Genetic and Molecular Analysis of the Proximal Region of the Mouse *t*-Complex Using New Molecular Probes and Partial *t*-Haplotypes

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ABSTRACT

The *t*-complex is located on the proximal third of chromosome *17* in the house mouse. Naturally occurring variant forms of the *t*-complex, known as complete *t*-haplotypes, are found in wild mouse populations. The *t*-haplotypes contain at least four nonoverlapping inversions that suppress recombination with the wild-type chromosome, and lock into strong linkage disequilibrium loci affecting normal transmission of the chromosome, male gametogenesis and embryonic development. Partial *t*-haplotypes derived through rare recombination between *t*-haplotypes and wild-type homologs have been critical in the analysis of these properties. Utilizing two new DNA probes, Au3 and Au9, and several previously described probes, we have analyzed the genetic structure of several partial *t*-haplotypes that have arisen in our laboratory, as well as several wild-type chromosomes deleted for loci in this region. With this approach we have been able to further our understanding of the structural and dynamic characteristics of the proximal region of the *t*-complex. Specifically, we have localized the *D17Tu1* locus as most proximal known in *t*-haplotypes, achieved a better structural analysis of the partial *t*-haplotype t^6, and defined the structure and lethal gene content of partial *t*-haplotypes derived from the lethal t^{w73} haplotype.

MUTANT forms of the *t*-complex, which comprises the proximal third of chromosome *17*, are found in high frequencies in wild mouse populations (KLEIN 1986). The initial identification of *t*-haplotypes was due to the fortuitous existence within them of a gene *tct* (*t-complex tail interaction factor*) that interacts with a spontaneous dominant mutation *Brachyury* (*T*). *Brachyury* produces short tails in *T*/+ mice (DOBROVOLSKAIA-ZAVADSKAIA 1927), but interacts with *tct* to produce a tailless phenotype in *T*/*t* mice (DOBROVOLSKAIA-ZAVADSKAIA and KOBOZIEFF (1932). This interaction identifies mice carrying *t*-haplotypes and has allowed investigators the opportunity to search wild populations for these naturally occurring polymorphisms and examine their unique properties.

Classical genetic and molecular analysis of *t*-haplotypes revealed the presence of two large inversions, referred to as proximal and distal (SILVER and ARTZT 1981; ARTZT, SHIN and BENNETT 1982; SARVETNICK *et al.* 1986; HERRMANN *et al.* 1986). A recent genetic study involving interspecific crosses between *Mus spretus* or *Mus abbotti*, and laboratory mice provided evidence for the presence of two additional nonoverlapping inversions. The first one is located between the centromere and the proximal inversion, and the second one centrally between the proximal and distal inversions (HAMMER, SCHIMENTI and SILVER 1989). Current nomenclature identified these inversions, starting at the centromeric end, as *In(17)1*, *In(17)2*, *In(17)3*, and *In(17)4* (Committee for the Mouse Chromosome *17*, 1990). These inversions, carried by all complete *t*-haplotypes, suppress recombination with the wild-type chromosome over a 15-centimorgan (cM) distance, and thus create strong linkage disequilibrium among mutant loci in *t*-haplotypes that affect the normal transmission of the chromosome, male fertility and embryonic development.

Embryonic lethal genes have been identified within the *t*-complex (BENNETT 1975; KLEIN, SIPOS and FIGUEROA 1984) and have been shown so far to fall into 16 complementation groups. Homozygosity for *t*-lethal genes results in the arrest of embryonic development at specific stages, different for each complementation group studied. Recent data have demonstrated that the transmission of *t*-haplotypes to progeny is controlled by several distinct loci, which interact in a complex fashion and result in a high ratio of transmission, approaching 100%, of the *t*-chromosome from heterozygous +/*t* males (LYON 1984; SILVER and REMIS 1987). Another effect of *t*-haplotypes on male germ cells results in sterility of those that are heterozygous for complementing *t*-haplotypes (t^x/t^y). The loci responsible for the sterility are thought to be identical to the loci responsible for transmission ratio distortion (TRD) (LYON 1986).

Partial *t*-haplotypes derived through rare recombinational events between *t*-haplotype and wild-type

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chromosomes have been important tools in the analysis of the diverse properties associated with the *t*-complex. While recombination is generally suppressed between *t* and wild-type chromosomes, the rare recombination events that create partial haplotypes occur on average one in a thousand times. These partial *t*-haplotypes are defined as proximal, central or distal depending upon the portion of *t*-chromatin retained, and the *t*-specific phenotypes observed. Characterization of partial *t*-haplotypes provides valuable information concerning the organization of the chromosome, and the location of genes within the *t*-complex.

In the first part of the study presented here, we attempted to generate random genomic clones mapping within the *t*-complex. This was accomplished using two moderately repetitive mouse specific probes, the intracisternal A particle (IAP) genes and the virus-like 30 (VL30) sequences. These two sequences have copy numbers of approximately 1000 and 250, respectively, and are randomly distributed throughout the mouse genome (PIKO, HAMMONS and TAYLOR 1984; COURTNEY *et al.* 1982). These probes were used to screen a cosmid library made from a mouse-hamster hybrid somatic cell line R4 4-1, which contains mouse chromosomes *17* and *18*. Preliminary analysis of a subset of the cosmids isolated from the R4 4-1 library generated two single copy probes, Au3 and Au9, mapping to the proximal region of the mouse *t*-complex.

Utilizing these two probes, and several previously described probes, we have analyzed the genetic structure of several partial *t*-haplotypes that have arisen in our laboratory, as well as several wild-type chromosomes deleted for loci in this region. With this approach we were able to show that the centromeric *D17Tu1* locus found in wild-type chromosomes is also centromeric to the start of the *t*-complex. Additionally, our analysis of three partial *t*-haplotypes derived from the complete t^{w73} haplotype, and our reexamination of the t^6 partial *t*-haplotype has furthered our understanding of the molecular structure and functional properties of the proximal region of the *t*-complex.

MATERIALS AND METHODS

Mice: All mice used in this study were bred in our mouse colony. The *t*-haplotypes were maintained heterozygous with *T qk tf* (*BTBR*) in balanced lethal, brother-sister, but not strictly inbred matings. The *quaking* (*qk*) and *tufted* (*tf*) loci are phenotypic markers located approximately 3 and 10 cM, respectively, distal from *T*. Other *t*-haplotypes are congenic, or in the process of being made congenic, on the C3H/DiSn background. The complete *t*-haplotypes were reviewed by BENNETT (1975). The partial haplotypes t^6- (LYON and MEREDITH 1964; HERRMANN, BARLOW and LEHRACH 1987), Tt^{s6} (SILVER and ARTZT 1981; HERRMANN *et al.* 1986), and the deletions T^{Hp} and T^{Or} were previously described (BENNETT *et al.* 1975; BABIARZ 1983). The T^{22H} deletion was a gift of MARY LYON, and will be described elsewhere (M. F. LYON, unpublished data). The t^{AE5} partial haplotype (VOJTISKOVA *et al.* 1976) was a gift from SALOME WAELSCH, Albert Einstein College of Medicine, New York, New York.

TABLE 1

Description of recombinant *t* haplotypes

Complementation group	Members	Exceptions	Reference
t^{12}	t^{12}	$t^{w111}tf$	SILVER, WHITE and ARTZT (1980)
	t^{w32}	$t^{w82}tf$	SILVER, WHITE and ARTZT (1980)
t^{w73}	t^{w73}	$t^{w73j}tf$	Present study
		$t^{w73}tf$*	Present study
	t^{w121}	$p^{w121a}tf$	Present study
t^{w5}	t^{w130}	$t^{w130a}tf$	Present study
	t^{w5}	t^{w5x}*	Present study
t^{w1} and t^{w5}	t^{w75}	$t^{w124}tf$	Present study
		$t^{w75q}tf$	Present study

* Not viable as homozygotes.

