

Deletion and duplication of DNA sequences is associated with the embryonic lethal phenotype of the t^9 complementation group of the mouse t complex

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We have analyzed the genomic structure of three mouse t haplotypes of the t^9 complementation group. Each of these t haplotypes, t^{w18} , t^t , and t^{ks1} , is known to have resulted from a rare recombination event between a complete t haplotype and a wild-type chromosome. Using molecular probes that identify sequences in the distal portion of the t complex, we have shown that each of these t haplotypes contains a similar (perhaps identical) deletion of one group of t complex sequences, and duplication of another group. These data suggest that the recombination events that produced these three t haplotypes involved similar unequal crossovers within the distal inversion. The deletion and duplication of genetic material associated with all members of the t^9 complementation group tested provides a molecular explanation for the recessive lethal mutation associated with these t haplotypes.

[Key Words: Mouse t complex; t^9 lethal factor; structural analysis; deletion; duplication]

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The t complex of the mouse is a large region (12–15 cM) of the proximal third of chromosome 17, which includes the major histocompatibility (H-2) complex. Variant forms of the t complex known as complete t haplotypes are found in wild mouse populations (Frischauf 1985; Silver 1985b). Such t haplotypes have a number of features in common that distinguish them from their wild-type counterparts. These include the suppression of recombination with the wild-type chromosome over the entire length of the t complex, and the selective transmission of the variant form by heterozygous males to their progeny. Although complete t haplotypes are indistinguishable from one another in nearly all other properties, different t haplotypes can carry different lethal mutations that complement each other in genetic tests. At present, 16 different complementation groups are known (Klein et al. 1984). Although few have been

studied in any detail, it appears that each member of a particular complementation group causes the same distinctive syndrome of disturbances leading to embryonic death, and that members of the different groups interrupt development at different times (Bennett 1975; Sherman and Wudl 1977; Magnuson 1983).

In recent years remarkable progress has been made in understanding the genetic and molecular basis of the unusual properties of t haplotypes. For example, an explanation for the almost complete suppression of recombination between t haplotype and wild-type chromosomal segments has been provided by the demonstration that in t haplotypes, two regions of the t complex are inverted. One of these inversions is in the proximal portion, spanning the region between the T and qk loci (Herrmann et al. 1986; Sarvetnick et al. 1986), and the other is in the distal portion, spanning the interval between the tf locus and the $H-2$ complex (Artzt et al. 1982b; Shin et al. 1983; Pla and Condamine 1984). Rare recombination events do occur, however, and the resulting recombinant chromosomes are known as partial t haplotypes, to indicate that part of the t complex is derived from the variant t haplotype and part from the wild-type parental chromosome. The crossovers that

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create partial t haplotypes must occur either at sites between or within the inversions. In the latter case, the formation of chromosomes containing deleted and/or duplicated regions can be expected, as first observed by Silver et al. (1980). Further studies have led to the identification of several partial t haplotypes in which recombination within the proximal inversion has resulted in coupled deletions and duplications or to duplication of almost the entire inverted region (Herrmann et al. 1986; Sarvetnick et al. 1986; Herrmann et al. 1987).

With respect to t haplotype effects on sperm function, nothing is known as yet about the genes responsible, but it has been demonstrated that at least five loci distributed throughout the t complex are involved (Lyon 1984; Silver and Remis 1987). Similarly, nothing is known about the functions of the genes represented by the lethal mutations, but it has been shown that representatives of different lethal complementation groups map to different positions in the t complex, suggesting that they are nonallelic (Artzt et al. 1982a; Artzt 1984; Shin et al. 1984).

Among the lethal t mutations, members of the t^9 complementation group are exceptional in that none have been found in wild populations. Instead, they have all arisen in laboratory stocks, with only six alleles identified in over 50 years of laboratory breeding: t^9 and t^4 (Dunn and Gluecksohn-Waelsch 1953; Moser and Gluecksohn-Waelsch 1967), t^{w18} (Bennett and Dunn 1960), t^{w30} (Dunn et al. 1962), t^{w52} (Bennett et al. 1976), and t^{ks1} , described for the first time in this report. It was Lyon (1960) who first appreciated that the generation of such new alleles results from a rare recombination event between t haplotype-bearing and wild-type chromosomes. In the case of the members of the t^9 complementation group, the t complex of the recombinant chromosome that carries one of these alleles consists of a proximal region, from the markers T to qk , derived from the t -haplotype parent and a distal region, from the marker tf to the H-2 complex, derived from the wild-type parent (Dunn et al. 1962; Bennett et al. 1976; Artzt et al. 1982a).

In light of the evidence that recombination between t haplotype and wild-type DNA can result in deletion and/or duplication of genetic material, we sought to determine whether the members of the t^9 complementation group contain such genomic alterations, which might be responsible for the lethal phenotype common to these t haplotypes. This investigation relied on the use of random DNA clones originally isolated from the proximal portion of mouse chromosome 17 by microdissection and microcloning (Röhme et al. 1984). Using these clones as probes in the analysis of t^{w18} , t^4 , and t^{ks1} haplotype DNA, one set of sequences was found to be deleted and another set duplicated in each of the t haplotypes tested. The map positions of the deleted and duplicated sequences on the wild-type chromosome were determined. The data from these studies indicate that the recombination events that produced these three t haplotypes involved similar unequal crossovers within the distal inversion.

