Genetic Evidence for Two *t* Complex Tail Interaction (*tct*) Loci in *t* Haplotypes

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ABSTRACT

The *t* complex on chromosome *17* of the house mouse is an exceptional model for studying the genetic control of transmission ratio, gametogenesis, and embryogenesis. Partial haplotypes derived through rare recombination between a *t* haplotype and its wild-type homolog have been essential in the genetic analysis of these various properties of the *t* complex. A new partial *t* haplotype, which was derived from the complete t^{w71} haplotype and which is called t^{w71Jr1}, was shown to have unexpected effects on tail length and unique recombination breakpoints. This haplotype, either when homozygous or when heterozygous with the progenitor t^{w71} haplotype, produced short-tailed rather than normal-tailed mice on certain genetic backgrounds. Genetic analysis of this exceptional haplotype showed that the recombination breakpoints were different from those leading to any other partial *t* haplotype. Based on this haplotype, a model is proposed that accounts for genetic interactions between the brachyury locus (*T*), the *t* complex tail interaction (*tct*) locus, and their wild-type homolog(s) that determine tail length. An important part of this model is the hypothesis that the *tct* locus, which enhances the tail-shortening effect of *T* mutations, is in fact at least two, genetically separable genes with different genetic activities. Genetic analysis of parental and recombinant haplotypes also suggests that intrachromosomal recombination involving an inverted duplicated segment can account for the variable orientation of loci within an inverted duplication on wild-type homologs of the *t* haplotype.

THE *t* complex in the house mouse provides an exceptional model for studying the genetic control of transmission ratio, male fertility and embryogenesis (SILVER 1985). The first known phenotypic property of the *t* complex, however, was its effect on tail length. Dominant mutations at the brachyury locus (*T*) result in a short-tailed phenotype in *T*/+ mice (DOBROVOLSKAIA-ZAVADSKAIA 1927). The *t* complex tail interaction (*tct*) locus is found in many *t* haplotypes and enhances the tail-shortening effect of brachyury (*T*) mutations, so that *T*/*t* mice lack a tail (DOBROVOLSKAIA-ZAVADSKAIA and KOBOZIEFF 1932). The *tct* locus does not, however, shorten tail length in the absence of a *T* mutation, so that *t*/+ and *t*/*t* mice have a tail of normal length (Table 1). *T* and *tct* are believed to be allelic (JUSTICE and BODE 1988). The reason why *tct* enhances the tail-shortening effect of *T* mutations, while not having any effect on tail length by itself, is not understood. None of the more than 75 genes that affect tail and appendages in the mouse are so complicated in their effects (GREEN 1981).

Partial *t* haplotypes resulting from rare recombination between the *t* complex and its wild-type homolog have been essential in mapping and characterizing genes responsible for the various properties of the *t* complex (LYON and MEREDITH 1964a; ALTON, SILVER and ARTZT 1980; STRYNA and KLEIN 1981; LYON 1984, 1986; FOX *et al.* 1985; HERRMANN *et al.* 1986). Some partial *t* haplotypes retain *tct*, and thus enhance the tail-shortening effect of *T* mutations, while others lose *tct* (LYON and MEREDITH 1964a; FOX *et al.*, 1985). Certain partial *t* haplotypes, modify this interaction between *T* and *tct* in unusual ways and illustrate additional complexity in the manner in which *T* and *tct* influence tail length (Table 1). One of these exceptional haplotypes, t^{h7}, suppresses rather than enhances the tail-shortening effect of *T*, so that T/t^{h7} mice have a tail of normal length rather than a short or missing tail (LYON and MEREDITH 1964b). Two other partial *t* haplotypes, t^{wLub2} (WINKING and SILVER 1984) and t^{Tu3} (STYRNA and KLEIN 1981), also produce mice with a tail of normal length in combination with *T*. Another *t* haplotype, t^{AE5}, is exceptional because homozygotes have a short tail rather than a normal tail (VOJTISKOVA *et al.* 1976). t^{AE5} retains its expected interactions with *T* and +, however, because T/t^{AE5} mice are tailless and t^{AE5}/+ mice have a tail of normal length (VOJTISKOVA *et al.* 1976). These novel interactions between *T* mutations, the *tct* locus and their wild-type homolog(s) provide an interesting model for studying the genetic control of the seemingly simple phenotype of tail length.

