semilethal *t* haplotypes (Figure 1). All other E^o strains tested (with the possible exception of t^o, see Discussion) failed to hybridize with the probe and thus apparently lack E_α-encoding mRNA.

To determine the size of the E_α-mRNA in t^{w2} and CRO437, we performed Northern blot analysis. Poly(A)$^+$ RNA was electrophoresed in formaldehyde/agarose gels, transferred from the gel to a nitrocellulose filter, and hybridized with the E_α probe. Both t^{w2} and CRO437 showed two bands, one ~1.25 kb (presumably the mature E_α mRNA) and the other ~2.8 kb (possibly a precursor form of the E_α mRNA) (Figure 2). In t^{w2} the 2.8-kb band was of a considerably higher intensity (comparable with that found in

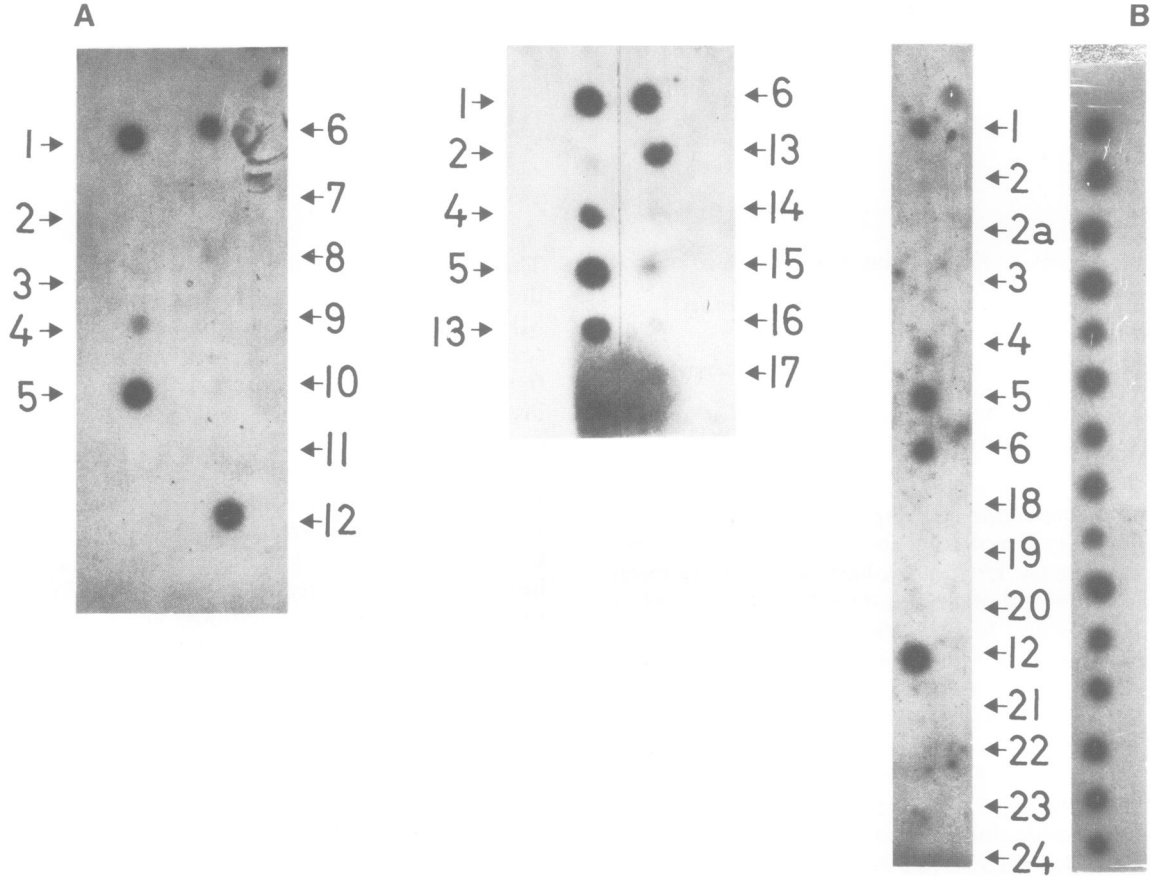

Fig. 1. Dot blot analysis of the poly(A)$^+$ RNA from E^o strains. Approximately 1 μg of poly(A)$^+$ RNA for each dot was used, and the filters were then hybridized with the E_α (**A**) and the E_β (**B**) probes. The latter served as a control for the quality of the RNA preparation. (**1**) DBA/2; (**2**) C57BL/10Sn; (**2a**) C57BL/6J; (**3**) B10.S; (**4**) B10.Q; (**5**) A.CA; (**6**) C3H/HeJ; (**7**) (BNK761 x B10.CAS2)F_1; (**8**) (GPC183 x B10.CAS2)F_1; (**9**) (PLD826 x B6.K2)F_1; (**10**) (t^{w5} x B10.CAS2)F_1; (**11**) (BNK266 x 129)F_1; (**12**) t^{w2}; (**13**) CRO437; (**14**) (MSW251 x B6.K2)F_1; (**15**) (t^{w32} x B6.K2)F_1; (**16**) B10.A(4R); (**17**) STS/A; (**18**) B10.CAS2; (**19**) ISL33; (**20**) CRO435; (**21**) (ERP1465 x B6.K1)F_1; (**22**) (LGN925 x B10)F_1; (**23**) (ROD1455 x B6.K1)F_1; (**24**) (t^{Lub7} x B6.K1)F_1.

H-2^f or *H-2^d*, see Mathis *et al.*, 1983) than the same band in CRO437. This pattern is different from that found in *H-2^q*, in which the 1.25-kb band is shifted to a higher mol. wt. region. Thus, the t^{w2} and CRO437 E_α mRNA resembles E_α^f mRNA more than E_α^q mRNA.