Results

Identification of sequences that are deleted or duplicated in the t^{w18} haplotype

In this analysis we made use of 25 of the cloned DNA probes that were obtained by microdissection of the proximal third of chromosome 17; these probes were selected on the basis of the finding that they represent unique or low-copy-number sequences that map to chromosome 17 (Röhme et al. 1984). To determine whether any of these sequences are deleted in the t^{w18} haplotype, we took advantage of the availability of an embryonic stem (ES) cell line isolated from mouse embryos homozygous for the t^{w18} lethal haplotype (Martin et al. 1987). Genomic DNA was isolated from these t^{w18}/t^{w18} ES cells, as well as from ES cells homozygous for a complete t haplotype, t^{w5} , and from wild-type ES cells (Martin et al. 1987), digested with restriction endonucleases, and analyzed by the Southern blot hybridization technique.

Clone Tu443, which hybridizes to a single *MspI* fragment, of approximately 15 kb in wild-type DNA and 8 kb in t^{w5}/t^{w5} DNA, did not hybridize to any *MspI* fragment in DNA from t^{w18}/t^{w18} cells (Fig. 1A). Probe Tu94 which hybridizes to a single *TaqI* fragment of approximately 4.6 kb in both wild-type and t^{w5}/t^{w5} DNA, did not hybridize to any *TaqI* fragments in DNA from the t^{w18}/t^{w18} cells (Fig. 1B). Similarly, clone Tu180, which hybridizes to a 7-kb *TaqI* fragment in wild-type and t^{w5}/t^{w5} DNA, did not hybridize to any *TaqI* fragment in t^{w18}/t^{w18} DNA (Fig. 1C). Rehybridization of the blots with other probes, such as Tu111 (Röhme et al. 1984) demonstrated that other DNA fragments are readily detectable in the lane containing t^{w18}/t^{w18} DNA (Fig. 1D and data not shown). To eliminate the possibility that the sequences represented by the Tu443, Tu94, or Tu180 probes were undetectable in the endonuclease digests of t^{w18}/t^{w18} DNA because they were contained within very small DNA fragments that ran off the gels, these probes were hybridized to t^{w18}/t^{w18} DNA fragments obtained by digestion with other endonucleases; again the results were negative (data not shown). From these results it is clear that the D17Leh443, 94, and 180 loci identified by the Tu443, Tu94, and Tu180 probes, respectively, are deleted in the t^{w18} haplotype.

Similar results obtained with probe p54M provided evidence that the D17Leh54 locus represented by this probe is also deleted in t^{w18} haplotype DNA. The p54M probe, a subclone of a cosmid clone isolated using the microclone Tu54, hybridizes strongly to three *TaqI* fragments of approximately 5.2, 2.4, and 1.6 kb in wild-type DNA and two fragments of 8.4 and 1.6 kb in t^{w5}/t^{w5} DNA, and very weakly to at least one other fragment in both genotypes (Fig. 1E). When this probe was hybridized to *TaqI* digests of DNA from t^{w18}/t^{w18} cells, the only hybridization signal detectable was very faint and localized to three bands corresponding to the three prominent wild-type bands and to two faint bands in t^{w5}/t^{w5} DNA. Since the t^{w18} lane contained an amount of DNA equivalent to that present in the wild-type lane (data not

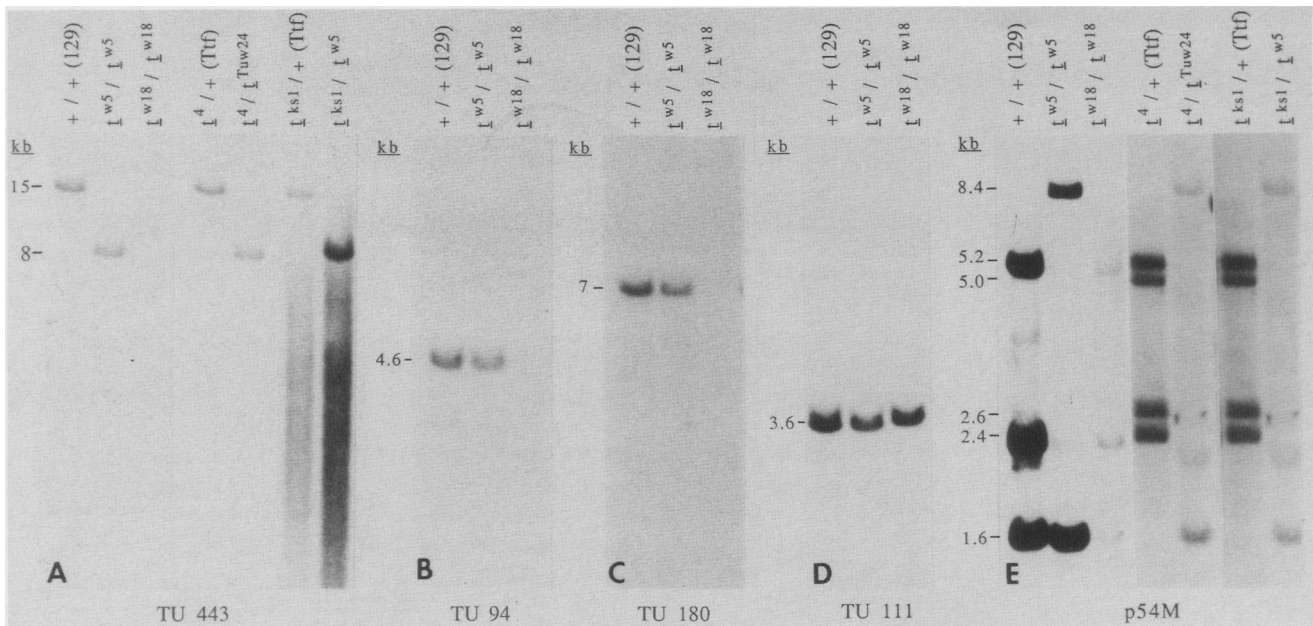


Figure 1. Deletion of sequences in the t^{w18} , t^4 , and t^{ks1} haplotypes. Southern blot hybridization analysis of genomic DNA from cells or animals of the designated genotypes. The genotype of the wild-type chromosome is indicated in parentheses. Each lane contains 10 μ g of genomic DNA digested with *Msp*I (A) or *Taq*I (B,C,D,E), and was hybridized with the designated probes Tu443, 94, 180, 111, or p54M. The smear in lanes $t^{ks1}/+$ and t^{ks1}/t^{w5} (A) is due to incomplete competition with mouse DNA of repetitive sequences present in the probe Tu443.

