ANALYSIS OF MAJOR HISTOCOMPATIBILITY COMPLEX HAPLOTYPES OF *t*-CHROMOSOMES REVEALS THAT THE MAJORITY OF DIVERSITY IS GENERATED BY RECOMBINATION

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t-haplotypes are natural polymorphisms of chromosome 17 that occur in feral mice, and are thought to descend from a single ancestral chromosome. A current reason for thinking this is that they show strong linkage disequilibrium with a restricted number of related H-2 serotypes (1), and have minimal restriction site polymorphisms around the H-2 class I genes (2, 3). Most *t*-haplotypes that have been isolated from wild populations carry one, or occasionally two lethal mutations at any of several different but related loci, although a substantial proportion contain no specific lethal gene, but behave as semilethals. Traditionally, *t*-haplotypes have been classified by their content of lethal genes, and on that basis, 16 different lethal complementation groups have been defined, in addition to the semilethal category (4). Members of some complementation groups have been recovered repeatedly from wild mice separated by considerable geographical distances; for example, the t^{w5} haplotype has been found in North and South America. Other complementation groups, such as t^{wPA-1}, have been isolated only once.

All wild lethal *t*-haplotypes, regardless of their content of lethal genes, share some unusual properties. Among these is the distortion of their own transmission through sperm, a phenomenon that is no doubt responsible for their maintenance in wild populations (5–7). Another notable property is the suppression of recombination between *t*-bearing and wild-type chromosomes. We know now that all *t*-haplotypes carry an inversion of at least 10 centimorgans (cM) surrounding the major histocompatibility complex (MHC)[1] that effectively prevents crossing over in that region in *t*/+ heterozygotes. However, in heterozygotes for two different complementing *t*-haplotypes, recombination occurs at an apparently normal rate, presumably because they share the same inversion (8, 9).

The opportunities for *t*-chromosomes to undergo recombinational exchanges in nature must be severely limited. Several factors contribute to this limitation. First, the overall frequency of *t*-haplotypes in wild populations is only ~10%,

[1] *Abbreviation used in this paper:* MHC, major histocompatibility complex.

and furthermore, mice live in small inbred demes in which, most likely, only one lethal *t*-haplotype would be segregating. The geographical distribution of different *t*-haplotypes is also not entirely random. For example, in North American mouse populations, the t^{w5} and t^{w1} chromosomes predominate, while in eastern Europe, members of the t^{w73} group are most abundant (4). This situation obviously reduces the opportunities for two complementing haplotypes to encounter one another. Nevertheless, migration between demes that occurs at a low but measurable frequency must provide some opportunity for two complementing haplotypes to meet in the same zygote. Even then, however, the opportunities for contributing recombinant chromosomes to the population will be restricted. First of all, complementation between two different *t*-haplotypes is never complete, in the sense that viability of heterozygotes is reduced, ranging between 18% and 85% of normal, depending on the specific mutations involved (10). Also, male heterozygotes for two different *t*-lethal genes are completely sterile, and therefore cannot generate recombinant progeny. Thus it is clear that *t*-haplotypes are almost completely isolated from the normal genome of the mouse, and also partially isolated from one another. It is impossible to give solid estimates of their deprivation of crossover events relative to wild type chromosomes, but a reasonable guess based on *t*-gene frequencies, complementation, etc. would be that they are involved in recombination only to about $10^{-3}-10^{-5}$ as often as normal.

We thought we could exploit the relative recombination isolation of *t*-haplotypes to provide a pedigree of their derivation from one another by analyzing in detail the structure of the MHC in all available *t*-haplotypes, using serological and molecular methods. In addition to ordering *t*-haplotypes by relatedness, these observations provided data which suggest strongly that class I and class II genes possess mechanisms, different from most genes, for generating sequence diversity within and around them.