Analysis of the E^o genes by Southern blotting

Mice of the *H-2^b* and *H-2^s* haplotypes have a deletion of ~650 ± 50 bp in the E_α gene, which includes all or part of the E_α promoter region, the RNA initiation site and the first exon (Mathis *et al.*, 1983; Hyldig-Nielsen *et al.*, 1983). Since the majority of the tested E^o strains resembled *H-2^b* and *H-2^s* in their lack of mRNA coding for the E_α protein, we asked whether these strains carry a deletion similar to that carried by these two haplotypes. To answer this question, we performed Southern blotting analyses with DNA isolated from these strains or from their F_1 hybrids with either B10.BR(*H-2^k*) or one of the *H-2^b* strains when *H-2* homozygotes could not be produced. The isolated genomic DNA was digested in separate experiments with one or two of six selected restriction endonucleases, the resulting fragments were separated by electrophoresis in agarose gels, transferred to nitrocellulose and hybridized with the E_α probe. Examples of some of the patterns obtained are given in Figures 3 and 4; all the patterns are diagrammatically depicted in Figures 5 and 6, and in Table I.

The digestion with *Eco*RI resulted in two E_α-hybridizing fragments in all the strains tested, a monomorphic 3.0-kb fragment and a polymorphic fragment which was 8.6 kb long in *H-2^k* DNA digests and 8.0 kb long in *H-2^b* digests. All strains that lacked E_α mRNA possessed the 8.0-kb (*H-2^b*-like)

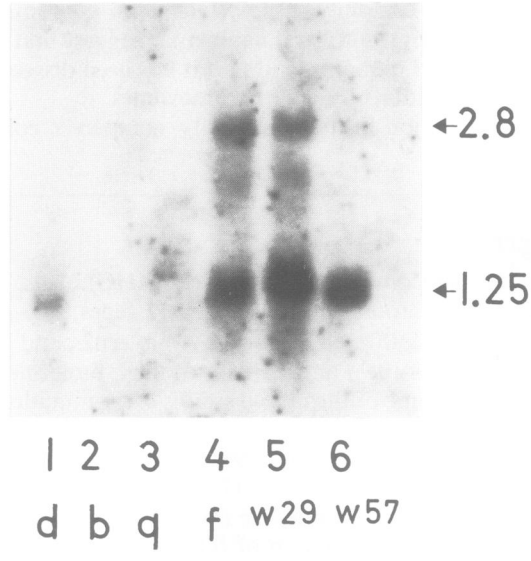

Fig. 2. Northern blot analysis of E_α mRNA from E^o strains. Poly(A)$^+$ RNA was isolated from spleens and hybridized to the E_α probe. Sizes of RNA molecules are in kb. (**1**) DBA/2; (**2**) C57BL/10Sn; (**3**) B10.Q; (**4**) A.CA; (**5**) t^{w2}; (**6**) CRO437. (Letters indicate *H-2* haplotypes.)

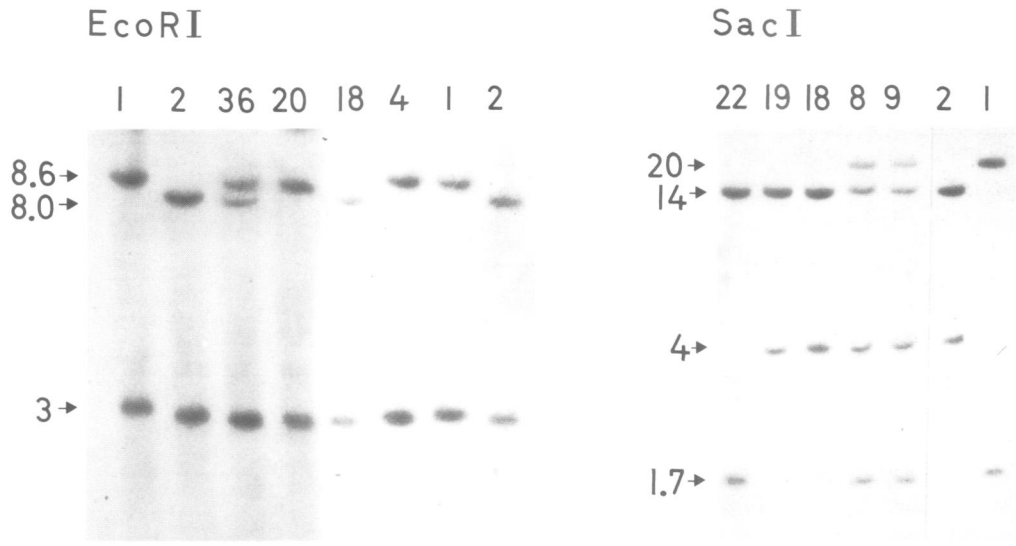

Fig. 3. Southern blot analysis of the E_α gene from some of the mouse strains. Kidney DNA was analysed after digestion with the restriction enzymes shown. Fragment sizes are in kilobases. **(1)** C3H/HeJ; **(2)** C57BL/6J; **(4)** B10.Q; **(8)** (t^{Lub7} x B10.BR)F_1; **(9)** (BNK266 x B10.BR)F_1; **(18)** CRO435; **(19)** ISL33; **(20)** CRO437; **(22)** t^{w2}; **(36)** (BRU382 x C57BL/10Sn)F_1.