We recently discovered a partial *t* haplotype that

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TABLE 1

Effect of *T* mutations, *tct* loci, and wild-type homolog(s) on tail length

Haplotype	*T*	+	t^y	t^*	Reference
+	ST	NT	NT	—	Standard
T	D	ST	OT	—	Standard
t^{w5}	OT	NT	NT	D	Standard
t^{h45}	OT	NT	NT	NT	LYON and MEREDITH (1964a)
t^{h7}	NT	NT	NT	D	LYON and MEREDITH (1964b)
t^{wLub2}	NT	NT	(NT)	D	WINKING and SILVER (1984)
t^{Tu3}	NT	(NT)	(NT)	(NT)	STYRNA and KLEIN (1981)
t^{AE5}	OT	(NT)	ST/NT	ST/NT	VOJTISKOVA *et al.* (1976)
t^{w71Jr1}	OT	NT	(ST)/NT	ST/NT	Present study

If empirical data have not been published, the predicted tail length is given in parentheses. The following abbreviations are used: OT for mice lacking a tail, ST for mice with a short tail, NT for mice with a normal tail, and D for mice that die as embryos. t^y refers to a complementing *t* haplotype and t^* to the same haplotype, *e.g.*, t^{AE5}/t^y refers to the genotype of mice heterozygous for the t^{AE5} haplotype and any other complementing *t* haplotype, whereas t^{AE5}/t^* refers to mice homozygous for the t^{AE5} haplotype. "—" indicates not done. t^{h45} is provided as an example of a proximal partial *t* haplotype.

interacts with the progenitor t^{w71} haplotype in novel ways to determine tail length. In this paper, effects of this haplotype on tail length are reported, the recombination breakpoints are mapped, and a model proposed to account for the manner in which *T* mutations and the *tct* locus influence tail length.

MATERIALS AND METHODS

Mice: All mice were obtained from the research and production colonies of The Jackson Laboratory.

GLO-1 electrophoretic assay: Methods described by NADEAU (1986) were used.

DNA probes: Probes for the *D17Leh48, D17Leh54, D17Leh66* and *D17Leh119* loci, which were provided by HANS LEHRACH, are microclones obtained by microdissection of the proximal portion of mouse chromosome *17* (ROHME *et al.* 1984). Mapping and restriction fragment analysis of these clones has been described (ROHME *et al.* 1984; FOX *et al.* 1985; HERRMANN *et al.* 1986; HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987). The p66M-RT probe, which was obtained from BERNHARD HERRMANN, is a subclone of the *D17Leh66* locus (HERRMANN *et al.* 1986; HERRMANN, BARLOW and LEHRACH 1987). The probes Bb-40, Bb-59 and Ca-45, which were obtained from LEE SILVER, are subclones of *D17Leh66* loci (SCHIMENTI *et al.* 1987).

Southern blotting, probe labeling and hybridization: These methods have been described previously (NADEAU and PHILLIPS 1987).

RESULTS

Origin of the t^{w71Jr1} haplotype: Because *T*/*T* and *t*/*t* homozygotes die during embryonic development, intercrosses between *T*/*t* mice usually produce tailless, progeny only. Occasionally exceptional progeny that have a tail of normal length are produced. These exceptional progeny, which are found in 1/500–1/1000 mice, result from recombination between the *t* haplotype and its wild-type homolog.

One intercross in our C3H-*T* tf/t^{w71} + colony produced an exceptional number of progeny with a tail of unexpected length. (The tufted mutation (*tf*) is included as a visible marker in most *T*/*t* balanced lethal stocks.) The intercross of female 125 x male 126 produced 10 progeny that lacked a tail and 9 progeny that had a short tail. Subsequent genetic crosses and molecular analysis demonstrated that these exceptional short-tailed mice were heterozygous for the complete t^{w71} haplotype and a partial *t* haplotype derived from t^{w71} through recombination (see below). The formal name for this partial haplotype is t^{w71Jr1} and is abbreviated t' for brevity in this paper. (The "Jr" in the haplotype symbol indicates "*J*ackson *r*ecombinant" and the "1" indicates that this is the first partial t^{w71} haplotype at The Jackson Laboratory.)