Materials and Methods

Mice. All *t*-chromosomes used are available on two backgrounds: (*a*) maintained heterozygous with *T qk tf H-2^b* in balanced lethal, brother-sister matings whenever possible, but not strictly inbred; (*b*) congenic (or in the process of being made congenic) with C3H/DiSn (*H-2^k*). Thus, in panel typing of *t*-heterozygotes, the trans H-2 type was either b or k, as appropriate to the antiserum, i.e. if the reagent crossreacted with k, the mouse tested was b/*t*, and vice versa. Certain inbred strains were from the colony of E. A. Boyse (B6 and A.TL⁻), or bought from the Jackson Laboratory (Bar Harbor, ME) (DBA/1, B10, and RIII).

Serology. Complement-mediated cytotoxicity tests on mesenteric lymph node cells were done as previously described (11). The source of complement was a 1:1:3 mixture of guinea pig serum, rabbit serum, and medium. These sera were selected for low toxicity on lymphocytes. Absorptions were for 45 min on ice, at a 1:2 ratio of cells to serum at the appropriate dilution. All but two of the reagents listed in Table I killed 100% of lymph node cells. The two exceptions were anti-H-2 mid^w5, which gave a maximal cytotoxic lysis of 50–80%, and could, therefore, be detecting an I-region antigen, and anti-H-2 mid^w75, which, after absorption, lysed 80% of the cells.

All reagents used are listed in Table I along with the reference to their original definition. Of the reagents referred to in this paper, two detect determinants defined previously: anti-H-2D^t0 is identical to anti-H-2D^t12, and monoclonal 13A1 is identical to anti-H-2K^w1.

TABLE I

Serological Reagents

Reagent	Type*	Reference	Description	Reciprocal titer	Number in Table III
10B6	M	This paper	A × B10 F$_1$ anti-b/t^{w5}	128‡	1
H-2D^{t12}	C	14	C3H/t^{K115} anti-C3H/t^{S510}	200	2
H-2D^{t0}	C	This paper	BALB/c anti-b/t^0 abs§ b, k, and t^{w1}	200	Same as 2
H-2D^{w5} (1)	C	9	C3H anti-C3H/t^{w73} abs b/t^{w1}	100	3
H-2D^{w5} (2)	C	9	C3H.t^{w1} anti-C3H.t^{w75}	400	4
H-2D^{w1}	C	This paper	C3H/t^{S510} anti-C3H.t^6	300	5
H-2 mid^{w75}	C	This paper	C3H.t^{w5} anti-C3H.t^{w75} abs C3H.t^{w1}	80	6
H-2 mid^{w5}	C	9	BALB/c anti-b/t^{w5} abs b and k	80	7
3B5	M	9	BALB/c anti-b/t^{w5}	64‡	8
H-2K^{w32}(1)	C	11	BALB/c anti-b/t^{w32} abs b/t^{w12}	200	9
H-2K^{t12} (2)	C	This paper	C3H anti-C3H.t^{12} abs b/Tt^{t505}	300	10
H-2K^{w1}	C	11	A × B10 F$_1$ anti-b/t^{w1}	500	11
13A1	M	This paper	C3H anti-C3H.t^{w12}	250‡	Same as 11
M4	M	28	C3H anti-C3H.t^{w12}	5,000^I	12
11.4.1	M	29	BALB/c anti-CKB (K^k)	>10,000¶	13

* M, monoclonal; C, conventional serum.
‡ Supernatant was used.
§ abs, absorbed with.
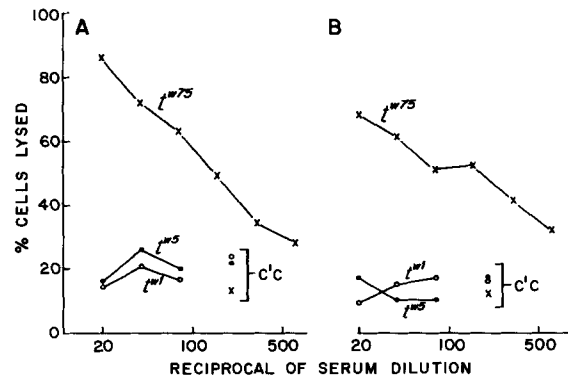^{I} Protein A–purified supernatant was used.
¶ Ascites was used.

FIGURE 1. t^{w75} unique specificity. C3H.t^{w5} anti-C3H.t^{w75} absorbed with C3H.t^{w1} lymphoid cells (A) cytotoxicity tested on all three cell types. C3H.t^{w1} anti-C3H.t^{w75} absorbed with C3H.t^{w5} lymphoid cells (B) tested on all three cell types. C'C, complement control.