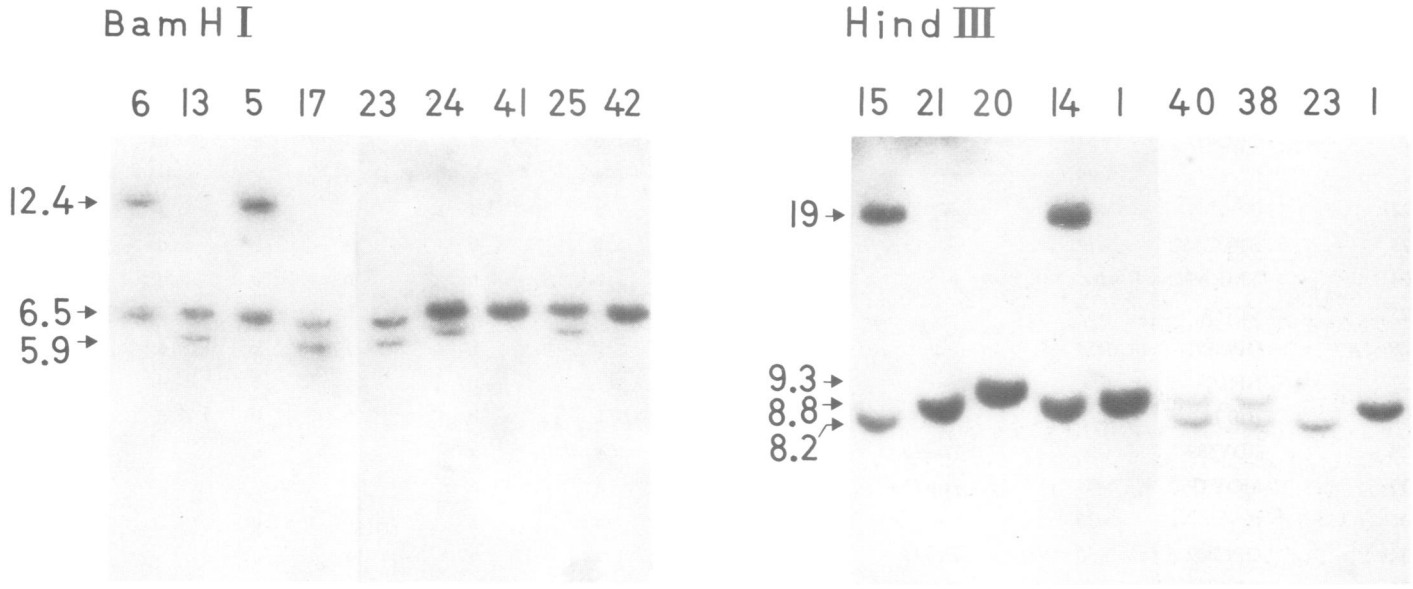

Fig. 4. Southern blot analysis of the E_α gene from some of the mouse strains. **(1)** C3H/HeJ; **(5)** (t^{w5} x B10.BR)F_1; **(6)** (t^{w94} x B10.BR)F_1; **(13)** (ROD1455 x B6.K1)F_1; **(14)** (ERP1465 x B10.BR)F_1; **(15)** (PLD826 x B6.K2)F_1; **(17)** (MSW251 x B6.K2)F_1; **(20)** CRO437; **(21)** (t^o x B10.BR)F_1; **(23)** B10.CAS2; **(24)** NRMI/Navy; **(25)** STS/A; **(38)** (t^{Lub9} x B10.CAS2)F_1; **(40)** (t^{Lub1} x B10.CAS2)F_1; **(41)** STU; **(42)** B10.D2.

fragment (heterozygotes with *H-2*^{k} also possessed the 8.6-kb fragment). The CRO437 strain possessed the 8.6-kb fragment; the t^{w2} mice the 8.0-kb fragment. However, the shortening of the fragment in t^{w2} does not result from a deletion (in digests with all other enzymes, the t^{w2} behaved as if it had a complete E_α gene; see below); rather, it resulted from the presence of an additional *Eco*RI site ~0.6–0.8 kb downstream from the original site.

Digestion with *Hind*III yielded four different patterns: *k*-like (t^{w2} and t^o); *b*-like (t^{w5}, GPC183, t^{Lub7}, t^{12}, MSW251, CRO435, and ISL33); CRO437-type; and BNK266-type (BNK266, BNK761, OBL984, LGN925, ROD1455, PLD826, ERP1465). The CRO437 pattern arises because of loss of the second *Hind*III site (counting sites from left to right in Figure 5) and consequent appearance of a fragment that is ~500 bp longer than the 8.8-kb fragment in *H-2*^{k}. The BNK266 pattern arises because of loss of the third *Hind*III site and consequent appearance of a fragment that is ~19 kb long.

DNA of *H-2*^{k} mice digested with *Bam*HI exhibits one strongly hybridizing band ~6.5 kb long. From previous restriction mapping, however, this band is known to consist of two different fragments of approximately the same size. In *H-2*^{b} mice, one of the two fragments is ~600 bp shorter than the other, so that, in this case, two fragments are seen. CRO437, t^o, and t^{w2} mice yielded the *k*-like pattern. Four strains (t^{w5}, t^{w94}, GPC183, t^{Lub7}) yielded one fragment approximately double the size of the fragments from *H-2*^{k}. This large fragment results from a loss of the second *Bam*HI site – an interpretation confirmed by double digestion with *Bam*HI and *Hind*III (data not shown). All remaining E° strains showed the *b*-like pattern.