The collection of partial *t*-haplotypes analyzed in this study arose as exceptional recombinants from standard balanced lethal matings (Table 1). The t^{w73} haplotype is the only haplotype studied that has an embryonic lethal mutation in the proximal inversion; the remainder are found in distal locations within the *t*-complex.

We have adopted a new nomenclature for designating exceptional recombinants from existing complete lethal *t*-haplotypes. Previously, according to the usage of DUNN (BENNET, DUNN and ARTZT 1976), once such a recombinant was characterized and established as a stock it was renamed with the next available number in the series of t^{w}- (from wild-derived chromosomes) or *t*- (for laboratory derived chromosomes). We now designate exceptional recombinants with the number of the parent allele and a superscript letter, that is, t^{w121a} is the first recombinant established from the lethal chromosome t^{w121}, and t^{w121b} will be the second, etc. This system is simpler, and has the advantage of immediately identifying the parent allele.

DNA probes and nomenclature: The IAP probe is a 7.0-kb full length clone, provided by L. PIKO, derived by *Eco*RI digestion of the IAP 11 plasmid (PIKO, HAMMONS and TAYLOR 1984). The VL30 probe, provided by M. J. GETZ, was also a full length clone (5.0 kb), and was prepared by *Xho*I digestion of the pVL47 plasmid (COURTNEY *et al.* 1982). The probe Tctex-1 is a 600-bp cDNA insert, derived by *Eco*RI digestion of plasmid 3-1 (LADER *et al.* 1989). Probes for the *D17Leh48* (locus: *T48*; probe: Tu48), *D17Leh66* (loci: *T66A*, *T66B*, *T66C*, *T66D*, *T66E*; probe: Tu66), *D17Leh119* (loci: *T119I*, *T119II*, *T119t*; probe: *Tu119*) and *D17Leh122* (locus: *T122*: probe: Tu122) loci were obtained by microdissection of the proximal region of mouse chromosome *17* (RÖHME *et al.* 1984). The mapping and characterization of these clones, kindly provided by HANS LEHRACH, has been described (RÖHME *et al.* 1984; FOX *et al.* 1985; HERRMANN *et al.* 1986; HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987). The Tcp-1 probe, provided by KEITH WILLISON, is a 800-bp cDNA insert, derived by *Bam*HI digestion of the plasmid pB1.4 (WILLISON, DUDLEY and POTTER 1986). The mouse SOD-2 probe, provided by GRAEME BELL, is a 900-bp cDNA insert, derived by *Eco*RI digestion of the plasmid pmMnSOD-1 (HALLEWELL *et al.* 1986; FIGUEROA *et al.* 1988). The Tu1

probe (locus: *D17Tu1* or *Tu1*), provided by FELIPE FIGUEROA, is a 6.4-kb *Eco*RI-*Sal*I DNA fragment cloned into pUC8 (FIGUEROA *et al.* 1987).

Construction and screening of the R4 4-1 cosmid library: The R4 4-1 Chinese hamster × mouse hybrid cell line contains only one mouse chromosome, *M1* (SMILEY *et al.* 1978). The *p* arm of this chromosome corresponds to mouse chromosome *17*, and the *q* arm corresponds to chromosome *18* and some unidentified mouse chromosome material (RICHARDS *et al.* 1985). High molecular weight DNA from the R4 4-1 hybrid cell line was prepared by standard methods. DNA was partially digested with the restriction enzyme *Mbo*I and used to prepare a cosmid library in the vector pTL5 (LUND, GROSVELD and FLAVELL 1982). The conditions for library construction and screening have been described (STEINMETZ *et al.* 1982; GROSVELD *et al.* 1981). The IAP and VL30 probes were independently nick translated to specific activities of at least 10^8 cpm/μg, and 2×10^6 cpm of each probe per ml of hybridization buffer were mixed together and hybridized to the library.

Southern blotting, probe labeling and hybridization: All Southern blots and library screenings were performed using Hybond-N membranes (Amersham). Hybridization and washing conditions used were those recommended by the manufacturer. Typically, 2×10^6 cpm of nick translated probe was used per ml of hybridization solution. For probes Tu1 and Au9, prior to hybridization, the ^{32}P-labeled DNA was pre-hybridized with 200–300 μg/ml sheared total mouse DNA in order to block repetitive sequences contained in these probes. The final washing conditions were at high stringency, 0.1 × SSC/0.1% SDS at 65° for 10 min. In the Southern blot analyses, when possible, DNA was prepared from homozygotes for the proximal partial haplotypes. The proximal recombinants still carrying lethal mutations were analyzed opposite a complementing complete *t*-haplotype, and also when heterozygous with a wild-type C3H chromosome.

RESULTS

Isolation of cosmid clones mapping to the *t*-complex: Southern blot analysis of genomic DNA from the R4 4-1 hybrid cell line with the IAP probe identifies at least 50 restriction fragments, and VL30 shows more than 20; no hybridization signals were detected in lanes containing just Chinese hamster DNA (data not shown). The R4 4-1 cosmid library was screened with both probes simultaneously. Approximately 100 clones gave positive signals of heterogeneous intensity on the primary screening. Analysis of a subset of these cosmids resulted in the identification of one low copy probe, Au3 (officially named D17Au3I and D17Au3II), and one that detects a unique sequence, Au9 (officially named D17Au9). Neither probe detected RNA transcripts on Northern blots of total RNA samples from a variety of somatic and germ cell tissues. They were shown to map within the *t*-complex by probing Southern blots containing DNAs from mice carrying various complete *t*-haplotypes congenic on C3H/DiSn.

Probe Au3, a 1.5-kb *Eco*RI fragment, hybridizes to two *t*-specific *Rsa*I fragments (2.7 kb and 2.35 kb), and two fragments derived from the C3H wild type chromosome (2.0 kb and 2.4 kb) (Figure 1A). Probe

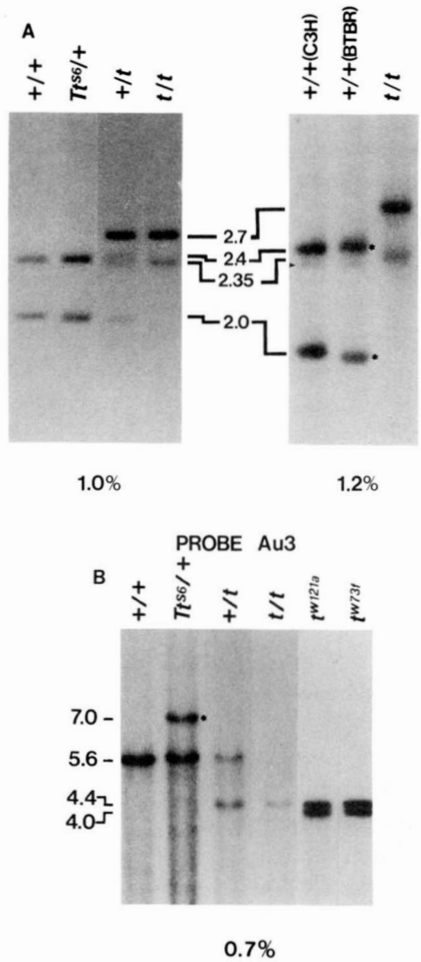

FIGURE 1.—Mapping of probes Au3 and Au9 to the *t*-complex by Southern blotting. Mouse genomic DNAs (10 μg) were digested with the appropriate restriction enzyme, electrophoresed on 0.7–1.2% agarose gels, blotted, probed with ^{32}P-labeled probes, washed and exposed to X-ray film. DNAs have been abbreviated as follows: +/+ (C3H); Tt^{v6}/+ (Tt^{v6}/C3H); +/*t* (C3H/t^{12}); *t*/*t* (C3H.t^{12}/t^{w5}); +/+ (BTBR—a brother-sister mated, wild-type chromosome 17); t^{w121a} ($t^{w121a}tf/t^{w121a}tf$); t^{w73f} ($t^{w73f}tf/t^{w73f}tf$). The sizes of fragments are shown in kilobases (kb). (A) *Rsa*I digested DNA probed with the 1.5-kb genomic probe Au3. Asterisks indicate wild type polymorphic fragments, which are 1.95 kb and 2.45 kb in size (see text). An additional cross-hybridizing 2.3-kb fragment is resolved in all DNAs when electrophoresed on 1.2% agarose gels for extended running time (arrowhead). (B) *Taq*I-digested DNA probed with the 3.9-kb genomic probe Au9. Asterisk indicates a wild-type polymorphic fragment 7.0 kb found in the proximal region of Tt^{56} (see text).