Inheritance of the short-tailed phenotype: Control crosses showed that the t^{w71} progenitor haplotype in our colony showed transmission ratio distortion, homozygous lethality and interaction with *T* mutations, as expected for a complete *t* haplotype (Table 2). Additional control crosses showed that t' retained its expected interactions with *T* mutations and its wild-type homolog because *T*/t' mice lacked a tail and t'/+ mice had a tail of normal length (Table 2).

Anomalous short-tailed mice were observed in six of the seven different experimental crosses that were used to study inheritance of the short-tailed phenotype (Table 2). In each of these six crosses, the genotype of mice with a short tail could be t'/t^{w71} or t'/t'. One of the more important crosses was the T/t^{w71} × *T*/t' cross that produced 10 short-tailed progeny, but failed to produce any of the expected normal-tailed progeny. The most likely genotype of these exceptional progeny is t'/t^{w71}, because *T*/*T* segregants are embryonic lethals and *T*/t' and T/t^{w71} segregants are tailless (Table 2). Another cross, $+/t^{w71}$ × *T*/t', produced too few normal-tailed progeny and too many short-tailed progeny. Again the most likely explanation is that t'/t^{w71} mice have a short tail rather than a

TABLE 2

Segregation of tail length in various crosses

Cross[a]	Expected Nos.[b] NT:ST:OT	Observed Nos. NT:ST:OT	Comment
Control crosses			
$t^{w71}/+\times T/t^{w71}$	Majority NT	22:3:1	Expected transmission distortion
$t^{w71}/+\times t^{w71}/+$	Majority NT	20:0:0	No short-tailed mice
$T/+\times T/t'$	1:1:1	28:13:12	Expected *T* and + interactions
$T/+\times t'/+$	2:1:1	90:21:29	Expected *T* and + interactions
Experimental crosses			
$T/t^{w71}\times T/t'$	1:0:2	0:10:9	Short-tailed exceptions and no normal-tailed mice
$+/t^{w71}\times T/t'$	2:1:1	13:17:12	Excess short-tailed mice and too few normal-tailed mice
$t'/+\times t^{w71}/+$	1:0:0	9:4:0	Short-tailed exceptions
$t^{w71}/+\times t'/+$	1:0:0	7:1:0	Short-tailed exception
$t'/t^{w71}\times T/+$	1:0:1	22:0:12	No short-tailed mice
$T/t'\times T/t'$	0:1:2	0:8:10	Short-tailed exceptions and no normal-tailed mice
$t'/+\times t'/+$	3:1:0	7:2:0	Short-tailed exceptions

[a] By convention, maternal genotype precedes paternal genotype.
[b] Expected numbers are based on the hypothesis that t'/t' mice, like t/t mice, should have a tail of normal length, and on the assumption that t' does not distort transmission. All crosses involved C3H congenic mice. NT is used as an abbreviation for mice with a tail of normal length, ST for mice with a short tail, and OT for mice that lack a tail.

tail of normal length. The only experimental cross that did not produce short-tailed progeny, the $t'/t^{w71} \times T/+$ cross, could not produce t'/t^{w71} or t'/t' progeny. Together, these results suggest that the anomalous short-tailed phenotype occurred only in t'/t^{w71} and t'/t', mice and not in other segregants of these experimental crosses.

The previous analyses suggested that t'/t' homozygotes are viable and have a short tail. Because most complete and many partial *t* haplotypes cause embryonic lethality when homozygous (LYON and MEREDITH 1964a; BUCAN *et al.* 1987), it is essential to directly test viability of t'/t' homozygotes. For this test, (SWR/J[*Glo-1*^{b}] × C3H-*T tf Glo-1*$^a/t'$+ *Glo-1*^{a})F_1 mice that had a tail of normal length and thus were assumed to be $t'/+$ heterozygotes were intercrossed. (Sibs with a short tail were assumed to be $T/+$ heterozygotes.) The glyoxalase-1 locus, *Glo-1*, is located within the *t* complex, encodes readily detectable isozyme variants (NADEAU 1986) and was used to identity the genotype of progeny of this cross. Seventy-seven progeny from nine litters of three intercross matings were typed for GLO-1 (Figure 1). Twenty-three GLO-1A homozygotes, 40 GLO-1AB heterozygotes, and 15 GLO-1B homozygotes were observed. The inferred genotype of these three GLO-1 phenotopic classes was t'/t', $t'/+$ and $+/+$, respectively. The occurrence of GLO-1A homozygotes in the expected frequency ($\chi^2 = 1.68$, $P > 0.05$) demonstrated that t'/t' homozygotes are fully viable and that the t' haplotype lost the t^{w71} lethal gene. Unexpectedly, all 23 GLO-1A homozygotes had a tail of normal length indicating that homozygosity for the t' haplotype did not affect tail length on the (C3H × SWR)F_1 genetic background as it did on the C3H background. Thus, genetic background can modify the short-tail phenotype associated with the t' haplotype.