*Kpn*I splits the E_α^d gene into two fragments, a smaller frag-

Table I. Restriction enzyme patterns of the E_α gene in the tested strains

Number	Strain	H-2 haplotype	*t* haplotype	E_α allele	Pattern					
					*Eco*RI	*Hind*III	*Bam*HI	*Kpm*I	*Sac*I	*Eco*RV/*Bam*HI
1	C3H	*k*	–	*k*	a[a]	a	a	a	a	a
2	C57BL/6J	*b*	–	b	b	b	b	b	b	b
3	B10.M	*f*	–	*f*	a	a	a	a	c	a
4	B10.Q	*q*	–	*q*	a				c	
5	t^{w5}	*w31*	*w5*	*w28.1*	b	b	c	b	b	
6	t^{w94}	*w31*	*w94*	*w28.1*	b	b	c		b	
7	GPC183	*w31*	*Tuw23*	*w28.1*	b	b	c	b	b	c
8	t^{Lub7}	*w63*	*Lub7*	*w28.1*	b	b	c	b	b	c
9	BNK266	*w36*	*Tuw2*	*w28.2*	b	c	b	b	b	b
10	BNK761	*w36*	*Tuw21*	*w28.2*	b	c	b	b	b	
11	OBL984	*w36*	*Tuw25*	*w28.2*	b	c	b		b	
12	LGN925	*w36*	*Tuw24*	*w28.2*	b	c	b	b	b	
13	ROD1455	*w36*	*Tuw27*	*w28.2*	b	c	b	b	b	
14	ERP1465	*w60*	*Tuw28*	*w28.2*	b	c	b	b	b	
15	PLD826	*w59*	*Tuw26*	*w28.2*	b	c	b	b	b	
16	t^{12}	*w28*	*12*	*w28*	b	b	b	b	b	
17	MSW251	*w38*	*Tuw20*	*w28*	b	b	b	b	b	b
18	CRO435	*w37*	*Tuw7*	*w28*	b	b	b	b	b	b
19	ISL33	*w56*	*Tuw32*	*w28*	b	b	b	b	b	b
20	CRO437	*w57*	*Tuw8*	*w29.1*	a	d	a		c	a
21	t^{o}	*w29*	*o*	*w29*	b	a	a	a	c	
22	t^{w2}	*w29*	*w2*	*w29*	b	a	a	a	c	a
23	B10.CAS2	*w17*	–	*w17*	b	b	b	b	b	d
24	NMRI/Navy	*dx*	–	*w17*		b	b	b	b	d
25	STS/A	*w67*	–	*w17*		b	b	b	b	d
26	GPC151	*w53*	–	*w17*	b	b	b		b	d
27	NFS/N	*sq4*	–	*w17*	b		b		b	d
28	B10.LG	*dx*	–	*w17*		b	b	b	b	d
29	EDY589	*w2*	*Tuw10*	*w2*	a	a	a		c	
30	MOY336	*w65*	*Tuw15*	*w2*	a	a	a			a
31	t^{6}	*w30*	*6*	*w2*	a	a	a	a	c	
32	GPC882	*w30*	*Tuw11*	*w2*	a	a	a	a	c	
33	LRA410	*w66*	*Tuw12*	*w2*	a	a	a		c	
34	t^{w1}	*w30*	*w1*	w2	a		a			
35	BRW942	*w61*	*Tuw29*	*w2*	a	a	a	a	c	
36	BRU382	*w58*	*Tuw6*	*w2*	a	a	a	a	c	
37	t^{Lub4}	*w64*	*Lub4*	*w2*	a	a	a	a	c	
38	t^{Lub9}	*w64*	*Lub9*	*w2*	a	a	a	a	c	
39	t^{w73}	*w32*	*w73*	*w2*	a		a		c	a
40	t^{Lub1}	*w33*	*Lub1*	*w2*	a	a	a	a	c	
41	STU	*w34*	–			a	a	a	c	a
42	B10.D2	*d*		*d*		a	a	a	c	a

[a]Each letter represents a specific band-pattern, as deduced from the Southern blot analysis of the DNA digested with the indicated restriction enzyme.

ment of ~4.3 kb encompassing the promoter region, as well as the first and second exons; and a larger fragment of ~10 kb containing the rest of the gene (Hylding-Nielsen *et al.* 1983). In *H-2*^{b} and all other E^o strains that do not transcribe the E_α gene, the smaller fragment is ~0.6 kb shorter than that in *H-2*^{k}. The CRO437, t^o, and t^{w2} yielded two fragments of the same size as *H-2*^{k}.

On the gels with double digests with *Eco*RV and *Bam*HI four different patterns could be distinguished. A *k*-like pattern, consisting of 2.7 kb and 5.0 kb fragments, is present in CRO437 and t^{w2}. (The 2.7-kb fragment encompasses the first exon and the 5.0-kb fragment the rest of the E_α genes.) A *b*-like pattern, in which the smaller fragment has been shortened to 2.0 kb by the deletion, is present in CRO435, ISL33,