Au9 is a 3.9-kb *Eco*RI fragment that hybridizes to two bands in *Taq*I digests (Figure 1B). A 5.6-kb fragment is detected in the C3H chromosome, and a 4.4-kb fragment was detected in all *t*-haplotypes examined. An additional 4.0-kb fragment was detected in the t^{w73} haplotype and its derivatives. Using high percentage agarose gels and extended running times, the C3H wild-type 2.0-kb and the 2.4-kb fragments, detected by Au3 (Figure 1A), are observed to be polymorphic in mice carrying the *T* to *tf* interval from BTBRTF/Nev made congenic on C3H. The fragment

sizes detected in these DNAs are 1.95 kb and 2.45 kb, and are shown by asterisks in Figure 1A. A similar wild-type polymorphism is observed on Southern blots of wild-type DNAs probed with Au9 (Figure 1B). A 7.0-kb fragment is detected, instead of the 5.6-kb fragment, in the inbred strains AKR and BTBRTF/Nev mice. This polymorphism is associated with several other chromosomes including *TR190*, t^{low}, T^{Hp}, T^{Orl} and t^{wLub2}, all of which contain non-C3H wild-type chromatin at their proximal ends.

Genetic mapping of probes Au3 and Au9 in wild-type and recombinant chromosomes: Mapping of these two probes to the proximal region of the *t*-complex can be determined from the Southern blots shown in Figure 1. The C3H/Tt^{s6} lanes show only the wild-type fragments for these two probes. The Tt^{s6} chromosome is known to be a partial distal haplotype that contains wild-type chromatin throughout the region of the proximal inversion, and contains *t*-chromatin from the *T66B* locus through the distal inversion (Fox *et al.* 1985) (Figure 2C). Thus, in *t*-haplotypes these two probes must map centromeric to the *T66B* locus; and in the wild-type chromosomes they map centromeric to the *T66D* locus.

To further map probes Au3 and Au9 in both *t*-haplotypes and wild-type chromosomes, these probes were hybridized to Southern blots of DNAs from various proximal partial *t*-haplotypes as well as wild-type chromosomes carrying deletions within the proximal region of the *t*-complex. The results of this analysis, and the structural organization of the haplotypes and deletions used are shown in Figure 2.

The molecular structure of the deletions used in this study are shown in Figure 2C. The T^{Hp} deletion extends the furthest distally, deleting *qk* and *Tcp-1*, with its distal breakpoint mapping between the *T66D-g1* locus and the distal *T66D* subregions (Bullard and Schimenti 1990). The T^{Or} and T^{22H} distal breakpoints are between *T* and *qk*. The proximal breakpoint in the T^{Hp} deletion is between an inverted duplication (Herrmann, Barlow and Lehrach 1987) indicated by facing arrows in the +-chromosome in Figure 2C. This region, which is duplicated in wild-type chromosomes, is defined as the region between and including the *T119* and *T66E* loci. All complete *t*-haplotypes contain a unique homologous region defined by the $T119^t$ and *T66A* loci, indicated by the single arrow in the *t*-chromosome (Figure 2C). The proximal breakpoint in the T^{Or} mutant has been mapped to a region between the *T48* and *Tctex-1* loci (Lader *et al.* 1989). The T^{22H} deletion extends the furthest proximally, with a breakpoint mapping centromeric to the *Tu1* locus (Figure 2C).

Probe Au3: Deletion analysis localized the two wild-type fragments hybridizing to probe Au3 to different locations on wild type chromosomes. As shown in Figure 2A the C3H lane has the expected wild-type-specific 2.4-kb and 2.0-kb fragments. The compound heterozygote, $t^{w5}/t^{w12}tf$, contains only the *t*-specific 2.7-kb and 2.35-kb restriction fragments. Southern blot analysis of mice carrying three deletions, hybridized with Au3 showed that the T^{Or}/t^{w5} and T^{22H}/t^{w5} DNAs lack both wild type fragments, while $T^{Hp}/t^{w12}tf$ contains only the 2.0-kb wild-type fragment. Thus, both wild-type bands recognized by probe Au3 are deleted from T^{Or} and T^{22H}, while only the 2.4-kb fragment is deleted from T^{Hp}. This maps probe Au3 to two regions in the wild-type chromosome (Figure 2C). The 2.0-kb restriction fragment (defining a wild-type *Au3* locus, which we designate *D17Au3I*) maps between the proximal breakpoints of T^{Hp} and T^{Or}; while the 2.4-kb fragment (defining the wild-type *D17Au3II* locus), which is deleted, must map distal to the T^{Hp} breakpoint.

Preliminary pulse field gel electrophoresis (PFGE) analysis of genomic DNA hybridized with probe Au3 and two other probes (Tu119 and Tu66) in the inverted repeat region, detects a wild-type-specific 550-kb *Nru*I restriction fragment that hybridizes to all three probes (data not shown). This is close to the distance reported between the *T119* and *T66E* loci in the 129/Sv wild type strain (Herrmann, Barlow and Lehrach 1987). This association is good evidence for the localization of *Au3* in the region of the inverted duplication and consistent with the duplication of this region and the finding of two distinct loci detected with the Au3 probe.

Analysis of the proximal *t*-haplotype t^{AE5} became relevant to this study because it provided evidence for the mapping of the *Au3* loci between *T119* and *T66E*. Molecular analysis of t^{AE5} has shown the breakpoints to be proximal to the *T66A* locus in *t*-chromatin and between *T119I* and *T66E* loci in the wild type chromosome (Herrmann, Barlow and Lehrach 1987). The *T66EI* locus is retained and is found in association with the $T119^t$ locus (Figure 2C). Southern hybridization of t^{AE5} DNA with probe Au3 detects the 2.7-kb and 2.35-kb *t*-specific fragments, as well as the 2.4-kb wild-type fragment. However, the 2.0-kb wild-type fragment was not detected (Figure 2A). This further defines the position of the *Au3I* locus in wild-type chromosomes to the region between the *T119I* and *T66EI* loci (Figure 2C). In addition, the *t*-specific fragments can now be mapped centromeric to the breakpoint of t^{AE5}, which is proximal to the *T66A* locus in *t*-haplotypes. Southern hybridization of pulse field gels probed with Au3, Tu119 and Tu66 detects a *t*-specific 780-kb *Not*I fragment, suggestive of a map position of the 2.35-kb and 2.7-kb *t*-specific fragments between $T119^t$ and *T66A* (NOTE: locations of *Au3* loci in *t*-haplotypes illustrated in Figure 2C includes data presented in the following section, which maps the 2.7-kb fragment to a more proximal region of the chromosome).