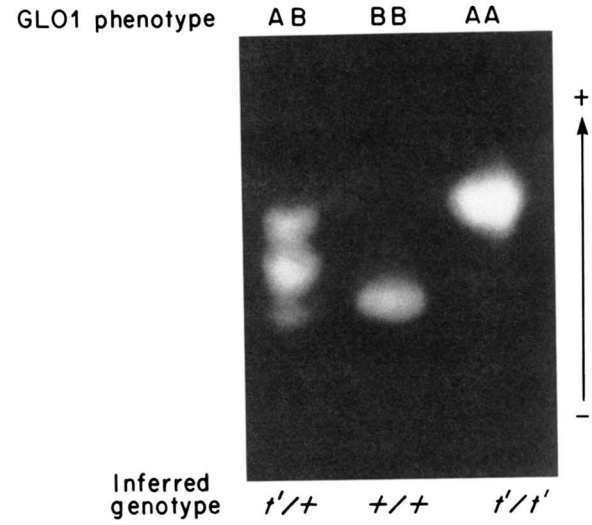

FIGURE 1.—GLO-1 phenotypes demonstrating viability of t'/t' homozygotes.

Mapping of recombination breakpoints that led to the *t'* haplotype: To map the recombination breakpoints, genomic DNA from t'/t' homozygotes, t'/t^{w71} heterozygotes, $t'/+$ heterozygotes, and various control mice was digested with the appropriate restriction endonuclease and analyzed with probes for various regions of the *t* complex (Figure 2). This analysis was based on determining for each locus whether the t' haplotype had restriction fragment variants associated originally with the t^{w71} haplotype or with the *T tf*

haplotype. A listing of these haplotype-specific variants, the probes used to detect them, and the locus to which they map is provided in Table 3. Representative variants are illustrated in Figure 3. In addition, because the published nomenclature for loci and alleles within the *t* complex sometimes does not correspond to accepted rules of mouse gene nomenclature, revised nomenclature is proposed (Table 3).

The *t'* haplotype had restriction fragments characteristic of the *D17Leh48* locus associated with the t^{w71} haplotype and fragments characteristic of the *D17Leh54* locus associated with the *T tf* chromosome in C3H-*T tf*/++ mice (Table 3, Figure 3A). The *t'* haplotype therefore resulted from rare recombination between the t^{w71} haplotype and its wild-type *T tf* homolog with recombination breakpoints located between *D17Leh48* and *D17Leh54* (Figure 2). The proximal portion of the *t'* haplotype was derived from the t^{w71} progenitor haplotype and the distal portion from the wild-type chromosome. The *t'* haplotype is thus one