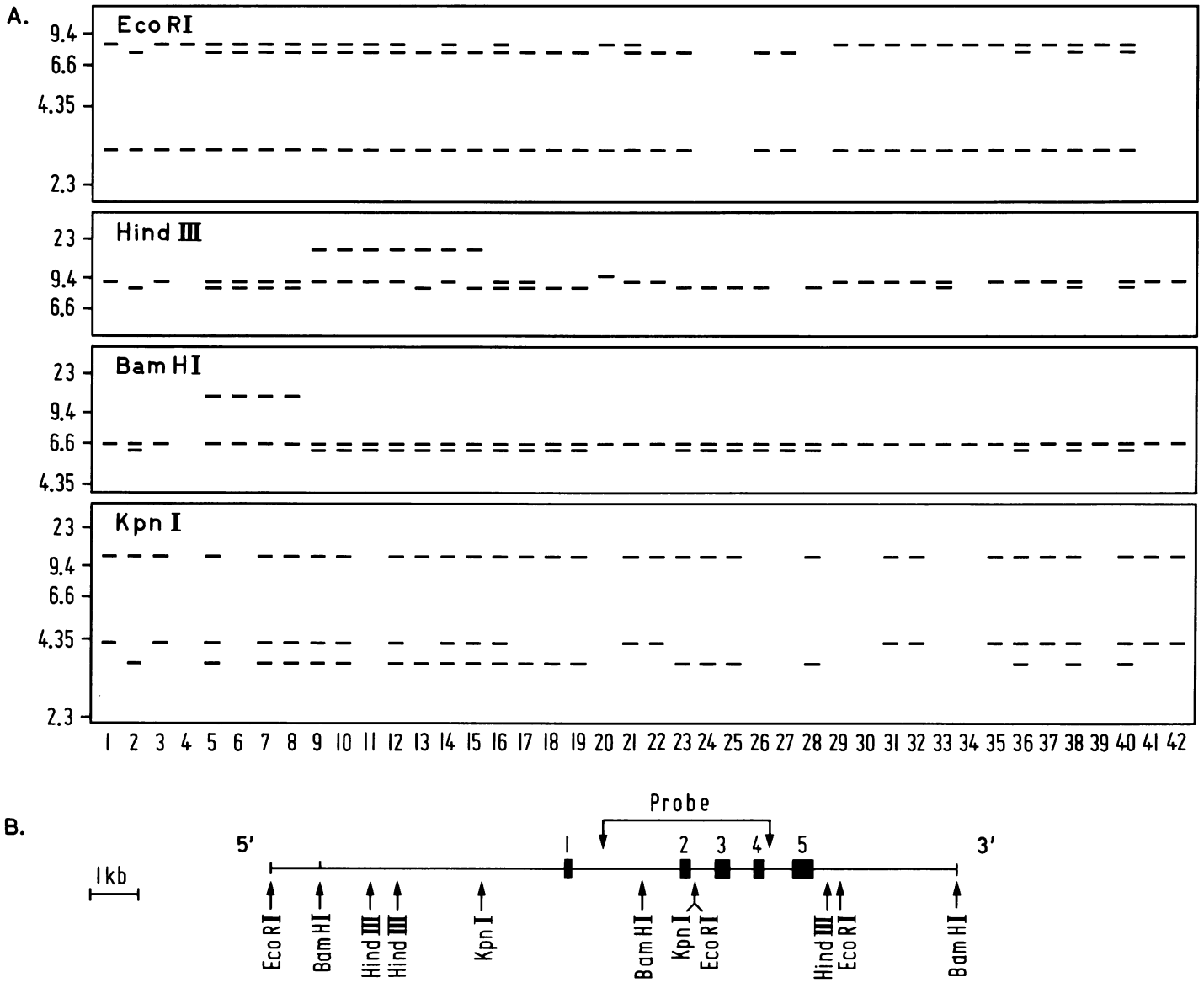

Fig. 5. Summary of restriction enzyme analysis of the E_α genes in the haplotypes tested. **(A)** Schematic representation of the band pattern in the Southern blots of the DNA from various mouse strains, digested with the indicated restriction enzymes. Fragment size is in kb. **(B)** Restriction enzyme map of the E_α gene (based on Mathis *et al.*, 1983 and Hyldig-Nielsen *et al.*, 1983). **(1)** C3H/HeJ; **(2)** C57BL/6J; **(3)** B10.M; **(4)** B10.Q; **(5)** (t^{w5} x B10.BR)F_1 **(6)** (t^{w94} x B10.BR)F_1; **(7)** (GPC183 x B10.BR)F_1; **(8)** (t^{Lub7} x B10.BR)F_1; **(9)** (BNK266 x B10.BR)F_1; **(10)** (BNK761 x B10.BR)F_1; **(11)** (OBL984 x B10.BR)F_1; **(12)** (LGN925 x B10.BR)F_1; **(13)** (ROD1455 x B6.K1)F_1; **(14)** (ERP1465 x B10.BR)F_1; **(15)** (PLD826 x B10.BR)F_1, except when tested with *Eco*RI and *Hind*III was (PLD826 x B6.K2)F_1; **(16)** (t^{12} x B10.BR)F_1; **(17)** (MSW251 x B6.K2)F_1, except when tested with *Hind*III was (MSW251 x B10.BR)F_1; **(18)** CRO435; **(19)** ISL33; **(20)** CRO437; **(21)** (t^{o} x B10.BR)F_1; **(22)** t^{w2}; **(23)** B10.CAS2; **(24)** NMRI/Navy; **(25)** STS/A; **(26)** GPC151; **(27)** NFS/N; **(28)** B10.LG; **(29)** EDY589; **(30)** (MOY336 x B10.BR)F_1; **(31)** (t^{6} x C57BL/10Sn)F_1, except when tested with *Eco*RI, *Bam*HI and *Kpn*I was (t^{6} x B10.BR)F_1; **(32)** (GPC882 x B10.BR)F_1; **(33)** (LRA410 x B10.BR)F_1, except when tested with *Hind*III was (LRA410 x B10.CAS2)F_1; **(34)** (t^{w1} x B10.BR)F_1; **(35)** (BRW942 x B10.BR)F_1; **(36)** (BRU382 x C57BL/10Sn); **(37)** (t^{Lub4} x B10.BR)F_1; **(38)** (t^{Lub9} x B10.CAS2)F_1; **(39)** (t^{w73} x B10.BR)F_1; **(40)** (t^{Lub1} x B10.CAS2)F_1; **(41)** STU; **(42)**B10.D2.

MSW251, and BNK266. A new pattern appears in GPC183 and t^{Lub7} in which the 2.0-kb fragment is replaced by a 7.5-kb fragment apparently because of loss of the second *Bam*HI site in these two strains. Finally, in all the non-*t* strains lacking cell-surface E molecules, the 2.7-kb and 5.0-kb bands disappear and a new 6.5-kb band appears with the 2.0-kb band. This pattern can be explained by loss of an *Eco*RV site downstream from the fifth exon. The next *Eco*RV site lies ~3 kb further downstream in these haplotypes (Figures 6 and 7).

All these data are consistent with the interpretation that all the E° strains, except CRO437, t^{w2}, and t^{o}, have a deletion in their E_α gene of about the same length and in about the same position as in the *H-2*^{b} and *H-2*^{s} haplotypes. Further evidence that this interpretation is correct comes from a digestion experiment with *Sac*I, which recognizes a site in the first exon of the E_α gene and generates a fragment of ~1.7 kb in the *H-2*^{k} and *H-2*^{d} haplotypes but not in the *H-2*^{b} haplotype. Instead, a new fragment appears in *H-2*^{b}, ~4.0 kb in length, because the *Sac*I site in the first exon is deleted and the next site is ~2.5 kb further upstream. The same 4.0-kb fragment is also seen in all E° strains, except CRO437, t^{w2} and t^{o}, indicating that all these strains have lost the *Sac*I site, most likely because of the deletion.