13A1 and 10B6 are monoclonal antibodies made by standard procedures using P3-U1 (12) as the nonsecretor parental myeloma. Anti-H-2 mid^{w75} is described in the text and in Fig. 1.

Anti K^{t12}(2) recognizes three determinants: H-2D^{t12}, H-2K^{t12}, and a new determinant, which is also present in t^{w1}. The antibody against H-2D^{t12} can be absorbed out with an intra-H-2 recombinant, Tt^{t505}, which has D^{t12}, mid^{w5}, and K^{w5}. This absorbed serum, anti-K^{t12} (2), reacts with intra-H-2 recombinants that have K^{t12} or K^{w1}, and is listed as number 9 in Table IV.

C3H/T^{S510} F$_1$ anti-C3H.t^6 was an immunization designed to identify the D end of the H-2 haplotype carried by t^6 and the t^{w1} group. t^{S510} is an intra-H-2 recombinant that carries D^{t12}, mid^{w1}, and K^{w1}, and therefore this immunization should only recognize the D^{w1} antigen(s). This was confirmed by testing on two other intra-H-2 recombinants. t^{t660},

which is identical to t^{S510}, was negative, and Tt^{X925}, which carries D^{w1}, mid^{w1}, and K^{t12}, was positive. The determinant is thus named D^{w1} and is number 5 in Table IV.

One negative immunization is worth mention here. t^{w18} is the longest partial haplotype known (8.1 cM), and must end at or just short of the t-MHC (13). It is known to carry a wild-type MHC and recombine freely with the tufted locus. However, because of the closeness of its map position, it was possible that t^{w18} carried a recombinationally trapped portion of a t-MHC. Three C3H mice were hyperimmunized eight times at biweekly intervals with C3H/$t^{w18}tf$ H-2^k congenic lymphoid cells, and no cytotoxic antibody resulted.

Genomic Blotting. Preparation of genomic mouse liver DNA and insert probes, their nick translation, and hybridizing and washing of Southern blots were done as previously described (2, 14). The Ia gene probes E_α, E_β and A_β were the gift of L. Hood (California Institute of Technology, Los Angeles). The E_α probe is a 3.4 kilobase (kb) Sal I fragment from the cosmid clone 32.1; the E_β probe is a 1.8 kb Eco RI fragment from the cosmid clone 24.2; and the A_β probe is a 5.6 kb Eco RI fragment from the cosmid clone 34.2. All three probes were used in mapping studies of the MHC of t-chromosomes in reference 9.

Results

Serology of t-H-2 Antigens. During the recombinational analysis of t-chromosomes, we developed a panel of conventional and monoclonal reagents against t-specific H-2 antigens, as well as a panel of six intra-H-2 recombinants that allowed us to map the relevant genes to one of three regions: a proximal part containing H-2D, a middle region containing the I genes and some class I genes, and the H-2K region distal (9). Although we do not yet know the relative order or the allelic status of genes within any one region, we can compare individual regions from different t-haplotypes, and define their relationships to one another.

Table II shows the complementation groups of the chromosomes used. Table III presents panel typing of all the reagents on 17 t-chromosomes arranged according to complementation group. Data for a few inbred strains are shown at the bottom. Several points described by others (1, 15) are also evident in Table III. First and most important, t-chromosomes isolated over wide temporal and geographic distances share a common pool of antigenic specificities not generally found in inbred strains (except for t^{w5}, which carries two H-2^k specificities, and t^{w1}, which carries two H-2^d determinants). Second, members of the same complementation group often share the same haplotype; i.e. t^{w1}, t^{w12}, and t^{w71} are alike, as are t^{12} and t^{w32}, although members of the same complementation group may

TABLE II
Complementation Groups of t-chromosomes Studied

Complementation group	Members
t^{12}	t^{12}, t^{w32}
$t^{sl}*$	t^{w2}, t^{w8}
t^0	t^0, t^6
t^{w1}	t^{w1}, t^{w12}, $t^{w12}tf$, t^{w71}
t^{w5}	t^{w5}
t^{wPA-1}	t^{wPA-1}
t^{Lub-1}	t^{Lub-1}
t^{w73}	t^{w73}, t^{w121}
dual	$t^{w75} = t^{w5} + t^{w1}$
dual	$t^{w120} = t^{w5} + t^0$

* sl, semilethal.