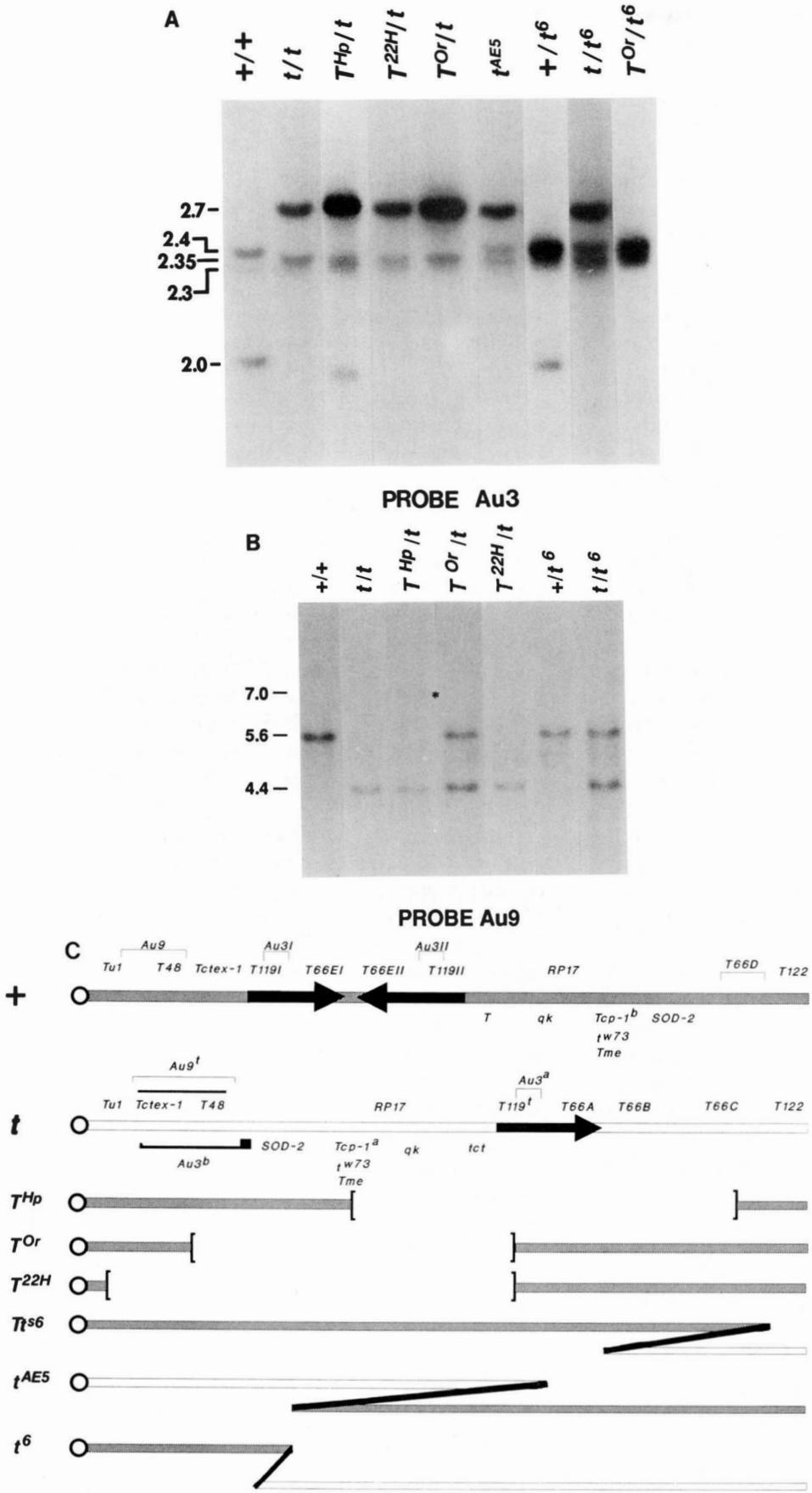

FIGURE 2.—Fine genetic mapping of probes Au3 and Au9 in wild-type and *t*-chromatin. (A) Genomic DNAs from various wild type deletion mutants and partial *t*-haplotypes were digested with *Rsa*I and probed with Au3. DNAs were abbreviated as follows: +/+ (C3H); *t*/*t* (C3H.t^{12}/t^{w5}); $T^{Hp}/t(T^{Hp}/t^{w12}tf)$; T^{22H}/t (T^{22H}/t^{w5}); T^{Or}/t (C3H.T^{Or}/t^{w5}); t^{AE5} (t^{AE5}/t^{AE5}); $+/t^6$ (C3H/t^6); t/t^6 ((t^{w5}/t^6). (B) Genomic DNAs digested with *Taq*I and probed with Au9. Asterisk indicates the presence of the 7.0-kb restriction fragment seen in T^{Hp} DNA, which does not show up well in this photograph. (C) Maps of the proximal region of the wild type and recombinant *t*-haplotypes used in the RFLP mapping of probes Au3 and Au9. The wild type and *t*-haplotype forms of the *t* complex have been aligned, and relevant gene loci and molecular markers indicated. The distances between loci are not drawn to scale. The large arrows show homologous regions, and their orientation. The line over the molecular marker T48 and adjacent loci indicates the order of these markers is not known. The map position of the *Au3* and *Au9* loci are indicated with brackets. The proposed location of the $Au3^b$ locus within the bracketed region is designated with a black box.

The t^6 haplotype, and all of its derivatives, have been shown to contain wild-type chromatin in a region defined by the molecular probe Tu48, although t^6-maintains the *t*-specific form of Tcp-1 (HERRMANN, BARLOW and LEHRACH 1987). Mapping data with various proximal probes revealed the wild-type chromosome breakpoint to be distal to T119I, and centromeric to SOD-2 in *t*-chromatin (Figure 2C). Southern hybridization of t^6-DNA with Au3 revealed additional information concerning the map position of loci detected in *t*-haplotypes and wild-type chromosomes.

Analysis of Southern blots of t^6 with Au3 detects the 2.4-kb wild-type fragment, but not the 2.0-kb fragment (Figure 2A). This is unexpected, since it has been shown that t^6 contains the *T119I* locus, which we find usually associated with the Au3I 2.0-kb restriction fragment. However, it suggests that the *Au3* wild-type loci are probably located nearer to the *T119* loci than *T66E* loci, due to the fact that the 2.4-kb fragment was found in association with *T119I*, and that only a small segment of wild-type DNA is distal to *T119I* in the t^6-haplotype (HERRMANN, BARLOW and LEHRACH 1987).

In the t^6-haplotype, the 2.35-kb fragment (defining the *t*-specific *D17Au3*^{a} locus) is found as would be expected, if it is located between *T119*^{t} and *T66A*, however the 2.7-kb fragment (defining the *D117Au3*^{b} locus) was not detected (Figure 2, A and C). In no other haplotype, either complete or partial, are these two *t*-specific Au3 fragments separated. This would suggest that the *Au3*^{b} locus maps centromeric to the t^6 breakpoint in *t*-chromatin, since it appears to be deleted. It is possible that the *Au3II* and *Au3*^{b} loci could have participated in the homologous but unequal crossover event that resulted in the formation of the distal t^6-haplotype.

Probe Au9: Southern blot analysis of the deletions T^{Hp}, T^{Or} and T^{22H} places the *Au9* locus centromeric to the *Tctex-1* gene in wild-type chromosomes (Figure 2, B and C). The wild-type fragments, either 7.0 or 5.7 kb, are seen in both T^{Hp}/t and T^{Or}/t DNA (Figure 2B). In contrast, T^{22H}/t DNA does not reveal any wild-type fragment, indicating that it is deleted for Au9. As previously described, the proximal breakpoint of the T^{Or} deletion is centromeric to *Tctex-1*; while the proximal breakpoint of the T^{22H} deletion is centromeric to the *Tu1* locus (Figure 2C). This places the wild type *Au9* locus between the *Tu1* and *Tctex-1* loci, in the region of the *T48* locus. Southern blot analysis of +/t^6 DNA revealed only a 5.6-kb wild-type fragment (Figure 2B). This result confirms the position of the *Au9* locus in wild-type chromosomes; and more importantly shows that in complete *t*-haplotypes the *Au9* locus (*Au9*^{t}) must map centromeric to the breakpoint of *t*-chromatin in t^6, and by inference from wild type, in the region of *T48* (Figure 2C).

Molecular analysis of new partial *t*-haplotypes: To extend the study of the proximal region of *t*-haplotypes, we analyzed a battery of proximal exceptions, with seven previously described proximal molecular probes (Tu1, Tu48, Tu119, Tu66, Tcp-1, SOD-2, and Tu122), and three that were isolated in our laboratory (Au3, Au9 and Tctex-1). The results are summarized in Figure 3.