Polymorphism of the E_α gene in E-expressor strains

To determine how related the E_α gene of the E° strains is

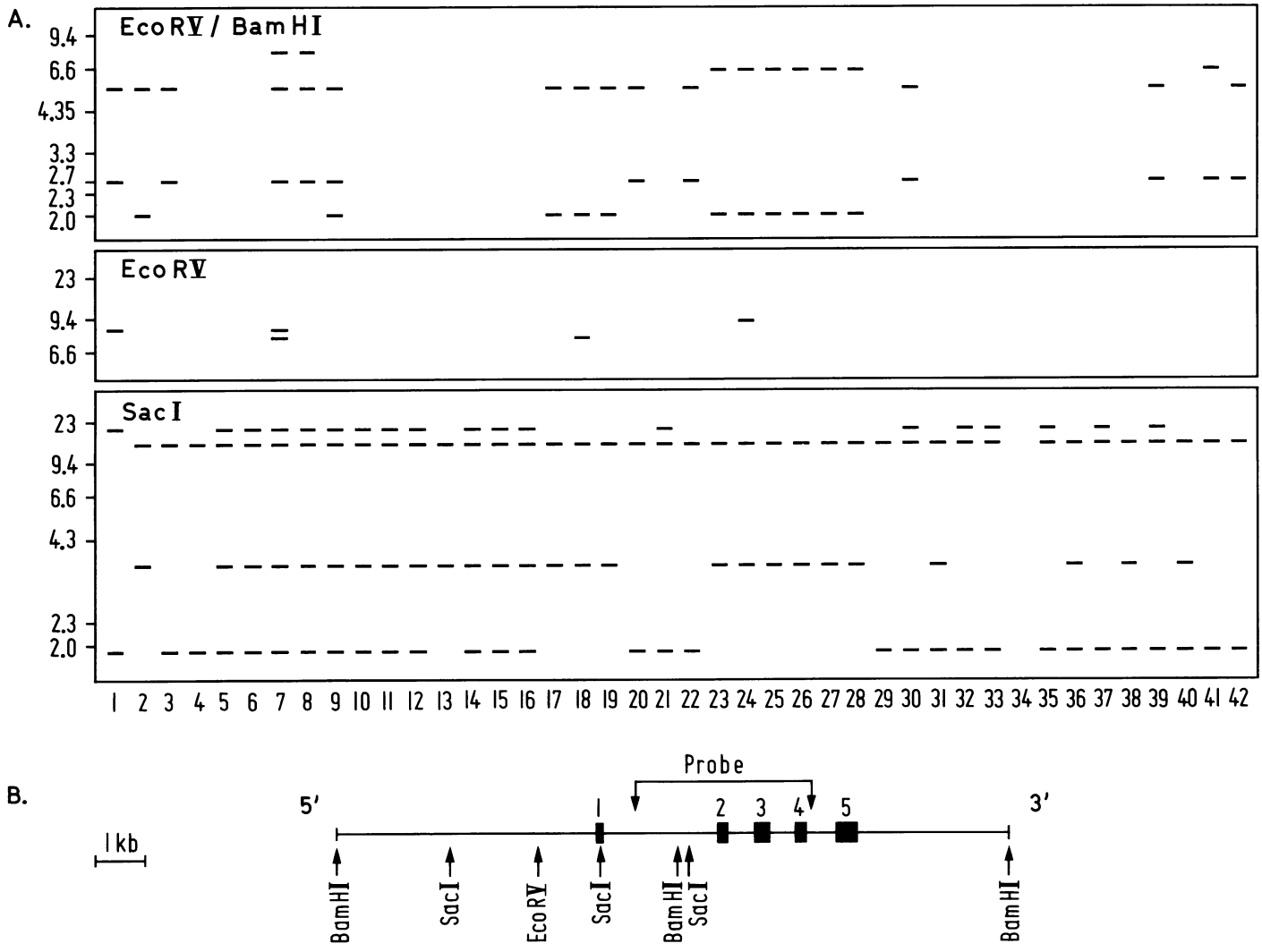

Fig. 6. Summary of restriction-enzyme analysis of the E_α genes: continuation. **(A)** Schematic representation of the band pattern in the Southern blots of the same DNAs as those used in Figure 5, digested with restriction enzymes shown and hybridized with the E_α probe. Fragment size is in kb. **(B)** Partial restriction enzyme map of the E^k_α gene. All numbers refer to the strains listed in Figure 5.

to the E_α gene of the expressor *t* strains, we also digested the DNA from the latter strains with the same set of enzymes. The results of this experiment are diagrammatically represented in the second half of Figures 5 and 6 (tracks 29–42) and summarized in Table I. The results indicate that all the E-expressor *t* strains carrying the same E_α allele (E^{w2}_α) which is, however, different from the alleles found in the E^o strains. The most closely related to the E^{w2}_α allele are the E^{w29}_α alleles of the three *t* strains that apparently lack the deletion but still fail to express cell surface E molecules (strains CRO437, t^{w2} and t^o). Altogether, we could distinguish seven alleles of the E_α gene in the strains tested. The restriction-enzyme maps of these seven genes are depicted in Figure 7.

Discussion

In the *t* strains and some other strains tested in this study, there are two situations that lead to non-expression of the *E* genes on the cell surface. In the first situation, which occurs in *t* lines CRO437, t^{w2}, and presumably also in t^o, both the E_α and E_β genes are transcribed but the message is not converted into a functional protein. (We have not tested the t^o strain for the presence of E_α mRNA, but because its *H-2* haplotype is indistinguishable from that of t^{w2} and because the two strains appear to have identical E_α genes, we presume that t^o also transcribes the gene but fails to express it on the cell surface.) A similar situation occurs in the inbred strains carrying *H-2* haplotypes *f* and *q* (Jones *et al.*, 1981; Mathis *et al.*, 1983; Hyldig-Nielsen *et al.*, 1983). In the abnormal size of its E_α mRNA, the t^{w2} strain resembles $H\text{-}2^f$, whereas the CRO437 strains resembles neither $H\text{-}2^f$ nor $H\text{-}2^q$. It will be possible to decide how these four mutations relate to one another only after the nature of the defect in these strains becomes known.