TABLE III

Serotyping of t Haplotypes

t-chromosome	D region						Middle region			K region					
	10B6	D^{t12}	D^{t0}	D^{w5}(1)	D^{w5}(2)	D^{w1}	Mid w75	Mid w5	3B5	K^{w32}(1)	K^{t12}(2)	K^{w1}	13A1	M4	11.4.1
t^{12}	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–
t^{w32}	–	+	+	–	–	–	–	–	–	+	+	–	–	–	–
t^{w2}	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
t^{w6}	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
t^{0}	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
t^{6}	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–
t^{w1}	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–
t^{w12}	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–
t^{w12}tf	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–
t^{w71}	–	–	–	–	–	+	–	–	–	–	+	+	+	+	–
t^{w5}	+	–	–	+	+	–	–	+	+	–	–	–	–	–	+
t^{w73}	+	–	–	+	–	–	–	–	–	–	+	+	+	+	–
t^{w121}	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–
t^{wPA-1}	–	+	+	–	–	–	–	–	–	–	–	–	–	–	–
t^{Lub-1}	–	+	+	–	–	–	–	–	–	–	+	+	+	+	–
t^{w75}	+	–	–	+	+	–	+	–	–	–	–	–	–	–	+
t^{w120}	+	–	–	–	+	–	–	+	+	–	–	–	–	–	+
Inbred strains															
BTBRTF/Nev (b)	–	C	C	–	+	–	C	–	–	–	–	–	–	–	–
C57/B6 (b)	–	–	–	–	+	–	+	–	–	–	–	–	–	–	–
C3H/DiSn (k)	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+
BALB/c (d)	–	–	–	–	–	–	–	–	–	–	+	–	–	+	–
A.TL$^-$ (K^{k}D^d)	–	–	–	–	–	ND*	–	–	+	–	+	–	–	+	–
DBA/1 (q)	ND	–	–	–	ND	ND	ND	+	+	–	ND	–	–	–	+
RIII (r)	ND	–	–	–	–	ND	–	–	–	–	ND	–	ND	–	+

* ND, not determined.

TABLE IV

Regional Haplotypes for Class I Antigens

t-chromosomes	D region						Middle region				K region					
	1	2	3	4	5	Type	6	7	8	Type	9	10	11	12	13	Type
t^{12}, t^{w32}	–	2	–	–	–	A	–	–	–	E	9	10	–	–	–	G
t^{0}, t^{wPA-1}, t^{w2}, t^{w8}	–	2	–	–	–	A	–	–	–	E	–	–	–	–	–	H
t^{Lub-1}	–	2	–	–	–	A	–	–	–	E	–	10	11	12	–	I
t^{w1}, t^{w12}, t^{w12}tf, t^{w71}, t^{6}	–	–	–	–	5	B	–	–	–	E	–	10	11	12	–	I
t^{w73}	1	–	3	–	–	C	–	–	–	E	–	10	11	12	–	I
t^{w121}	1	–	3	–	–	C	–	–	–	E	–	–	–	–	–	H
t^{w5}	1	–	3	4	–	D	–	7	8	F	–	–	–	–	13	J
t^{w75}	1	–	3	4	–	D	6	–	–	F/new	–	–	–	–	13	J
t^{w120}	1	–	–	4	–	D/A or B	–	7	8	F	–	–	–	–	13	J

have different H-2 haplotypes; i.e. t^0 and t^6, and t^{w73} and t^{w121}. Third, members of different complementation groups can also share the same H-2 haplotype, i.e. t^6 and t^{w1}, and t^0, t^{w8}, and t^{wPA}.