The nine proximal partial *t*-haplotypes fall into two groups based on Southern blot analysis. The first group consists of recombinants ($t^{w111}tf$, $t^{w75q}tf$, $t^{w73f}tf$, $t^{w121a}tf$ and t^{AE5}) that have breakpoints in an inverted region in the *t*-haplotype. This causes duplications and deletions of loci in the relevant region. It is worth pointing out that in such cases, any marker to the left of the breakpoint in *t*-chromatin and to the right of the breakpoint in +-chromatin will be duplicated. For example, this whole first group have duplications of the region from *SOD-2* through the *T119* locus (Figure 3). Recombinants of this first class resulted from homologous unequal recombination between sequences from the *T66A* and *T66B* region of the *t*-haplotype and the *T66E* region of the wild-type chromosome. The second group have breakpoints outside of any inverted region, and therefore do not involve the duplication of markers. This class includes $t^{w130a}tf$, $t^{w73}tf$, $t^{w124}tf$ and $t^{w82}tf$. Members of this group are the result of homologous recombination between sequences from the *T66ABC* region and the *T66D* region of the wild-type chromosome. Similar partial proximal *t*-haplotypes of both groups have been previously described (HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987).

Partial proximal *t*-haplotypes derived from the complete t^{w73} or t^{w121} haplotypes: These chromosomes are members of the same complementation group, and are unique in that they carry a lethal gene in the extreme proximal end of the *t*-complex. We have collected three new exceptional proximal haplotypes from maintaining this chromosome in the balanced lethal configuration (*e.g.*, T tf/t^{w73}+ × *T tf*/t^{w73}+). Two of the exceptional recombinants, t^{w121a} and t^{w73f}, arose as normal tailed nontufted animals, which when analyzed proved to be t^{w121} +/$t^{viable}tf$ and t^{w73} +/$t^{viable}tf$. Homozygotes for the recombinant chromosome were derived from these exceptions. The third exception arose as a tailless tufted mouse (*T tf*/$t^{w73}tf$), and the recombinant *t tf* chromosome was still lethal in homozygotes.

The homozygous viable recombinant $t^{w121a}tf$ contains *t*-specific restriction fragments of T119 and Au3, however T66A associated fragments are not present. Thus the breakpoint in *t*-chromatin is between the *Au3*^{a} locus and *T66A*, and in the wild-type chromosome, between the *Au3I* locus and *T66E* (Figure 3). The $t^{w73f}tf$ viable recombinant includes the *T66B* locus and recombines at a similar place with the wild-type chromosome. Significantly, the $t^{w73}tf$ recombinant re-

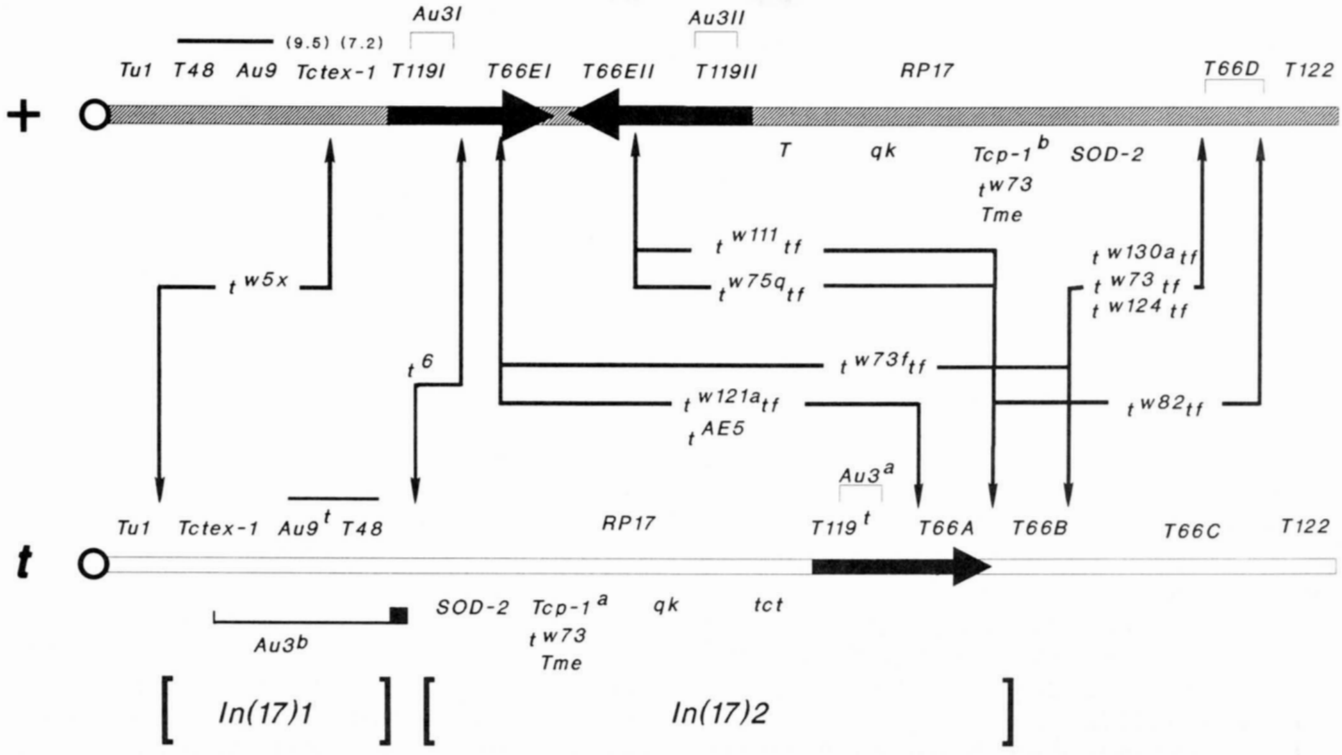

FIGURE 3.—Analysis of recombination breakpoints in new partial *t*-haplotypes. Maps of the proximal region of wild-type and *t*-haplotype forms of chromosome *17* have been aligned, and relevant gene loci and molecular markers indicated. The location of the *Au3* and *T66D* loci is indicated with brackets. The numbers above the *Tctex-1* locus in the wild-type chromosome (9.5 and 7.2), represent the sizes of restriction fragments, and their relative map order within the *Tctex-1* region (see text). The large brackets below the *t*-chromosome represents inverted regions in *t*-haplotypes; *In(17)1* [centromeric inversion], *In(17)2* [proximal inversion]. The arrow connecting the chromosomes indicate the breakpoints of the recombinant chromosomes analyzed in this study.

tains *t*-chromatin through the *T66B* locus and recombined proximal to the *T66D* locus in wild type. This chromosome retains the t^{w73} lethal phenotype.

Previous experiments showed that the wild-type allele of *tcl-*t^{w73} maps within the T^{Hp} deletion, but outside the T^{Orl} deletion (BABIARZ 1983). This places it between *qk* and *T66D*. Genetic analysis of the partial distal t^{wLub2} *t*-haplotype gives a more precise location of the wild type *tcl-*t^{w73} locus. The exceptional crossover event that generated it resulted in a deletion including the *T*-associated maternal effect locus (*Tme*) and *Tcp-1* (WINKING and SILVER 1984). The t^{wLub2} deletion also includes the wild-type *tcl-*t^{w73} locus, because t^{wLub2}/t^{w73} heterozygotes are inviable (SARVETNICK *et al.* 1986). This maps the wild-type *tcl-*t^{w73} locus near *Tcp-1*, *SOD-2* and *Tme* in wild-type chromosomes, and by association near *Tcp-I*, and *SOD-2* in *t*-haplotypes. Since the homozygous viable recombinants derived from the complete t^{w73} haplotype are duplicated for the region surrounding *Tcp-1* and *SOD-2* (Figure 3), they appear to retain the t^{w73} lethal gene, but have also acquired the wild-type allele as a duplication.