shown), it seems likely that the absence of any strongly hybridizing fragments was due to deletion in the t^{w18} haplotype of sequences represented by the p54M probe. The faint bands could either be due to cross-hybridizing sequences elsewhere in the genome or could result from hybridization of the probe to fragments derived from the DNA of the wild-type feeder cells with which the t^{w18}/t^{w18} ES cells were co-cultivated, and which may not have been completely removed from the ES cell sample.

To determine whether sequences are duplicated in the t^{w18} haplotype, an analysis was carried out with a subset of the remaining probes, which detect restriction fragment length polymorphisms (RFLPs) between wild-type and t -haplotype DNA (Fig. 2). The Tu89 probe hybridizes strongly to three *Msp*I fragments of approximately 14–18 kb in wild-type DNA and to two *Msp*I fragments of 45 and 3 kb in DNA from t^{w5}/t^{w5} cells (Fig. 2A). When the Tu89 probe was hybridized to *Msp*I digests of t^{w18}/t^{w18} DNA, the hybridization pattern observed indicated that the t^{w18} haplotype contains the three wild-type-specific and the two t -specific Tu89 fragments (Fig. 2A). It thus appears that the D17Leh89 sequences, represented by the Tu89 probe, are duplicated in the t^{w18} haplotype. Similar results were obtained with the Tu467 probe, which hybridizes strongly to two *Taq*I fragments, of approximately 3.0 and 2.3 kb in wild-type DNA, two fragments of approximately 4.3 and 2.6 kb in t^{w5}/t^{w5} DNA, and all four fragments in t^{w18}/t^{w18} DNA (Fig. 2B). These data indicate that the D17Leh467 sequences, represented by the Tu467 probe, are also duplicated in the t^{w18} haplotype. Results with the Tu525 probe were essentially the same, but in this case the t^{w18}/t^{w18} DNA was found to contain not only

the 6.7-kb *Eco*RI fragment found in wild-type (strain 129) DNA and the 4-kb *Eco*RI fragment observed in t^{w5} -haplotype DNA, but also a 2-kb fragment (Fig. 2C). The simplest explanation for the observed pattern of hybridization is that the Tu525 sequences are duplicated in the t^{w18} haplotype, and that the 6.7- and 2-kb fragments are wild-type alleles for which the t^{w18}/t^{w18} cells are heterozygous. Consistent with this interpretation is the observation that in the blot of t^{w18}/t^{w18} DNA, the intensities of hybridization of the 6.7- and 2-kb fragments are each approximately half that of the 4-kb t -specific fragment.

Deletions and duplications in the t^4 and t^{ks1} haplotypes

To determine whether the loci that are deleted and duplicated in the t^{w18} haplotype are similarly altered in other members of the t^9 complementation group, a study of the t^4 and t^{ks1} haplotypes was carried out. However, in this case the analysis was more complicated because no cell lines homozygous for these t haplotypes are available, and embryos homozygous for t haplotypes in this complementation group die by approximately 10 days of gestation (Bennett and Dunn 1960; Moser and Gluecksohn-Waelsch 1967), making it very difficult to obtain sufficient t^4/t^4 or t^{ks1}/t^{ks1} embryonic DNA for these studies. We therefore analyzed DNA from t^4 and t^{ks1} animals heterozygous with the wild-type form of the t complex ($t^4/+$ and $t^{ks1}/+$) and heterozygous with a complete t haplotype (t^4/t^{Tuw24} and t^{ks1}/t^{w5}) using molecular probes which show RFLPs between t -haplotype and wild-type sequences (see Materials and methods). In this case deletion of a sequence represented by a given probe would be demonstrated if in $t^4/+$ and $t^{ks1}/+$ DNA only