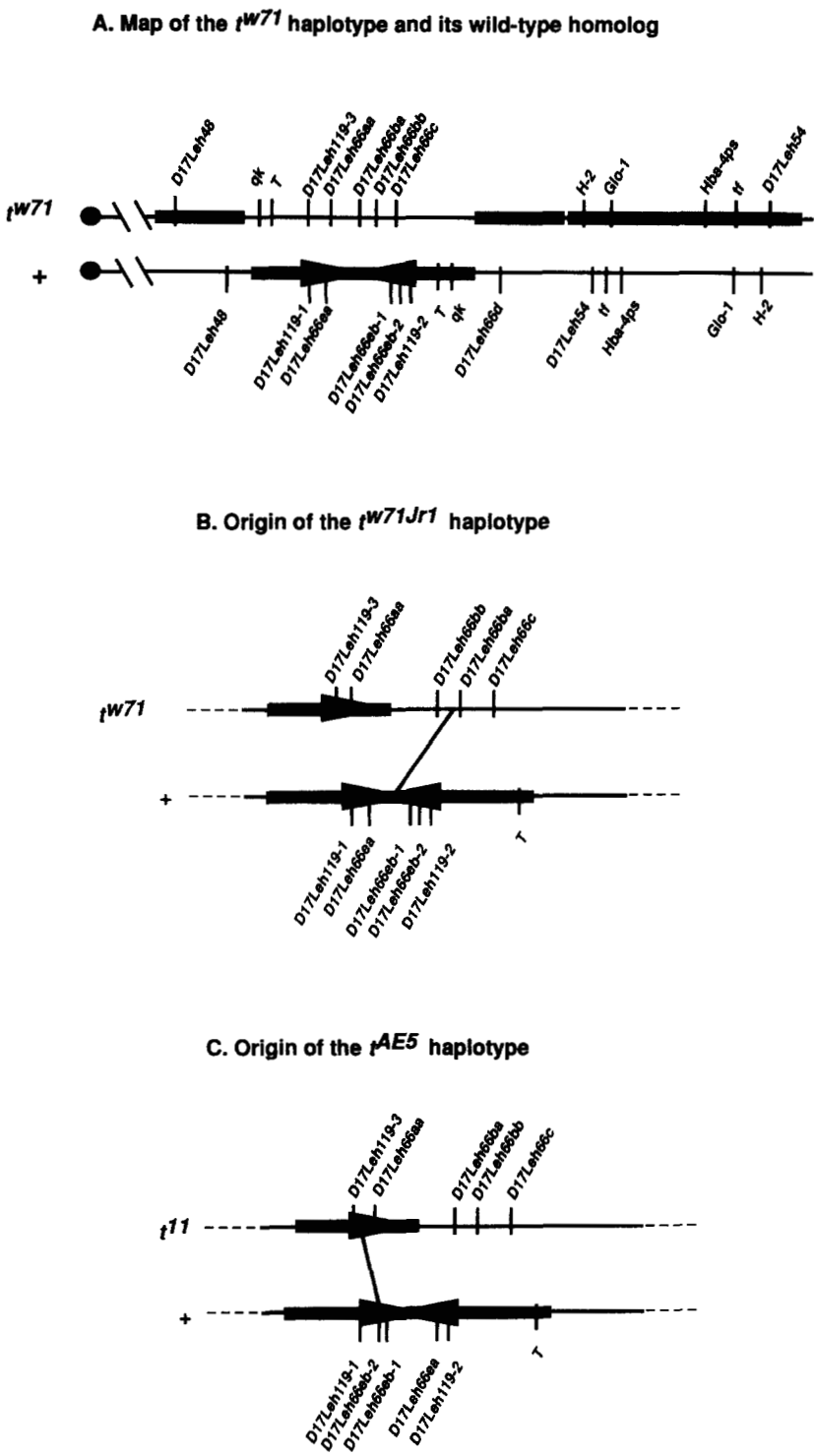

FIGURE 2.—Location of marker loci in the t^{w71} haplotype and its wild-type homolog (+). Although the order of loci shown is consistent with current information, the distances shown are not intended to be accurate. The reader should consult the original literature to obtain information about genetic and physical distances between loci.