An exceptional distal haplotype, t^{w5x}: The final recombinant analyzed, t^{w5x}, arose in our congenic C3H.t^{w5} stock as a phenotypically silent exception, because the recombination event occurred proximal to the *Brachyury* (*T*) and *tail interaction* (*tct*) loci. It is thus formally analogous to the t^6-haplotype, which is an extremely long distal partial haplotype (Figure 3). This recombinant chromosome must have been segregating in the stock, since it was detected serendipitously in DNAs banked at about the same time from three mice when they were analyzed with proximal region probes. The importance of this recombinant was understood only after it was extinct. The three DNA samples available were from two compound heterozygotes, C3H.t^{12}/t^{w5x}, and a C3H.*T tf*/t^{w5x} heterozygote.

Molecular analysis of the t^{w5x} recombinant with proximal probes detected *t*-specific restriction fragments for each probe except Tu1. In addition, wild type fragments were also detected with probes Tu1, Au9, Tu48 and Tctex-1, providing evidence for a recombinational event involving the duplication and deletion of loci in the formation of the t^{w5x} recombinant chromosome. The results of Southern blot analysis of the t^{w5x} containing DNAs with probes Tu1 and Tctex-1 are shown in Figure 4.

The Tu1 probe hybridizes with two *Taq*I fragments (5.5 kb and 5.7 kb) in C3H wild-type DNA, and with four *t*-specific fragments (3.7, 4.3, 4.7 and 6.1 kb) as shown in the compound heterozygote C3H.t^{w5}/t^{w12}*tf* DNA (Figure 4A). The *t*-specific fragments were found to be polymorphic between the t^{w5} and t^{12} or

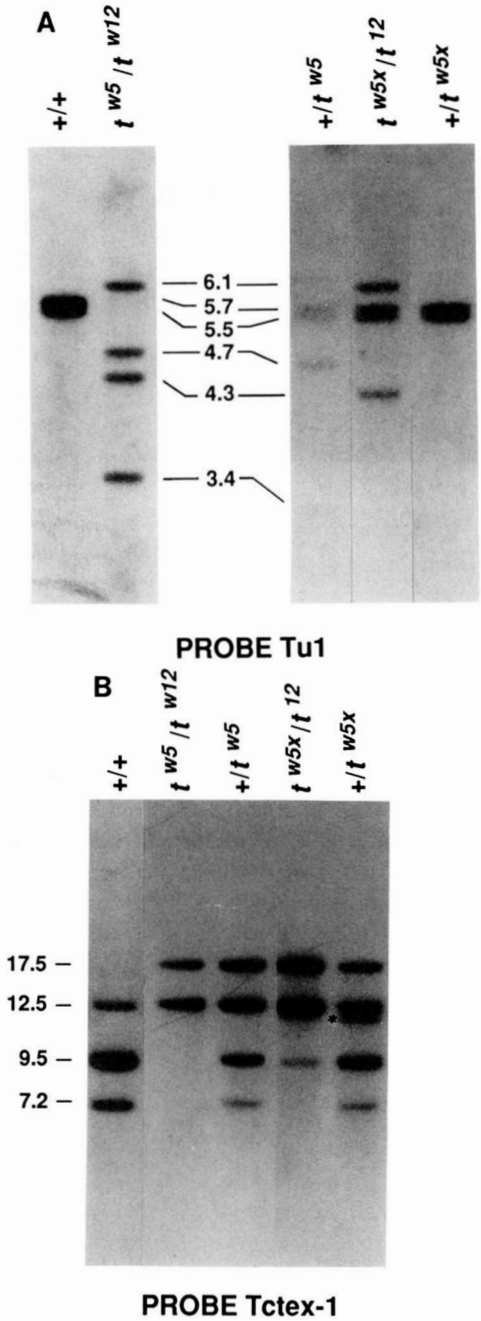

FIGURE 4.—Analysis of the t^{w5x} recombinant. Genomic DNAs were digested with the appropriate restriction enzyme, electrophoresed on 0.7% agarose gels, Southern blotted and probed with ^{32}P-labeled probes. The DNAs analyzed are shown at the top of each lane. The wild type DNA from C3H/DiSn was abbreviated (+). The t^{w5} and t^{w5x} containing DNAs are from contemporary animals, further supporting our finding that the t^{w5x} recombinant was segregating in our t^{w5} stock. (A) *Taq*I digested DNA probed with Tu1. (B) *Eco*RI-digested DNA probed with Tctex-1. The asterisk indicates an additional 12.4-kb restriction fragment that is seen in t^{w5x} DNA, and has also been observed occasionally in other *t*-DNAs.

t^{w12} haplotypes, with t^{12} and t^{w12} being identical. The C3H.t^{w5} lane contains the two expected wild-type fragments, but only the 3.4-kb and 4.7-kb *t*-specific fragments. Thus, these two fragments are specifically associated with t^{w5}, while the t^{w12} (or t^{12})-specific fragments are the additional 4.7-kb and 6.1-kb bands observed in the compound heterozygote C3H.t^{w5}/$t^{w12}tf$. The existence of this *t*-specific polymorphism allowed us to specifically identify the t^{w5x} recombinant chromosome. In the C3H.t^{w5x} lane, only the two wild-type fragments were detected. The t^{w5}-associated fragments had been deleted. Furthermore, in the C3H.t^{w5x}/t^{12} lane, the only *t*-specific fragments were those typical of t^{12}. The wild-type fragments in this lane must be coming from the t^{w5x} recombinant chromosome.

Southern blot analysis of C3H.t^{w5x} DNA with the Tctex-1 probe detected the expected 7.2-kb and 9.5-kb wild-type fragments, as well as the 12.5-kb and 17.5-kb *t*-associated fragments (Figure 4B). The C3H.t^{w5x}/t^{12} DNA contains the two *t*-sssociated bands, but unexpectedly contains one of the two wild-type specific fragments detected by this probe. The t^{w5x} recombinant chromosome therefore is duplicated for the *Tctex-1* locus. Similar results were obtained with the probes Au9 and Tu48 (data not shown).

The crossover event that generated the t^{w5x} recombinant may have occurred between the homologous *Tctex-1* loci in wild-type and *t*-chromosomes (Figure 3). The *Tctex-1* gene is known to be present in at least four copies in both wild-type and *t*-chromosomes (LADER *et al.* 1989). The t^{w5x} recombinant chromosome, having duplicated and deleted loci, provides good evidence that the *Tctex-1*, *T48* and *Au9* loci are associated with the genetically defined centromeric inversion, with the *Tctex-1* locus being proximal to *T48* and *Au9* in *t*-haplotypes. More importantly, this recombinational event provides evidence that the *Tu1* locus is outside of the centromeric inversion, and thus proximal to the beginning of the *t*-complex.

DISCUSSION

We have performed a molecular and genetic analysis of the proximal region of the mouse *t*-complex with two new and several previously described DNA probes, and several new partial proximal *t*-haplotypes that arose in our laboratory. The existence of partial *t*-haplotypes, derived from rare recombinational events between *t*-haplotypes and wild-type chromosomes, have allowed investigators to determine the relative order of a large number of DNA markers on both *t*-type and wild-type chromosomes. In addition to providing valuable information concerning chromosome organization, these recombinants are important to our understanding of the diverse properties associated with *t*-haplotypes, and the location of genes controlling these processes within the *t*-complex.

Genetic mapping of molecular probes Au3 and Au9: The new DNA probes Au3 and Au9, isolated and characterized in this study, have been valuable in our analysis of the proximal region of *t*-haplotypes. The low copy probe Au3 was found to map to two locations in both *t*-haplotypes and wild-type chromo-

somes. In wild-type chromosomes, the Au3 probe detects two loci (*Au3I* and *Au3II*, Figure 3) that map within an inverted duplication defined by the *T119* and *T66E* loci (HERRMANN, BARLOW and LEHRACH 1987). This region has been found to be extremely dynamic in organization as a result of intrachromosomal recombination occurring within the region of this duplication (this study; HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987; NADEAU, VARNUM and BURKART 1989). Furthermore, this region has also been shown to be involved in rare recombinational events with complete *t*-haplotypes, which generated many of the partial proximal *t*-haplotypes characterized to date (this study; FOX *et al.* 1985; HERRMANN *et al.* 1986).