In the second situation, the non-expression of E molecules on the cell surface is caused by a deletion of the promoter region as well as some other regions of the E_α gene. This deletion, which occurs in all remaining E^o strains tested, appears to be identical with that found previously in inbred strains carrying $H\text{-}2^b$ and $H\text{-}2^s$ haplotypes (Mathis *et al.*, 1983; Hyldig-Nielsen *et al.*, 1983). Two interpretations of the occurrence of this deletion in the different strains are possible. Either the E_α gene contains two mutational hot spots at its 5′ end that result in repeated excisions of the same DNA segment in different *H-2* haplotypes, or the E_α genes of all these strains derive from the same ancestral gene. We consider the second interpretation more likely because the *H-2* haplotypes

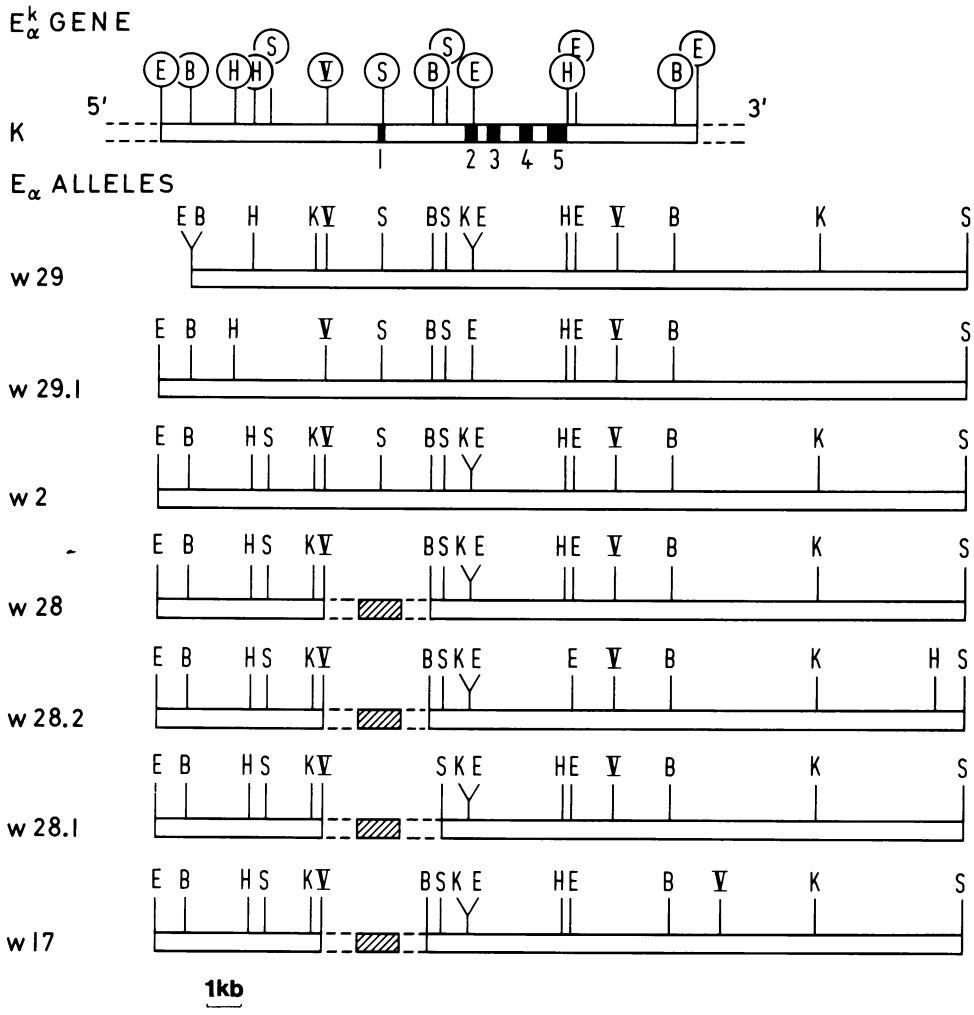

Fig. 7. Restriction enzyme maps of the identified E_α genes. Shaded box – maximum limits possible for the 650 ± 50 bp deletion; full boxes, E_α exons; E, *Eco*RI; H, *Hind*III; B, *Bam*HI; K, *Kpn*I; V, *Eco*RV; S, *Sac*I. The numbers (*w29, w29.1*, etc.) indicate E_α alleles.

of most of the deletion-bearing strains appear to be related. These strains fall into three groups: the *t* lines, European inbred strains and others. In other publications, we provide strong evidence that the *H-2* complexes of all the *t* chromosomes derive from a single common ancestor (Sturm *et al.*, 1982; Nižetić *et al.*, 1984; F. Figueroa, M. Golubić, D. Nižetić, and J. Klein, in preparation). Relatedness of *H-2* haplotypes in *t* strains has also been pointed out by other investigators (Silver, 1982; Shin *et al.*, 1982; Rogers and Wilson, 1983), and it is also suggested by the restriction mapping analysis of the E_α gene in this communication (see below).

The European inbred strains (NMRI/Navy, STS/A, STU and NFS/N) are related in origin [they all derive from a colony of 'Swiss mice' at Lausanne, Switzerland (Staats, 1980) and the first three carry similar *H-2* haplotypes (K^{dv4}, D^{dv1}; F. Figueroa and J. Klein, unpublished data) and similar alleles at enzyme loci (Rice and O'Brien, 1980)]. The B10.LG strain, although no longer traceable to the Lausanne Swiss colony, carries an *H-2* haplotype frequent in that colony. Similarly, the $H\text{-}2^s$ and $H\text{-}2^q$ haplotypes were also present among Swiss mice and, in addition, the origin of $H\text{-}2^s$ strains from the Lausanne colony is documented (Staats, 1980).

In the third group ($H\text{-}2^b$ strains, GPC151, B10.CAS2) are miscellaneous strains for which direct evidence connecting them with the previous two groups is lacking. There is, however, indirect evidence for such a connection. Thus, the E^s_β and E^b_β alleles are serologically very similar to each other (Klein *et al.*, 1983a) and alleles related to K^b and D^b occur among various *t* strains (Nižetić *et al.*, 1984). The B10.CAS2 strain, derived from the Asian species *Mus castaneus*, has a *D* allele identical with that found in STS, STU, NMRI/Navy and B10.LG. The only strain that cannot be connected with the other groups even indirectly is GPC151 which carries an *H-2* haplotype of a wild mouse captured in Chile (Zaleska-Rutczynska *et al.*, 1983).

All these observations suggest that the *H-2* haplotypes of the different E° strains are related and that the E_α deletions carried by these strains might be the same. If so, the E_α deletion has been present in the mouse population for a long time, at least before the population split into the *M. musculus* and *M. domesticus* species more than 1 million years ago (Sage, 1981), and possibly before *M. casteneus* separated from it. The non-expression of the E molecule must be a deleterious trait because some antigens are recognized in the context of this molecule and individuals lacking it are non-responders to these antigens. One must ask, therefore, why the mutation (if it really is one mutation) has been preserved in the mouse population for such a long time. We suggest that the answer lies in the *t* haplotypes. Because of high transmission ratios,

these haplotypes have spread throughout the world, and the E^o deletion might have spread with them in a piggy-back fashion.