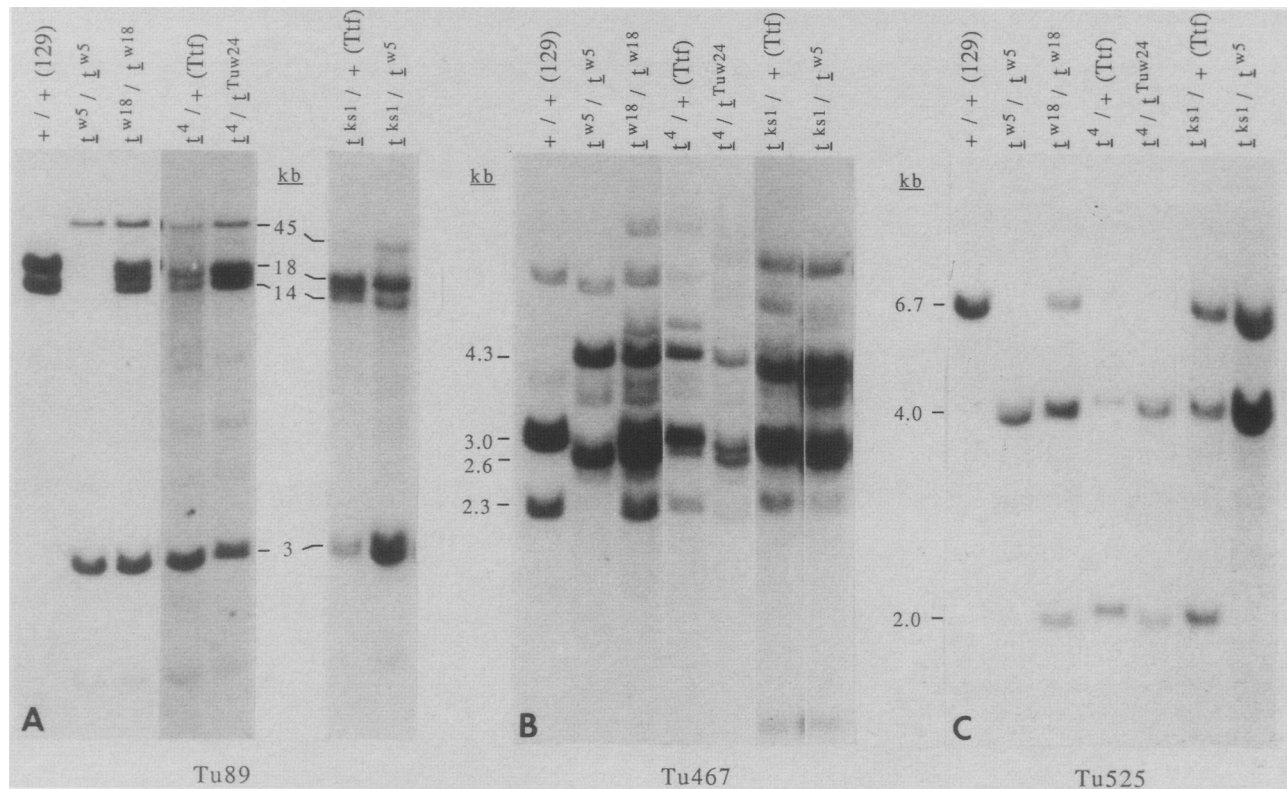


Figure 2. Duplication of sequences in the t^{w18} , t^4 , and t^{ks1} haplotypes. Southern blot hybridization analysis of genomic DNA from cells or animals of the designated genotypes. The genotype of the wild-type chromosome is indicated in parentheses. Each lane contains 10 μ g of genomic DNA digested with *MspI* (A) or *TaqI* (B,C) and was hybridized with the designated probes, Tu89, 467, or 525.

the wild type-specific fragment(s) were detectable, and if in t^4/t^{Tuw24} and t^{ks1}/t^{w5} DNA only the t -specific fragment(s) were observed. A duplication would be demonstrated if both the wild-type and t -specific fragments were detected in all the heterozygote samples.

Of the four loci deleted in t^{w18} , only two could be studied in this analysis because only the Tu443 and p54M probes detected RFLPs between t -haplotype and wild-type DNA. Probe Tu443 was found to hybridize to a 15-kb *MspI* fragment specific to wild type in $t^4/+$ and $t^{ks1}/+$ DNA (Fig. 1A), and to an 8-kb *MspI* t -specific fragment in t^4/t^{Tuw24} and t^{ks1}/t^{w5} DNA (Fig. 1A). This hybridization pattern indicates that the D17Leh443 locus is deleted in the t^4 and t^{ks1} haplotypes. An analogous result was obtained using the probe p54M. In this case, four hybridizing fragments of approximately 5.2, 5.0, 2.6, and 2.4 kb were detected in $t^4/+$ and $t^{ks1}/+$ DNA (Fig. 1E). Although different in size from the fragments detected in strain 129 DNA (Fig. 1E), these fragments were found to be specific to the wild-type chromosome present in these heterozygotes (data not shown); no t -specific fragments were detected. In t^4/t^{Tuw24} and t^{ks1}/t^{w5} DNA probe p54M hybridized to two t -specific fragments of approximately 8.4 and 1.6 kb; no wild-type fragments were detected (Fig. 1E). Taken together these data indicate that the D17Leh54 locus is deleted in the t^4 and t^{ks1} haplotypes.

Analysis of the t^4 and t^{ks1} heterozygotes using the mo-

lecular probes representing the loci that are duplicated in the t^{w18} haplotype (D17Leh89, 467, and 525) showed hybridization to both t -specific and wild-type specific fragments in all samples (Fig. 2A,B,C). These data indicate that those loci are also duplicated in t^4 and t^{ks1} haplotypes.

Genetic mapping of deleted and duplicated sequences

The map positions of the sequences deleted in the t^{w18} haplotype were determined by recombinant inbred (RI) strain analysis (Taylor 1978). For each probe that represents a sequence deleted in the t^{w18} haplotype, a RFLP between the C57BL/6 (B) and DBA/2 (D) strains of mice was identified. The strain distribution patterns (SDPs) of the B and D alleles of the D17Leh443, D17Leh94, D17Leh180, and D17Leh54 loci were then determined by hybridization of the probes to Southern blots of genomic DNA from each of 26 strains in the BXD RI set. These SDPs are shown (Table 1) in comparison with the SDPs previously determined for other markers in the t complex. The data indicate that although the four deleted loci have not been separated from one another by recombination in any of the BXD strains, they have been separated from the α -globin pseudogene 4 (*Hba-4ps*) locus in one strain, from the α A-crystallin (*Crya-1*) locus in four strains, and from the T1 region of the H-2 complex in five strains. These results place these loci 1 cM proximal to *Hba-4ps* (95% confidence interval of