In *t*-haplotypes, the Au3 probe detects two loci ($Au3^a$ and $Au3^b$) mapping to two different regions (Figure 3). The $Au3^a$ locus appears to map between the $T119^t$ and *T66A* loci. This region is homologous to the *T66EII-T119II* region found in the wild-type chromosome (HERMANN, BARLOW and LEHRACH 1987), and by this association, the detection of the $Au3^a$ locus in this region of *t*-haplotypes was an expected result. However, this probe also detected a locus ($Au3^b$) that maps proximal to the *SOD-2* locus, probably near the proximal *t*-chromatin breakpoint in the long partial distal t^6-haplotype (Figure 3). The existence of two *Au3* loci in *t*-haplotypes brings up an interesting speculation concerning the structure of *t*-haplotypes. In *t*-haplotypes we find only one of the two homologous *T119-T66* regions found in wild-type chromosomes. The $Au3^b$ locus may be a remnant of a second such region, most of which might have since been deleted.

The unique sequence *Au9* was found to map proximal to the *Tctex-1* gene family in wild-type chromosomes, probably in the region of the *T48* locus (Figure 2C). The relative order of the *T48* and the *Au9* loci is not known. The mapping of the *Au9* locus in *t*-haplotypes to a location also in the region of the *T48* locus provides a new marker within the centromeric inversion that has been genetically defined by HAMMER, SCHIMENTI and SILVER (1989).

Recombination in the proximal region of the *t*-complex: Our analyses of several partial proximal *t*-haplotypes have shown that the rare recombinational events occurring between *t*-haplotypes and wild-type chromosomes are facilitated by the homologous and repetitive *T66* elements (FOX *et al.* 1985; HERRMANN *et al.* 1986) and the *Tctex-1* gene family (LADER *et al.* 1989). Eight of the nine recombinant chromosomes examined, derived from five different haplotypes, had their breakpoints within the T66ABC region of *t*-haplotypes and recombined with the wild-type chromosome at either the *T66D* or *T66E* loci (Figure 3). These results were similar to those previously reported. Recombinational events involving the *T66E* loci in wild-type chromosomes, and loci within the proximal inversion of *t*-haplotypes, resulted in duplication of the region from *T119* through the *SOD-2* locus, while recombinational events involving the *T66D* locus on wild-type chromosomes, and loci outside of the proximal inversion in *t*-haplotypes had no detectable duplications or deletions. The final recombinant, t^{w5x}, appears to have resulted from pairing between wild-type and *t*-copies of the *Tctex-1* gene family.

The t^{w73} haplotype and its derivatives: Proximal recombinant chromosomes derived from recombination between *T66* loci are generally viable since they have lost their lethal gene, which in all cases, except t^{w73}, maps to the distal inversion. Two classes of recombinants were derived from the complete t^{w73} haplotype, and were analyzed in this study. The first class, represented by the $t^{w73}tf$ recombinant, was shown to have retained lethality (Figure 3). The second class of recombinants, represented by $t^{w121a}tf$ and $t^{w73f}tf$, are viable as homozygotes, which suggests that they have gained a copy of the wild type locus. Both of these were found to contain duplications of the *T119* through *SOD-2* interval (Figure 3). Previous work by BABIARZ (1983), mapped the *tcl*-t^{w73} locus near the *Tcp-1* and *Tme* loci in wild-type chromosomes, and by analogy near *Tcp-1* in *t*-haplotypes. Consequently, it appears that these homozygous viable recombinants have retained the t^{w73} lethal gene, but in addition have acquired the wild-type allele.

The $t^{w121a}tf$ and $t^{w73f}tf$ homozygotes have four copies of the t^{w73} locus, two wild type and two mutant. Additionally, these animals carry four copies of all other genes within the *T119* through *SOD-2* region (Figure 3). Nevertheless, these animals are phenotypically normal in all respects.

In general, the duplication of the proximal region of chromosome *17* does not cause abnormalities of development. This was shown by the recovery of viable and fertile partial trisomics for the region proximal to H-2 (FOREJT, ČAPKOVÁ and GREGOROVÁ 1980). In contrast, trisomics for complete chromosome *17*s die quite early in development, at approximately 10–12 days after fertilization (GROPP, KOLBUS and GIERS 1975). Our findings, in addition to these previous studies, suggest that an overabundance of the gene products encoded in this region does not have detrimental effects upon development or that mechanisms exist that compensate for the effects of increased gene dosage. Furthermore, crosses to produce $t^{w73f}tf/t^{w73}$ animals demonstrate that development is normal in the presence of two lethal and one wild-type t^{w73} gene (K. ARTZT, unpublished results). Thus, it can be concluded that the t^{w73} lethal causes embryonic death as a result of a loss of or a defective gene product. Similar results have been observed for the embryonic lethals, t^6 and t^{12}. When triploid em-

bryos possessed a wild-type allele and two mutant alleles ($+/t^6/t^6$ or $+/t^{12}/t^{12}$), development and viability is indistinguishable from that of wild type (McGrath and Hillman 1982a,b).

The t^{w5x} partial *t*-haplotype: The recombinational event generating the t^{w5x} partial *t*-haplotype represents a recombinant chromosome not previously described. The *Tctex-1* gene family, present in at least four copies on both wild type chromosomes and on *t*-haplotypes may have facilitated this rare recombinational event in the centromeric region of the *t*-complex (Figure 3). The t^{w5x} distal haplotype has a relatively small region of wild-type chromatin at the centromeric end. This end of the chromosome is characterized by the wild type *Tu1*, *T48*, *Au9* and *Tctex-1* loci. The breakpoint in the wild type chromosome occurred within the *Tctex-1* gene family (Figure 3). The evidence for this is the presence of the 9.5-kb wild-type-specific restriction fragment, but the absence of the other (7.2 kb) wild-type-specific fragment normally associated with *Tctex-1* (Figure 4). These data also map the smaller restriction fragment distal to the 9.5-kb fragment within the *Tctex-1* gene cluster on wild-type chromosomes.

The breakpoint in the complete t^{w5} haplotype was between the *Tu1* locus and the *Tctex-1*, *T48* and *Au9* loci (Figure 3). The duplication of the *Tctex-1*, *T48* and *Au9* loci in the t^{w5x} recombinant suggests that these loci are in reverse orientation as compared to wild type. The order of these loci in the wild-type chromosome is *Tu1*, [*T48* and *Au9*] (order not known), and *Tctex-1*. The data presented here provide good evidence that the order of these loci in *t*-haplotypes is *Tu1*, *Tctex-1*, [*Au9* and *T48*]. Furthermore, the t^{w5x} recombinant defines the location of the *Tu1* locus in *t*-haplotypes to be proximal to the centromeric inversion containing the *Tctex-1*, *Au9* and *T48* loci. As already seen, recombinational events involving loci in inverted regions in *t*-haplotypes, results in the duplication and deletion of markers.

Analysis of the t^6 partial *t*-haplotype: The first molecular analysis showing that the centromeric end of t^6 was different from that of other "complete" *t*-haplotypes was done by Silver *et al.* (1983). They showed with 2-D gel analysis of testicular cell lysates that the protein products of genes mapping to this region showed *t*-specific polymorphisms. The t^6 haplotype and its derivatives all had the wild-type forms of these proteins, suggesting that its centromeric end was derived from a wild-type chromosome. The isolation and subsequent mapping of the DNA marker, Tu48, to the centromeric region of *t*- and wild-type chromosomes demonstrated that t^6 carried the wild-type locus, while all other *t*-haplotypes carried the *t*-specific form. Our results with probes Au9 and Tu1 have confirmed the work of others that the t^6 haplotype is actually a long distal haplotype, with its centromeric end marked by the wild-type loci of *Tu1*, *Au9* and *T48*.