An interesting observation is that virtually all the E_α expressor strains in our sample have E_α alleles with identical restriction enzyme patterns. In contrast, among the E^o strains different E_α alleles could be distinguished. An obvious explanation for this difference is that the E_α gene is strongly selected for monomorphism and that in the E^o strains it is allowed to vary because it is not expressed and hence not subject to selection.

Materials and methods

Reagents

Restriction nucleases were purchased from Boehringer, Mannheim, or New England Biolabs, Schwalbach, FRG, nick translation kit from BRL, Neu Isenburg, FRG, guanidinium thiocyanate from Fluka A.G., Neu Ulm, FRG, guanidinium chloride from BRL and cesium chloride and phenol from Merck, Darmstadt, FRG. Oligo(dT)-cellulose was from Collaborative Research, Lexington, KY. Proteinase K, RNase A, and RNase T1 were from Boehringer. [α-^{32}P]dCTP was obtained from NEN, Dreirach, FRG. The E_α probe was 3.4-kb *Sal*I fragment from the cosmid clone 32.1, subcloned into pBR325. The E_β probe is the 2-kb *Eco*RI fragment from the clone 24.2 (Steinmetz *et al.*, 1982).

Mice

All animals were produced in our breeding colony at the Max Planck Institute for Biology, Tübingen.

Preparation of the poly(A)$^+$ RNA

Total RNA was isolated from spleens of 5 – 10 mice by the guanidinium thiocyanate procedure (Chirgwin *et al.*, 1979). Poly(A)$^+$ RNA was then selected by two passages over oligo(dT)-cellulose.

Isolation of genomic DNA

DNA was isolated from kidney cells. Kidneys from 3 – 4 mice were removed into RPMI 1640 medium and pressed through a wire mesh. DNA was then extracted as described by Maniatis *et al.* (1982).

Southern, Northern and RNA dot blot analysis

Restriction endonuclease-digested genomic DNA was electrophoresed in 0.8% agarose gels and transferred to nitrocellulose filters (Southern, 1975). Poly(A)$^+$ RNA was bound to nitrocellulose filters by the dot blot method of Thomas (1980). Alternatively, denatured RNA was electrophoresed on a formaldehyde/agarose gel (1% agarose) and then transferred to nitrocellulose filter. All filters were hybridized to the nick-translated probe in 50% formamide, 5 x SSC, 10% dextran sulfate, 5 x Denhardt solution, 0.1% SDS at 42°C for 24 h. Southern filters were washed twice for 15 min each in 2 x SSC, 0.1% SDS at 52°C and finally once in 0.1 x SSC at 52°C. Northern and dot blots were washed twice, for 15 min each time, in 2 x SSC, 0.1% SDS, at 68°C, and finally once in 0.1 x SSC at 68°C. Filters were then exposed to DuPont Cronex film for 16 – 48 h using Dr. DeGoos intensifying screens.

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References

Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,W.J. (1979) *Biochemistry (Wash.),* **18**, 5294-5299.

Hyldig-Nielsen,J.J., Schenning,L., Hämmerling,U., Widmark,E., Helding, E., Lind,P., Servenius,B., Lund,T., Flavell,R., Lee,J.S., Trowsdale,J., Schreier,P.H., Zablitzky,F., Larhammar,D., Peterson,P.A. and Rask,L. (1983) *Nucleic Acid Res.,* **11**, 5055-5072.

Jones,P.P., Murphy,D.B. and McDevitt,H.O. (1978) *J. Exp. Med.,* **148**, 925-939.

Jones,P.P., Murphy,D.B. and McDevitt,H.O. (1981) *Immunogenetics,* **12**, 321-337.

Klein,J. and Hammerberg,C. (1977) *Immunol.Rev.,* **33**, 30-104.

Klein,J, Figueroa,F. and David,C.S. (1983a) *Immunogenetics,* **17**, 553-596.

Klein,J., Figueroa,F. and Nagy,Z.A. (1983b) *Annu. Rev. Immunol.,* **1**, 119-242.

Klein,J., Sipos,P. and Figueroa,F. (1984) *Genet. Res. (Camb.),* in press.

Lyon,M.F. (1981) *Symp. Zool. Soc. (London),* **47**, 71-104.

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

Mathis,D.J., Benoist,C., Williams,V.E., II, Kanter,M. and McDevitt,H.O. (1983) *Proc. Natl. Acad. Sci. USA,* **80**, 273-277.

Nižetić,D., Figueroa,F. and Klein,J. (1984) Immunogenetics, **19**, 311-320.

Rice,M.C. and O'Brien,S.J. (1980) *Nature,* **283**, 157-161.

Rogers,J.H. and Wilson,K.R. (1983) *Nature,* **304**, 549-552.

Sage,R.D. (1981) in Foster,H.L., Small,J.D. and Fox,J.G. (eds.), *The Mouse in Biomedical Research,* Vol. **I**, Academic Press, NY, pp. 39-90.

Shin,H.-S., Starnezer,J., Artzt,K. and Bennett,D. (1982) *Cell,* **29**, 969-976.

Silver,L.M. (1982) *Cell,* **29**, 961-968.

Southern,E.M. (1975) *J. Mol. Biol.,* **98**, 503-517.

Staats,J. (1980) *Cancer Res.,* **40**, 2083-2128.

Steinmetz,M., Minard,K., Horvath,S., McNicholas,J., Frelinger,J., Wake, C., Long,E., Mach,B. and Hood,L. (1982) *Nature,* **300**, 35-42.

Sturm,S., Figueroa,F. and Klein,J. (1982) *Genet. Res. (Camb.),* **40**, 73-88.

Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA,* **77**, 5201-5205.

Zaleska-Rutczynska,Z., Figueroa,A. and Klein,J. (1983) *Immunogenetics,* **18**, 189-203.

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