Table 1. Strain distribution pattern in BXD recombinant inbred strains of alleles of the loci that are deleted or duplicated in the *t* haplotypes of the *t*⁹ complementation group

Loci	BXD strains																										
	0 1	0 2	0 3	0 5	0 8	0 9	1 1	1 2	1 3	1 4	1 5	1 6	1 8	1 9	2 0	2 1	2 2	2 3	2 4	2 5	2 7	2 8	2 9	3 0	3 1	3 3	
D17 Leh 443																											
D17 Leh 94	D	B	D	D	B	B	B	D	B	B	B	B	D	B	B	B	D	B	D	D	B	D	B	D	D	D	D
D17 Leh 180																											
D17 Leh 54M																											
<i>Hba-ps4</i> ^a	D	B	D	D	B	B	B	D	B	-	B	B	D	B	B	D	D	B	D	D	B	D	B	D	D	D	D
<i>Crya-1</i> ^b	D	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	B	D	B	D	D	D	D
T1 region of <i>H-2</i> complex ^c																											
D17 Leh 89	D	B	D	D	B	D	D	D	B	B	B	D	D	B	B	D	D	B	D	D	D	D	B	D	D	D	D
D17 Leh 525																											

B, Allele carried by C57BL/6 mice.

D, Allele carried by DBA/2 mice.

^a Data from Mann et al. (1986).

^b Our data, obtained with a probe described by King et al. (1982). The finding that BXD strain 24 carried the *D* allele disagrees with the data published by Skow and Donner (1985).

^c Our data, obtained with a probe described by Winoto et al. (1983).

0.03–7 cM; Silver 1985a), in the vicinity of *tf* (Fox et al. 1984; Mann et al. 1986). The location of the deleted sequences proximal to *tf* in the wild-type chromosome has been confirmed in an independent study by conventional recombinational analysis of back-cross individuals (B.G Herrmann, M. Búcan and H. Lehrach, unpubl.).

RI strain analysis was also used to determine the map position of the duplicated loci. RFLPs appropriate for mapping these sequences using the BXD RI strains could be found only for the Tu89 and Tu525 probes, but not for Tu467. The SDPs of the *B* and *D* alleles of the D17Leh89 and D17Leh525 loci shown in Table 1 indicate that no recombination had occurred between these two sequences in the BXD RI strains, but that they are separated by recombination from the cluster of deleted sequences in five strains. These data indicate that the duplicated cluster is 6.8 cM distal to the cluster of deleted sequences (95% confidence interval 1.2 – 24 cM). Moreover, the SDP of the duplicated loci is the same as that of sequences in the T1 region of the *H-2* complex that are represented by the probe mp22.1 (Winoto et al. 1983), indicating that these loci map in or near the *H-2* complex.

To define better the position of the duplicated probes in this region, we took advantage of the existence of congenic intra-*H-2* recombinant strains between AKR and C57BL/6 (Fig. 3A; Flaherty 1976; Flaherty et al. 1981; Robinson et al. 1987) and the finding that appropriate RFLPs between these strains could be identified for each of the duplicated sequences. For example,

Tu467 hybridizes to a 4.4-kb *TaqI* fragment in C57BL/6 DNA, but this fragment is absent from AKR DNA; Southern blot analysis showed that the B6.K1 and B6.K2 recombinant strains do not carry a Tu467 hybridizing fragment (data not shown), indicating that these strains carry the AKR allele of D17Leh467 locus (Fig. 3B). Similarly, the B6.K1 and B6.K2 recombinant strains were found to carry the AKR alleles of the D17Leh89 and 525 loci (see Fig. 3B). Our data, in conjunction with previously published analysis of the *H-2* complex sequences carried by the congenic intra-*H-2* recombinant strains (see Fig. 3A), localize the sequences that are duplicated in the *t*^{w18} haplotype to a region of the *H-2* complex distal to the *Qa-2,3* region in wild-type chromatin. Further experiments have placed the group of duplicated probes distal to the T1 region (K. Fischer-Lindhahl, M. Búcan, H. Lehrach, unpubl.).

Discussion

It has been known for some time that the members of the *t*⁹ complementation group arose by recombination between *t*-haplotype and wild-type chromatin, and that in each case the resulting *t* complex consists of a proximal portion derived from the *t*-haplotype parent and a distal portion derived from the wild-type parent. We have taken advantage of the availability of DNA probes, obtained by microdissection and microcloning of fragments of mouse chromosome 17 (Röhme et al. 1984), to carry out a structural analysis of three *t* haplotypes from the *t*⁹ complementation group, *t*^{w18}, *t*⁴, and *t*^{ks1}, to gain