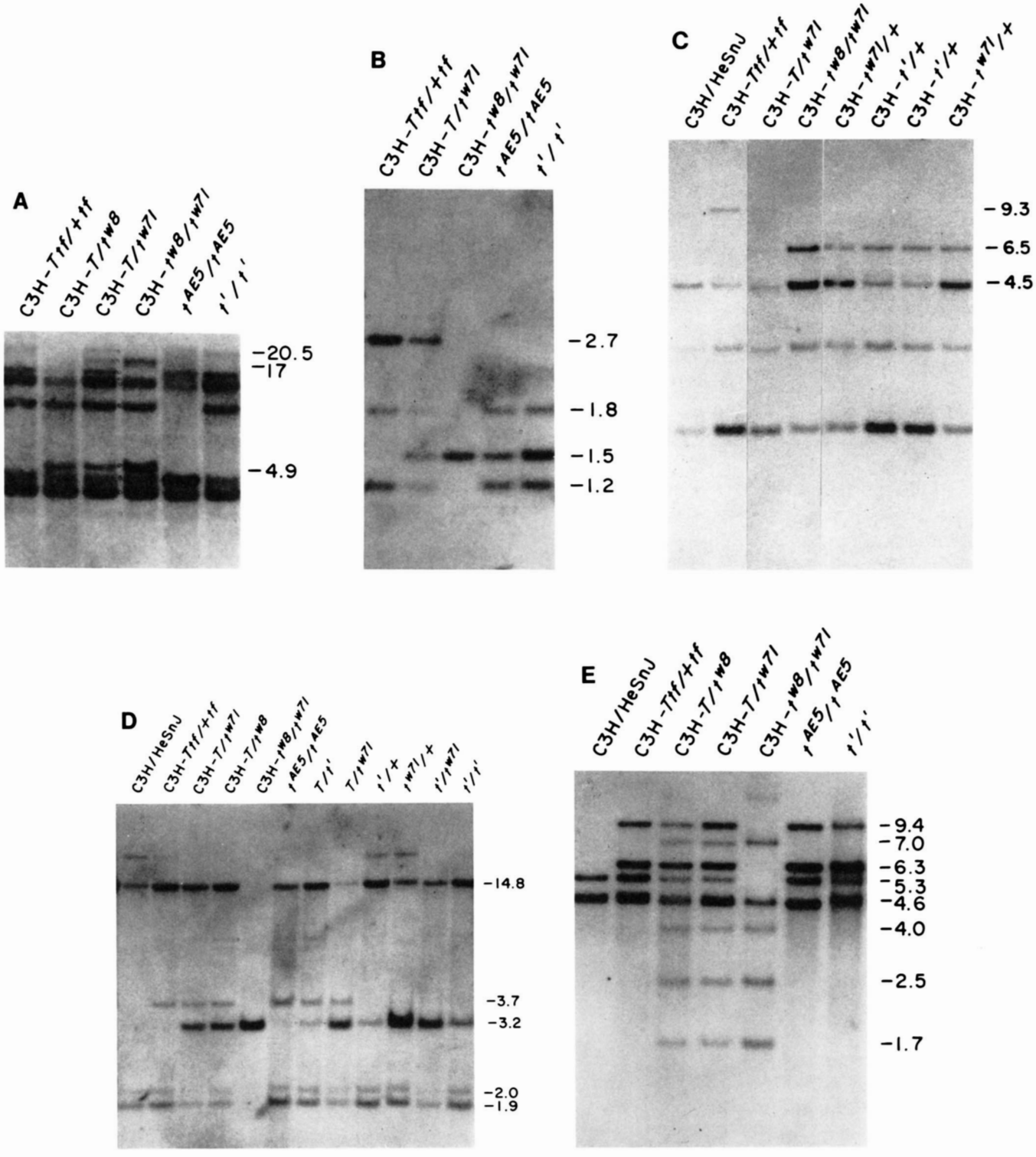

FIGURE 3.— Restriction fragment variation for representative loci used to characterize the *t'* haplotype. A, Locus—*D17Leh54*, probe—T54, enzyme—*Hin*dIII; B, locus—*D17Leh119*, probe—T119, enzyme—*Msp*I; C, locus—*D17Leh66*, probe—T66, enzyme—*Taq*I; D, locus—*D17Leh66*, probe—p66M-RT, enzyme—*Bam*HI; E, locus—*D17Leh66*, probe—Bb-40, *Taq*I.

of many proximal partial *t* haplotypes with breakpoints between these two loci (HERRMANN *et al.* 1986; SCHIMENTI *et al.* 1987).

Probes for *D17Leh119* and *D17Leh66* loci were used to map the recombination breakpoints more precisely. There are two *D17Leh119* loci on wild-type chromosomes; the proximal locus is called *D17Leh119–1*, the distal *D17Leh119–2* (Figure 2). The *t* complex has a single locus, *D17Leh119–3* (Figure 2). The *t'* haplotype not only had restriction fragments characteristic of the *D17Leh119–3* locus associated with the t^{w71} haplotype, but also had one but not both *D17Leh119*

TABLE 3

Diagnostic restriction fragments found in the *t'* haplotype or the *T tf* haplotype in C3H-*T tf*/++ mice and restriction fragments observed in the *t'* and t^{AE5} haplotypes