Table IV shows the data organized differently. The 13 determinants for D, "mid", and K regions are numbered arbitrarily across the top; t-chromosomes with identical H-2 haplotypes are listed on the same line. The vertical list is arranged according to regional haplotype, i.e. *D*, *Middle*, or *K* region. The obvious intraregional recombinants are listed below the line (to be discussed later). For the purpose of this discussion, each regional haplotype has been

named arbitrarily. The D region contains four (A–D), the middle region two (E and F), and the K region four (G–J). It is evident that, for the 15 chromosomes above the line, the three different regions are being exchanged intact by recombination among the different haplotypes. Excluding the fact that t^{w5} is unique in all regions, "DFJ" (and in other *t*-haplotypes the middle region class I antigens do not vary), the H-2D A type, can be associated with the H-2K G, H, or I type; the H-2K I type can be associated with the H-2D A, B, or C type; and the H-2K H type is associated with both H-2D A and C types.

In the lower part of Table IV are two chromosomes that appear to be intraregional recombinants. The t^{w75} haplotype deserves special discussion because this chromosome gave the first clue that *t*-chromosomes were shuffling their H-2 haplotypes by recombination. Isolated from the German Democratic Republic, it was the first chromosome retrieved from the wild that carried two *t*-lethal genes: t^{w5} and t^{w1} (16). Shortly after we had this information, we found that female compounds for two different complementing lethals had normal recombination, and that t^{w5} and t^{w1} were nonallelic and a considerable genetic distance apart (13). Thus, we could assume that t^{w75} simply represented a natural recombinational event, although we could not at that time define the precise recombinational breakpoint. In 1982, cDNA probes to class I antigens were used (2, 3) to study the structure of H-2 in *t*-haplotypes. All *t*-haplotypes proved to be remarkably similar, thus confirming earlier studies (17, 18). However, the data (2) showed that t^{w75} appeared to be a class I intragenic recombinant. It contained some restriction fragments typical of both t^{w5} and t^{w1} parents, but, in addition, a new restriction fragment polymorphism was found that was not present in any other *t*-haplotype, the only unique band we found in 10 *t*-chromosomes analyzed. Since the new restriction fragment is not found in other haplotypes, it is probably a recent event. To ask if this novel restriction fragment represented a new class I gene generated by the recombinational event, two reciprocal immunizations were made: $C3H.t^{w5}$ anti-$C3H.t^{w75}$, and $C3H.t^{w1}$ anti-$C3H.t^{w75}$. Both antisera, when absorbed with the appropriate immunizing parental chromosome, recognized a new serological specificity unique to t^{w75} (Fig. 1). The new antigen has the characteristics of a class I antigen; the antisera were cytotoxic for >80% of lymph node cells, and immunoprecipitated a molecule of 40 kilodaltons associated with β_2 microglobulin (data not shown). The new serological specificity (number 6 in Table IV) is assigned to the middle region by assumption only, since the rest of the middle region had lost the characteristic determinants of t^{w5}.

A second wild chromosome containing two lethals, t^{w5} and t^0, was analyzed shortly after this: t^{w120}, from Ithaca, NY. Its H-2 type also showed evidence of intraregional H-2 recombination, this time in the D region. The middle and K region serological specificities were all derived from t^{w5}, but the D region seemed partly derived from t^{w5} and partly from the A- or B-type D region. We know the relative order of the t-lethals and the three H-2 regions, and there is no way to obtain t^{w75} or t^{w120} in a single recombinational event. Thus, these naturally occurring intra-H-2 recombinants are probably not recovered as primary events, but most likely represent secondary or tertiary events from already recombinant chromosomes.

The semilethals are the second most frequent t-haplotypes found in North America (after t^{w5}). It is possible that they also represent recombinants between two t-lethal haplotypes, since, in laboratory recombination experiments, we have obtained many complete t^T chromosomes that contain none of the specific parental lethals. However, some but not all of these behave as semilethals, in that the viability of homozygotes is severely reduced. t^{w2} and t^{w8} are two naturally occurring semilethals from the eastern United States included in this analysis. Although they are serologically identical to t^0 and t^{wPA-1} (both from France), it is possible that they are recombinants in regions other than H-2 or between t-lethal–bearing chromosomes that did not differ at H-2.