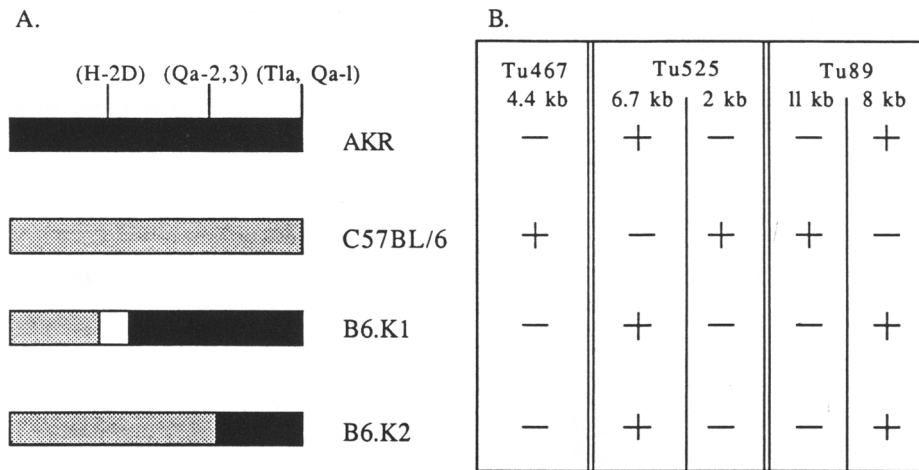


Figure 3. Segregation in congenic intra-*H-2* recombinant strains of loci that are duplicated in the *t* haplotypes of the t^9 complementation group. (A) Schematic representation of the *H-2* region of the intra-*H-2* recombinant strains, B6.K1 and B6.K2. The portions of the *H-2* region derived from each parental strain [AKR (black) and C57BL/6 (stippled)] are shown. The derivation of part of this region (white) in the B6.K1 strain is unknown. These maps are based on data from Flaherty (1976), Flaherty et al. (1981), and Robinson et al. (in press). (B) Distribution in these strains of the alleles of the loci represented by the Tu467, 525, and 89 probes. The table is based on the results of Southern blot hybridization analysis; (—) fragment not present, (+) fragment present.

insight into the nature of the recombination events that created them.

Previous genetic and molecular analyses of the t^{w18} lethal haplotype had shown that the crossover had occurred in the interval between the D17Leh122 and *tf* loci (Bennett et al. 1976; Fox et al. 1984). Since at least part of this region lies within the distal inversion characteristic of all complete *t* haplotypes, there are four possible ways in which recombination might have occurred, as illustrated in Figure 4. One possibility is that recombination between *t*- and wild-type chromatin occurred between homologous sequences, outside of and proximal to the distal inversion. If this were the case, the recombination event would not generate either deletions or duplications (see Fig. 4A). The three remaining possibilities all involve unequal crossing-over. Recombination could have taken place between sequences within the distal inversion in *t* chromatin, but outside the inversion in wild-type chromatin, which would result in duplication of sequences designated Y in Figure 4B, or the converse might have occurred, which would result in deletion of sequences designated X in Figure 4C. Finally, if recombination occurred within the inversion in both *t*- and wild-type chromatin, both deletion of sequences designated X and duplication of sequences designated Y would occur (Fig. 4D).

Our data showing that one group of probes is deleted and another duplicated in members of the t^9 complementation group are clearly consistent with the latter case. Moreover, if the hypothesis that the crossover occurred within the limits of the distal inversion is correct, one would predict that in the wild-type chromosome the deleted sequences would map between the proximal end of the distal inversion and the position of the crossover in the wild-type DNA. Conversely, the re-

gion between the position of the crossover and the proximal end of the inversion in the *t* chromosome should be duplicated in the recombinant chromosome. Again, our data are consistent with this hypothesis: The deleted loci were found to lie in a cluster that maps approximately 1 cM proximal to *tf* in wild type, and the duplicated loci were found to lie in a cluster that maps distal to the *Qa-2,3* region of the *H-2* complex in wild-type, or proximal to this region in *t* haplotypes (see Figs. 4 and 5).

What we now know about the structure of the members of the t^9 complementation group (see Fig. 5) provides some insight into the mechanism by which the recombination events that created them occurred. For the recombinant products to be duplicated for one end of the inverted region, deleted for the opposite end, and for the central region of the inversion to be present in single copy, recombination must have taken place at nonequivalent positions on the two chromosomes. This could have occurred when the inverted regions were paired in a linear and nonhomologous manner, mediated perhaps by chromosome breaks, or by homology resulting from short repetitive elements that occur at different positions on the two chromosomes, or from a sequence transposed and inverted on one of the two chromosomes. In contrast, other partial *t* haplotypes such as t^{w1ub2} and Tt^{or1} , are apparently deleted for one portion of the inverted region and duplicated for the remaining portion; no part of the inverted region is present in single copy (Herrmann et al. 1986; Sarvetnick et al. 1986). In these cases recombination presumably occurred at equivalent points on the *t*-haplotype and wild-type chromosomes, possibly mediated by a second, smaller inversion contained within the inverted region or by repetitive elements occurring in both orientations. In the case of another class of the partial *t* haplotypes t^{h45}