Herrmann, Barlow and Lehrach (1987) have suggested that t^6 arose as either a random breakage-reunion event or as a recombinational event between a wild type chromosome having its breakpoint in the *T119I-T66EI* region, and a *t*-haplotype having its breakpoint between *T48* and *Tcp-1*. Our studies demonstrated the existence of two *Au3* loci in wild-type chromosomes, mapping near to the duplicated *T119* loci. In our wild-type control DNA we found the order of these loci to be *T119I-Au3I-T66EI-T66EII-Au3II-T119II*, with the distal duplication being deleted in DNA from T^{Hp} mice (Figure 2C). It has been shown that t^6 and its derivatives contain the wild-type *T119I* locus plus only a small amount of wild-type DNA distal to *T119I*, before the start of *t*-chromatin (Herrmann, Barlow and Lehrach 1987). Therefore, we expected to find the *Au3I* locus present but unexpectedly we found the *Au3II* locus present instead, and probably contained within the short stretch of DNA distal to *T119I*. This was surprising since this locus was shown to be associated with *T119II* in other wild-type chromosomes analyzed. Previous analyses of the order of molecular markers on wild-type chromosomes revealed the presence of an inverted duplication, marked by the *T119* and *T66E* loci, and also showed that some variation in the order of these loci within the duplication does occur (Herrman, Barlow and Lehrach 1987; Schimenti *et al.* 1987). The order of loci is either *T119I-T66EI-T66EII-T119II* or *T119I-T66EII-T66EI-T119II*. These findings were addressed in a recent study (Nadeau, Varnum and Burkart 1989). In that report an explanation for the variable order of loci found in wild-type chromosomes was proposed, which involves intrachromosomal recombination between loci within the inverted duplication. The results of the molecular mapping of the *Au3* loci in wild-type chromosomes and the difference found in the t^6 partial *t*-haplotype can be explained by this mechanism, and is shown in Figure 5.

The wild-type chromosome, +, is shown with the orientation of loci similar to those in Herrmann, Barlow and Lehrach (1987), with the addition of the two *Au3* loci, in the orientation found in the wild type chromosomes used in this study. A second wild-type chromosome is also shown, +′, in which the orientation of the *Au3* and *T66E* loci within the inverted duplication are reversed, and is similar to the order found in Schimenti *et al.* (1987). These different chromosomes can be explained by an intrachromosomal recombination event occurring between homologous sequences within the duplicated regions. In this example, a crossover has occurred between the *T119* and *Au3* loci in the +-chromosome, and appropriately resolved to form the +′-chromosome now showing the *Au3II* locus in association with the *T119I*

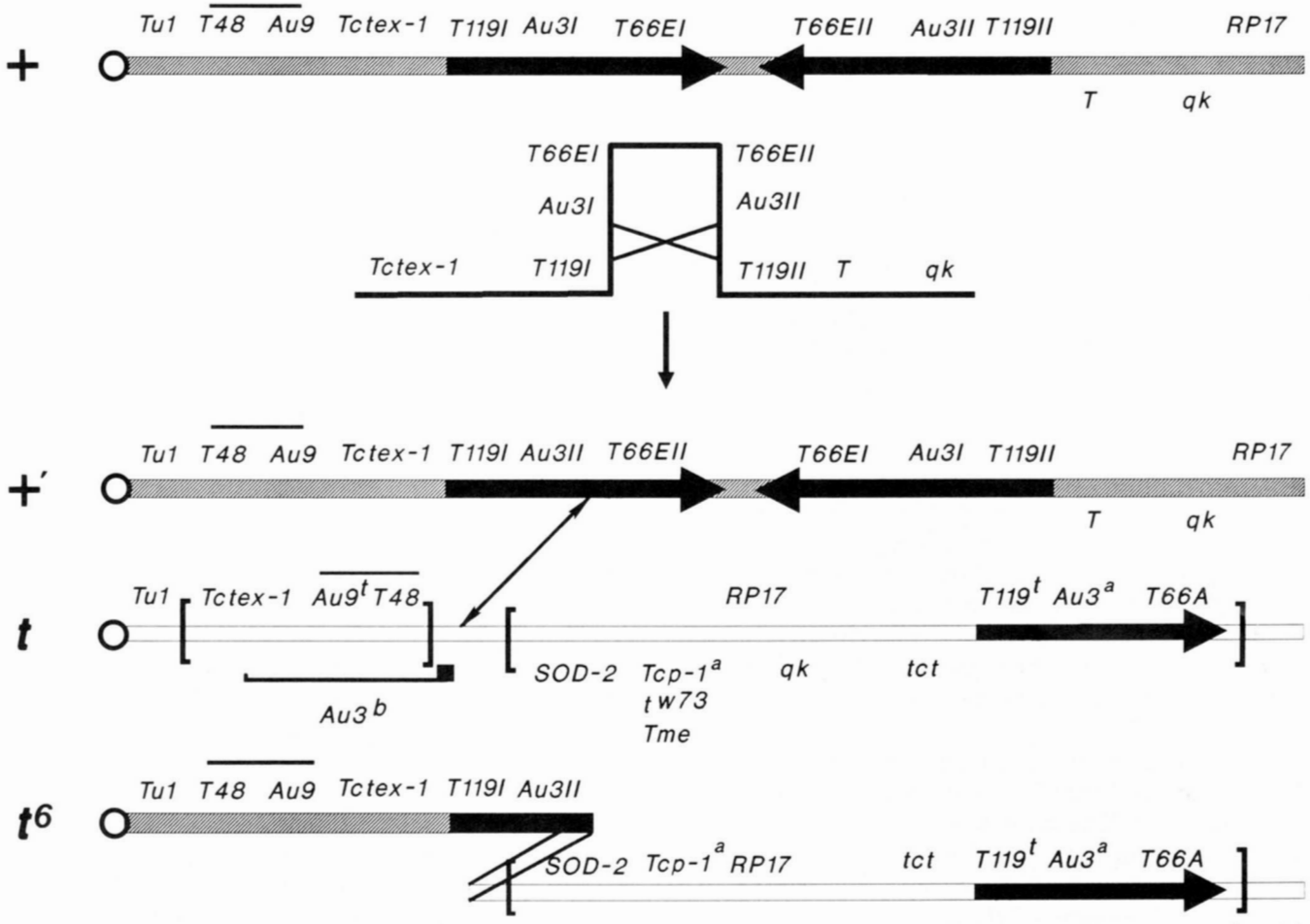

FIGURE 5.—Molecular organization of the t^6 partial *t*-haplotype. Maps of the proximal region of wild type, *t*-haplotype, and of the t^6 partial haplotype have been aligned and relevant gene loci and molecular markers indicated (also see Figure 3 legend for more details). The order of loci in the +-chromosome is converted to that in the +′-chromosome by a process of intrachromosomal recombination (illustrated between them) involving the inverted duplicated regions indicated by solid arrows in wild-type chromosomes. The solid lines between the *T119* and *Au3* loci in the stem loop structure, represents the crossover breakpoints. The arrow connecting the +′-chromosome and *t*-haplotype represents the breakpoints generating the t^6 recombinant chromosome. The bracket on the *t* and t^6 chromosomes indicate *In(17)1* and *2* as in Figure 3.

locus. This must have been the orientation of loci on the wild-type chromosome that was involved in the recombinational event that generated the t^6-haplotype.

The t^6 partial *t*-haplotype appears to have been generated by recombination as the result of pairing of homologous *Au3* sequences located on a +′-type chromosome and a complete *t*-haplotype. The breakpoints in wild-type and *t*-chromatin is shown in Figure 5. The breakpoint in the +′-chromosome is distal to the *Au3II* locus (deleting the *Au3I* locus), and is distal to the $Au3^b$ locus in the *t*-haplotype. Thus, the t^6-haplotype contains the $Au3^a$ locus, but the $Au3^b$ locus has been deleted. This places the $Au3^b$ locus centromeric to *SOD-2*, within the region shown by the bracket in Figure 5, and very likely near the breakpoint in *t*-chromatin generating the t^6 partial *t*-haplotype.

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Note added in proof: The correct designation for $Au3^a$ and $Au3^b$ should be *Au3A* and *Au3B*.

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