Southern Blotting of t-I Region Genes.   Analysis of DNA by Southern blotting gives a different level of resolution compared to serology of protein polymorphisms. Using DNA probes to three of the I region genes (E_α, E_β, and A_β), and three different restriction enzymes per haplotype (Hind III, Bam HI, and Eco RI) we analyzed the same 17 t-chromosomes. A typical blot is shown in Fig. 2A. The data were analyzed in the following way: all mice were heterozygous with C3H/DiSn. For any one enzyme and probe, the bands due to C3H were excluded. All other major bands were numbered and the haplotypes then grouped into arbitrary patterns. For example, in Fig. 2A, t^{w8} has bands 1, 3, and 5, as does t^{w1}; these were members of group 3 for A_β and Eco RI. t^{12} and t^{w32} have band 2 only; this constitutes group 5. t^6, t^{w73}, and t^{Lub-1} have band 5 only; this is group 6, etc. Thus, there is a group code for each haplotype, each enzyme, and each probe. The data are then expressed in Table V as a three-digit code for Bam HI, Hind III, and Eco RI, respectively, with the t-haplotypes arranged in the same relative order as Table IV (by similarity). An example from Table V is t^6, which, with E_β, was in group 423. The dashes in the three-digit code indicate there is inadequate information for that enzyme.

Haplotypes indistinguishable by serology are different by I-region blotting: t^{w8} (121 114 4-3) is completely different from t^0 and t^{wPA} (112 311 228); t^6 has a different I region (122 423 116) from the t^{w12} group (121 423 113); and, most surprising, t^{w1} is different from the rest of its group with one enzyme (Bam HI) and one probe (A_β). The 17 t-chromosomes seem very similar in their E_α, probably only 4 different ones, and very diverse in E_β (probably 7) and A_β (a minimum of 11). The difference in polymorphism between E_α on the one hand, and E_β and A_β on the other, is striking, and confirms other laboratories' results with inbred strains (see Discussion). In fact, A_β is the most intriguing, given the context of the limited origin of t-chromosomes and the constraints on recombination. t^{w1}, which is in every other way identical to other members of its group, can now be separated from them. When digested with Bam HI and probed with A_β, a band of 10.5 kb is replaced by two bands of 8.0 and 2.2 kb. Other examples are that t^{w120} still resembles its parental t^{w5} chromosome, but t^{w75} is entirely different. In fact, if the 17 haplotypes were analyzed only with A_β, the recombinational relationships established by the serology and blotting of class I genes would be obscured. Thus, the polymorphism of A_β is an order of magnitude above that of the already polymorphic class I genes. Its extreme variation implies a different mechanism(s) for generation of variation.
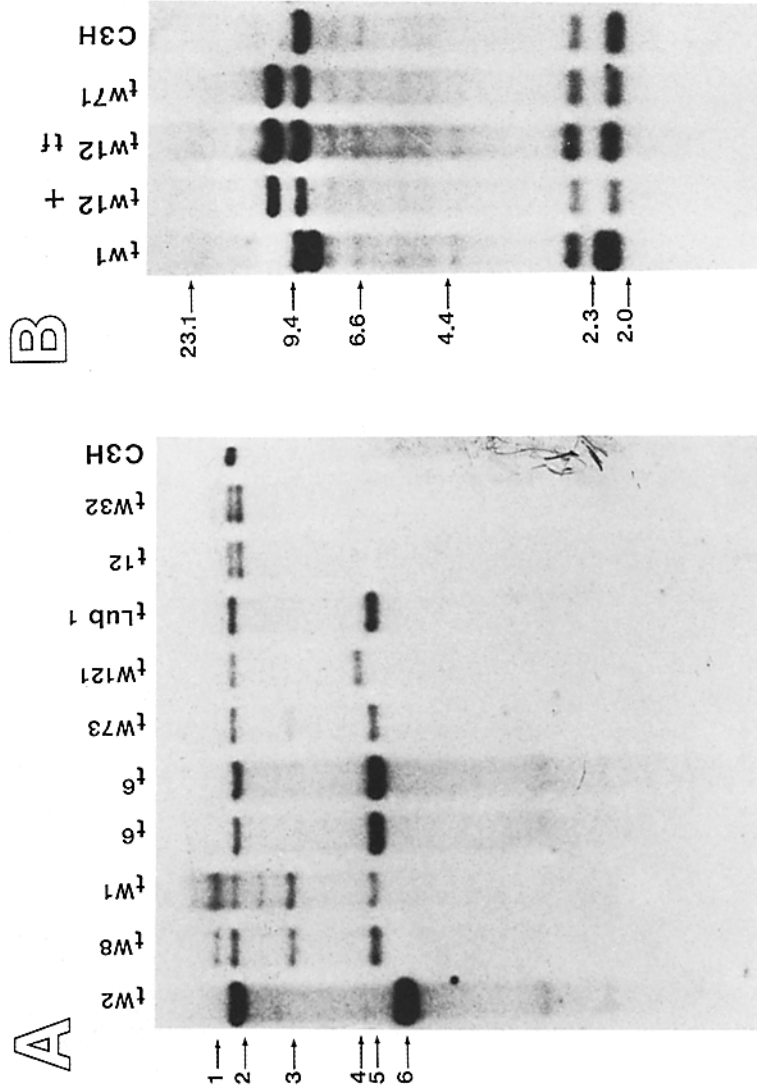
FIGURE 2.   Southern blots probed with A_β. The illustration of five different patterns of Eco RI–digested *t*-genomic DNA (*A*). The C3H band is not numbered; t^{w2} has band 6 only; t^{w8} and t^{w1} have bands 1, 3, and 5; t^6, t^{w73} and t^{Lub-1} have band 5 only; t^{w121} has band 4 only; t^{12} and t^{w32} have band 2 only. The t^6 lane is repeated at two DNA concentrations. (*B*) is a Bam HI digest illustrating the only difference ever found between t^{w1} and the rest of its complementation group (see text). The markers indicated are Hind III–digested phage λ.