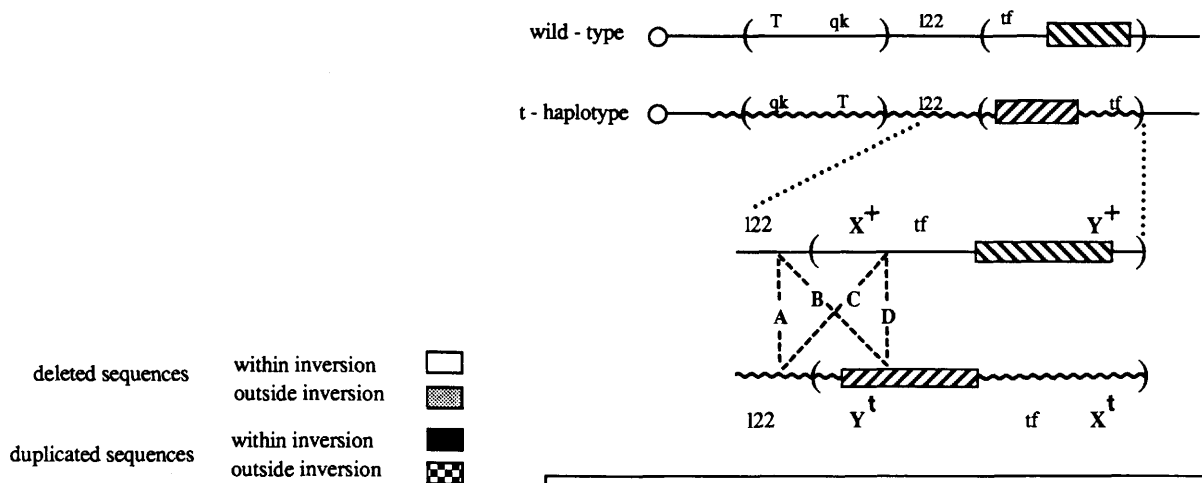
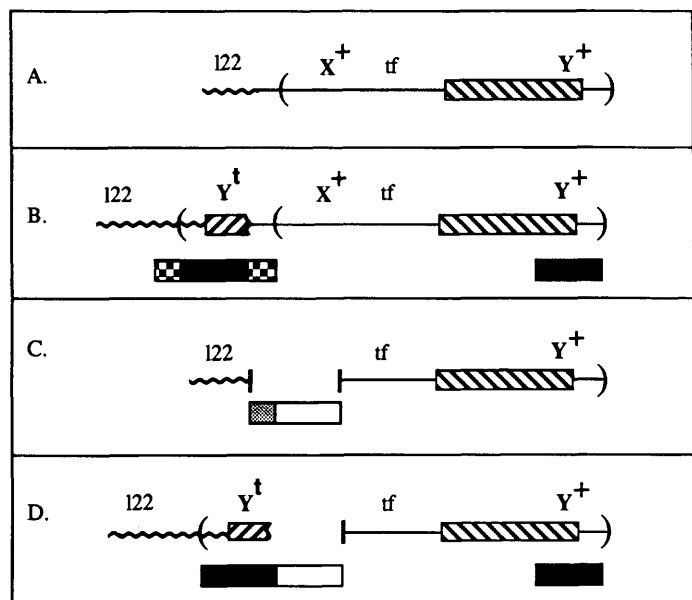


Figure 4. Four possible modes of recombination between a wild-type and a complete *t*-haplotype chromosome, leading to the formation of a distal partial *t* haplotype. Above are maps of the wild-type and *t*-haplotype forms of the *t* complex. The circle indicates the centromere of chromosome 17. The straight and wavy lines represent wild-type and *t*-haplotype chromatin, respectively. The parentheses indicate the positions of the regions that are inverted in *t* haplotypes, relative to wild-type. The "proximal inversion" contains the phenotypic markers *T* and *qk*, and the "distal inversion" contains the phenotypic marker *tf* and the *H-2* complex (cross-hatched box). The relative position of the D17Leh122 locus (122) is shown. Immediately beneath is shown an expansion of the region from D17Leh122 to the end of the *t* complex, including markers designated X and Y. Four possible modes of recombination in this region are designated A,B,C,D. The recombinant chromosomes that would result from each of these modes of recombination, and the consequent deletions and duplications, are diagrammed below.



and *t^{ae5}*, which are duplicated for virtually the whole of the inverted region, an extended region of inverted duplication provides the region of homology for recombination (Herrmann et al. 1987).

From our data it is not possible to obtain a minimal estimate for the size of the deletion in the members of the *t⁹* complementation group, since no recombination between any of the deleted markers was detected in 26 BXD RI strains or in the products of a high-resolution genetic cross (B.G Herrmann, M. Búcan, and H. Lehrach, unpubl.). However, the fact that the sequences represented by four probes out of 25 tested were found to be contained in the deletion might be an indication of a fairly large size. Similarly, the duplicated region, represented by three other probes may be relatively large.

At present it is uncertain whether the deletion or the duplication is responsible for the recessive lethal phenotype characteristic of members of the *t⁹* complementation group. If the latter is the case, however, then one would have to argue that the detrimental effects of the aneuploidy are manifested only in animals tetrasomic

for the region in question, since *+/t^{w18}*, *+/t⁴*, and *+/t^{ksl}* heterozygotes, which are trisomic for that region, are completely normal. Therefore, it seems most likely that the cause of the embryonic lethality of the *t⁹* complementation group homozygotes is nullisomy for a vital gene.

One approach to identifying the specific structural alterations responsible for the lethal phenotype is to determine which changes are common to all members of the complementation group. The fact that *t⁴* homozygotes appear to be more severely affected than *t^{w18}* homozygotes (Bennett and Dunn 1960; Moser and Gluecksohn-Waelsch 1967) might be an indication that different members of the *t⁹* complementation group contain different size deletions or duplications. With the probes available, however, we were unable to detect any differences among the three haplotypes tested, which are the only members of the *t⁹* complementation group still extant. We also attempted to narrow down the region of deletion potentially responsible for the lethal phenotype by carrying out an analysis of the *t^{h20}* haplotype. This *t*

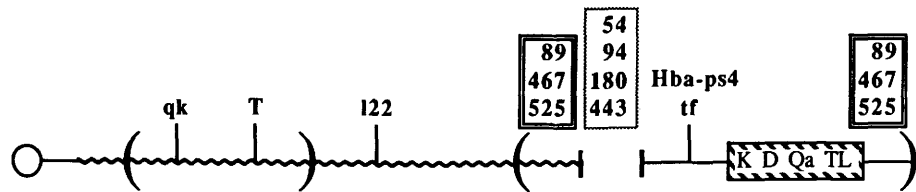


Figure 5. Genetic map of the t haplotypes of the t^9 complementation group. The circle indicates the centromere of chromosome 17. The straight and wavy lines represent wild-type and t -haplotype-derived chromatin, respectively. The parentheses indicate the positions of the regions that are inverted in t haplotypes, relative to wild-type. The relative order of the markers is shown. Loci present in single copy: quaking (qk), Brachyury (T), D17Leh122, α -globin pseudogene 4 ($Hba-ps4$), tufted (tf), $H-2$ complex (cross-hatched box). Loci deleted: D17Leh94, 180, 443, and 54. Loci duplicated: D17Leh89, 467, and 525.