Probe	Diagnostic fragment		Locus	Presence of fragment	
	t^{w71}	+		*t'*	t^{AE5}
T48	5.8 B		*D17Leh48*	+	+[a]
		6.4 B	*D17Leh48*	−	−[a]
T119	—	2.7 M	*D17Leh119-1*	−	−
	—	1.2, 1.8 M	*D17Leh119-2*	+	+
	1.5 M	—	*D17Leh119-3*	+	+
T66	—	9.3 T	*D17Leh66ea*	−	+
	6.5 T	—	*D17Leh66aa*	+	−
	4.5 T[b]	—	*D17Leh66ba*	−	−[a]
	4.5 T[b]	—	*D17Leh66ca*	−	−[a]
Bb-40	4.0 T	—	*D17Leh66cg-1*	−	−
	1.7 T	—	*D17Leh66cg-3*	−	−
	2.5 T	—	*D17Leh66cg-4*	−	−
	7.0 T	—	*D17Leh66cx*	−	−
	—	4.6 T	*D17Leh66eb-2*	+	−
		5.3 T	*D17Leh66dg-1*	+	+
	—	6.3 T	*D17Leh66db-1*	+	+
	—	9.4 T	*D17Leh66dg-2*	+	+
Bb-59	2.4 B	—	*D17Leh66bb*	+	−
	6.5 B	—	*D17Leh66cb-1*	−	−
	5.2 B	—	*D17Leh66cb-2*	−	−
	—	5.3 B	*D17Leh66eb-2*	+	−
		5.0 B	*D17Leh66db*	+	+
Ca-45	2.4 H	—	*D17Leh66aa*	+	−
	4.3 H	—	*D17Leh66ba*	−	−
p66M-RT	3.2 B[c]	—	*D17Leh66a*	+	−
	3.2 B[c]	—	*D17Leh66ba*	−	−
	—	14.8 B	*D17Leh66d*	+	+
	—	3.7 B	*D17Leh66ea*	−	+
	—	1.9, 2.0 B	*D17Leh66eb-1*	+	+
T54	4.9, 17 H	20.5 H	*D17Leh54*	+	ND

Fragment sizes are given in kilobases (kb). The following abbreviations were used for restriction endonucleases: B, *Bam*HI; H, *Hin*dIII; M, *Msp*I; and T, *Taq*I. Only fragments that distinguish *t* haplotypes and their wild-type homologs are listed; other fragments were invariable. ND indicates not done, + indicates fragment was observed, − indicates fragment was not observed. *D17LehE-alpha* and *D17Leh66E-beta* of SCHIMENTI *et al.* (1987) are equivalent to *D17T66EI* and *D17T66EII* of HERRMANN *et al.* (1986, 1987) and according to the accepted rules of gene nomenclature are here called *D17Leh66ea* and *D17Leh66eb*, respectively.

[a] FOX *et al.* (1985).

[b] The 4.5-kb *Taq*I fragment is found in *D17Leh66ba* and *D17Leh66ca* in *t* haplotypes and in *D17Leh66ea* in wild-type chromosomes. We know that the 4.5-kb fragment in the *t'* haplotype is not *D17Leh66ea* (note T66, 9.3T), that it is not *D17Leh66ca* (note T66, 1.5T), and that it is not *D17Leh66ba* (note p66M-RT, 5.2 B). The 4.5-kb *Taq*I fragment in *t'*/+ mice must be from the wild-type chromosome. Differences in hybridization intensity support this conclusion.

[c] As shown in Figure 3D, the hybridization intensity of the 3.2-kb *Bam*HI fragment is half as intense in t'/t' as in t^{w71}/t', suggesting that the *t'* haplotype has only one 3.2-kb fragment and that a complete *t* haplotype has two. The accumulated evidence suggests that *D17Leh66ba* is proximal to *D17Leh66bb* and is therefore probably the locus retained in the *t'* haplotype.

loci associated with wild-type chromosomes (Table 3, Figure 3B). The recombination breakpoints are therefore located distal to *D17Leh119–3* in the t^{w71} haplotype and between the two *D17Leh119* loci on the wild-type chromosome (Figure 2).