TABLE V

Group Identities of Class II Genes Determined by Southern Blotting

t-chromosomes	E_α			E_β			A_β		
	B*	H	E	B	H	E	B	H	E
t^{12}, t^{w32}	1	2	2	1	1	–	4	3	5
t^{0}, t^{wPA-1}	1	1	2	3	1	1	2	2	8
t^{w2}	1	2	2	3	1	4	2	–	2
t^{w8}	1	2	1	1	1	4	4	–	3
t^{Lub1}	1	2	–	4	2	–	3	1	6
t^{w1}	1	2	–	4	2	–	3	1	3
t^{w12}, $t^{w12}tf$, t^{w71}	1	2	1	4	2	3	1	1	3
t^{6}	1	2	2	4	2	3	1	1	6
t^{w73}	1	2	–	4	2	3	1	1	6
t^{w121}	1	2	1	5	3	2	3	4	7
t^{w5}	2	2	2	1	1	1	2	2	2
t^{w75}	1	1	2	2	1	1	3	–	1
t^{w120}	2	2	2	1	1	1	2	–	4
minimum number of regional haplotypes	–	4	–	–	7	–	–	11	–

* B, Bam HI; H, Hind III, E, is Eco RI.

Discussion

The evidence for the restricted origin of t-haplotypes is compelling: (a) Analysis of their class I genes by Southern blotting shows they diverge from one another by an average of only 3.5%, compared to 47% for inbred strains (2). (b) In a search for random protein differences between t and wild type using two-dimensional gels of testicular cell lysates from 129/J.t-chromosome congenic mice, none of the eight proteins identified is polymorphic between t-haplotypes. A ninth protein that was polymorphic proved to be the PGK-2 enzyme (testicular-specific phosphoglycerate kinase), known to be outside the T/t-complex (19). (c) Nine random genomic DNA clones were isolated from a wild-type chromosome 17; all had t-specific restriction fragment polymorphisms, but none of them varied among different t-haplotypes (20). (d) all attempts to find protein polymorphisms among known chromosome 17 markers within the T/t-complex have failed. Markers studied were: Glo-1, both for qualitative and quantitative variation, TL, and Qa (13). Additionally, we studied the Tla region at the DNA level. Using a probe from outside the TL gene, we found only one restriction fragment polymorphism using three different haplotypes and 23 endonucleases (our unpublished results). In fact, the only two components of the 20 cM T/t-complex that do vary significantly are the content of t-lethals, and parts of the MHC. This overall similarity can be construed as an apparent reflection of their unique restrained recombination situation. This makes them a laboratory in which to ask questions concerning the generation of diversity in polymorphic systems.