haplotype, which is known to contain a deletion encompassing both the tf and $Hba-ps4$ loci (Lyon et al. 1979; Fox et al. 1984), complements the t^{w18} haplotype (Lyon et al. 1979). Thus, if any of the sequences deleted in t^{w18} were found to be similarly deleted in t^{h20} , this would provide evidence that those sequences are not responsible for the lethal phenotype. However, all of the sequences deleted in t^{w18} were found to be present in t^{h20} (M. Búcan, G.R. Martin, F. Michiels, and H. Lehrach, unpubl.). Although further analysis of the molecular nature of the lethal mutation will be difficult, especially considering the potentially large size of the structurally altered regions, the data reported here provide a starting point for the search for the lethal gene.

Materials and methods

Animals and cell lines

The origin of the t^{w18} haplotype has been previously described by Bennett and Dunn (1960). All analyses of this haplotype were performed on DNA obtained from animals maintained in L. Silver's mouse colony or from the t^{w18}/t^{w18} ES cell lines isolated by Martin et al. (1987). The conditions of ES cell culture and separation from feeder cells are described in detail by Martin (1981).

The origin of the t^4 haplotype was previously described by Moser and Gluecksohn-Waelsch (1967). Animals carrying this haplotype were kindly provided by Dr. Salome Waelsch (Albert Einstein College of Medicine, New York, New York), and were mated to wild-type mice or mice carrying the t^{Tuw24} haplotype to obtain mice with a $+/t^4$ or a t^4/t^{Tuw24} genotype.

The t^{ks1} haplotype was first detected among the offspring of a brother \times sister mating of $Ttf/t^{w5}+$ mice in the colony maintained at Kansas State University (Manhattan, Kansas). This is a balanced lethal cross that normally produces only tailless, nontufted animals, and the new haplotype was first identified in a normal-tailed, nontufted female. She was mated back to her father ($Ttf/t^{w5}+$) and gave both tailless tufted ($Ttf/t^{ks1}tf$) and normal tailed, nontufted ($t^{w5}+/t^{ks1}tf$) offspring. The appearance of these offspring indicated that the new haplotype was derived by recombination, with a gain of the *tufted* mutation and loss of the t^{w5} lethal factor. When mated inter se, the tailless, tufted animals gave only tailless, tufted offspring, indicating that the recombination event had created a new lethal factor. In further complementation tests the recombinant chromosome was found to complement the t^{12} and Tt^{Ori} , but not the t^{w18} haplotype (0/38 offspring were normal-tailed). The tailless, tufted off-

spring were brother \times sister mated for 12 generations and then backcrossed to the inbred $Ttf/t^{w5}+$ line for six generations before sampling for this study. The t^{ks1} haplotype has a transmission ratio of only 37% (47/126) in one group of males.

DNA isolation and analysis

Total cellular DNA was isolated from mouse liver or spleen or from ES cells by standard techniques. BXD RI strain DNA was purchased from the Jackson Laboratory (Bar Harbor, Maine). Mouse genomic DNA was digested, fractionated by electrophoresis, and blotted onto a Gene Screen membrane by capillary transfer in denaturation solution (1.5 M NaCl and 0.5 M NaOH) as previously described by Herrmann et al. (1986). The filter was exposed to UV light and hybridized according to the methods described by Church and Gilbert (1984) using 2×10^6 cpm/ml of hybridization probe.

Identification of t -specific DNA restriction fragments

DNA from mice congenic with strain 129/Sv for the complete t haplotypes t^0 , t^{w2} , t^{w5} , t^{w12} , t^{w32} , and t^{wLub1} , as well as DNA from inbred mouse strains (129/Sv, C57BL/6, DBA/2, AKR, C57BL/10, C3H/He, C3H/Sw, BALB/c) were examined for the presence of restriction fragments specific to t -haplotype DNA. Only fragments that were detected in all t -haplotype DNAs analyzed, but not in any of the wild-type DNAs tested, are referred to as t specific.

Hybridization probes

Each of the following genomic clones is an *EcoRI* fragment inserted into pUC9: Tu443 (1.7 kb), Tu94 (0.4 kb), Tu180 (0.4 kb), Tu525 (2 kb), Tu467 (1.7 kb), and Tu89 (1.7 kb). They were derived by microdissection (Röhme et al. 1984). The probe p54M (2.9-kb *EcoRI* fragment), kindly provided by M. Zimmer and T.M. Pohl, was derived from a cosmid clone isolated from a genomic cosmid library using the clone Tu54, derived by microdissection, as a probe. Hybridization probes were prepared by isolation of insert DNA from agarose gels by agarase treatment (Búcan et al. 1986) and radiolabeled to a specific activity of 3×10^9 cpm/ μ g with 32 P[dCTP] using the Klenow fragment of DNA polymerase I and random hexamer priming (Feinberg and Vogelstein 1983). Probes Tu443 and p54M contain repetitive sequences and therefore are prehybridized against total genomic mouse DNA prior to hybridization (Ardeshir et al. 1983).

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