Whatever the evolutionary age of t-chromosomes (and it is believed they are at least one million years old [21]), in general the T/t-complex region has not had an extraordinary rate of mutation. For example, they have not (as would be

expected of homozygous lethal chromosomes) collected secondary deleterious mutations. Once a chromosome carries one early-acting recessive embryonic lethal and is driven by transmission ratio distortion, other later-acting parasitic mutations should be able to accumulate on that chromosome without further genetic detriment to the organism or population. In laboratory recombination experiments, we have freed nine t-chromosomes from their specific lethals, and in complementation studies, we examined the rest of their t-complex regions for accumulation of parasitic recessive mutations, with the result that only one was found (22). This represents the analysis of ~180 cM of t-region (9 × 20 cM). Thus, these recombinationally locked regions do not appear to accumulate a burden of specific mutations. How then can the polymorphism in the H-2D, -K, and -I regions of the MHC be explained?

Serological analysis of H-2 class I antigens presented here argues strongly for recombination as a source of variation, both for exchanging existing antigens among different t-chromosomes, and, in one instance, t^{w75}, creating a new antigen. Regardless of whether the recombination is standard (homologous equal), homologous unequal (23), or the result of gene conversion between members of the gene family, the MHC could be interpreted as representing a recombinational hot spot, compared to the rest of chromosome 17. Perhaps the unique factor is the high number of closely linked class I genes (30–35), which would make the MHC a large target in which recombination can promote diversity.

Southern blotting of three I region genes confirms the results of experiments with inbred strains (24, 25). There seems to be a boundary of polymorphism between E_α, which is relatively conserved, and E_β and A_β, which are much more polymorphic. Whatever the mechanisms operating to generate diversity in H-2D and H-2K genes, A_β must have an added source of variation unavailable to the class I genes, because in examining the A_β of the closely related t-chromosomes, their relatedness is almost totally obscured. Although both class I and class II undergo gene conversion at an unknown rate, the mutation rate of class I and II has been studied by skin grafting in inbred mice, and it is higher in class I by an order of magnitude (26), a result that does not fit with the greater polymorphism in A_β measured here. Either the previous assay is not effective at detecting class II mutations or, as suggested by McIntyre and Seidman (27), selection is the major force governing the polymorphism of A_β.

Other researchers (1) have examined an even larger but different selection of t H-2 haplotypes and organized them into a hypothetical evolutionary tree. Our data, on what are obviously not primary recombinational events, lead us to conclude that it is difficult to decide any lineage. In general, it seems impossible, at present, to say what the ancestral chromosome looked like, either with respect to its MHC or its content of t-lethals. However, we do draw three conclusions: (a) There is an increased rate of genetic change at H-2 class I loci compared to other chromosome 17 loci. (b) there is an increased rate of change at some class II loci compared to class I. And (c), these changes are shuffled by recombination that may be either homologous and reciprocol, or that, certainly in some cases, result from unequal exchanges or gene conversion.

Summary

t-chromosomes are natural polymorphisms in feral populations of mice that are thought to be descended from a single ancestral chromosome. They carry an inversion of at least 10 cM surrounding the major histocompatibility complex (MHC) that effectively prevents recombination between a t-bearing chromosome and wild type chromosomes. However, on the rare occasion when two different t-chromosomes meet in a wild female, recombination occurs at an apparently normal rate. Since they contain the highly polymorphic MHC, their limited origin and restricted chances for recombination make t-chromosomes a valuable tool for studying the relative contributions of mutation and recombination to the generation of diversity. Using 13 different serological reagents to class I antigens, and studying restriction enzyme polymorphisms detected with three molecular probes for class II genes examined with three endonucleases, we present data indicating that the major factor responsible for the diversity of class I antigens is recombination, but that for class II genes, mutation must play an important role in addition to recombination.

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