Characterization of *D17Leh66* loci was more complicated both because the large and variable number of loci occurring on each chromosome made homologies difficult to define and because these loci are differently arranged in *t* haplotypes and wild-type chromosomes (HERRMANN *et al.* 1986; HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987, 1988). The T66 probe demonstrated that the *t'* haplotype lacked the *D17Leh66ea* locus associated with wild-type chromosomes, but had the *D17Leh66aa* locus associated with the t^{w71} haplotype (Table 3, Figure 3C). Subclones Ca-45 and p66M-RT (Figure 3D) confirmed these results (Table 3). Subclones Bb-40, Bb-59, Ca-45 and p66M-RT also demonstrated that the *t'* haplotype lost the *D17Leh66bb* and *D17Leh66c* loci from the t^{w71} progenitor haplotype, and gained the *D17Leh66eb* and *D17Leh66d* loci from the wild-type *T tf* chromosome (Table 3, Figure 3E). Recombination breakpoints were therefore located between *D17Leh66ba* and *D17Leh66bb* loci on the t^{w71} haplotype and between *D17Leh66ea* and *D17Leh66eb-1* on the wild-type chromosome (Figure 2). These results also suggest that the order of loci on the wild-type chromosome (C3H-*T tf*) was centromere-*D17Leh66ea*-*D17Leh66eb*, as in HERMMANN *et al.* (1986) and HERRMANN, BARLOW and LEHRACH 1987), rather than the reverse, centromere-*D17Leh66eb*-*D17Leh66ea*, as in SCHIMENTI *et al.* (1987).

Comparison of the *t'* and t^{AE5} partial *t* haplotype: t^{AE5} is the only other partial *t* haplotype that is associated with a short-tailed phenotype. Like the *t'* haplotype, genetic background appears to influence expression of the short-tail phenotype because short-tailed mice are observed on some backgrounds but not on others. Although t^{AE5}/t^{AE5} homozygotes usually have a short tail (VOJTISKOVA *et al.* 1976), some t^{AE5}/t^{x} compound heterozygotes, *e.g.*, t^{AE5}/t^{3}, have a normal tail (L. SILVER, personal communication), while other compound heterozygotes, *e.g.*,t^{AE5}/t^{3}, have a short tail (S. WAELSCH, personal communication). Since these two haplotypes, t^{w5} and t^{3}, are maintained on different genetic backgrounds, it is more likely that variation in tail length results from differences in background genes than in specific interactions between the t^{AE5} haplotype and certain *t* haplotypes. t^{AE5} is also the only other partial *t* haplotype with recombination breakpoints similar to those described above for the *t'* haplotype (HERRMANN *et al.* 1986; HERRMANN, BARLOW and LEHRACH 1987; SCHIMENTI *et al.* 1987).

To compare directly the recombination breakpoints for the *t'* and t^{AE5} haplotypes, genomic DNA from a

t^{AE5}/t^{AE5} homozygote was digested with the appropriate restriction enzymes and analyzed with the probes T66, T119, Bb-40, Bb-59, Ca-45 and p66M-RT. Several important differences between the t' and t^{AE5} haplotypes were detected (Table 3, Figure 2). The breakpoints that produced the haplotype occurred proximal rather than distal to *D17Leh66aa* and between *D17Leh66eb-1* and *D17Leh66eb-2*. Our results for the t^{AE5} haplotype confirm previous typing by HERRMANN *et al.* (1986) and HERMANN, BARLOW and LEHRACH 1987) and show that breakpoints for the t' and t^{AE5} haplotypes are in similar but not identical locations (Figure 2).

DISCUSSION

Two of the most unusual features of the *t* complex are the unique combination of genetic and developmental properties affected by genes within the complex and the unexpected complexity in the manner these genes exert their influence. Because the tail-shortening effect of *T* mutations can be explained simply by postulating that these mutations are amorphs (LYON and MEREDITH 1964a), genetic interactions between *T* mutations and their wild-type alleles do not appear to be complicated. The influence of genes within the *t* complex on these interactions is less readily explained, however, because some *t* haplotypes enhance the tail-shortening effect of *T* mutations, others suppress the tail-shortening effect, and still others shorten tail length in combination with other *t* haplotypes. Partial *t* haplotypes that result from rare recombination between the *t* complex and its wild-type homolog have been essential for inferring the genetic basis for the various phenotypic properties of the *t* complex. Hundreds of partial *t* haplotypes have been at least partially characterized. Most involve recombination within the proximal inversion or between the proximal and distal inversions (LYON and MEREDITH 1964a; FOX *et al.* 1985; ERHART, PHILLIPS and NADEAU 1988). Most partial *t* haplotypes produce normal-tailed mice in *t/t* homozygotes and in t^x/t^y and *t/+* compound heterozygotes. The only exceptional haplotypes are t^{h7} (LYON and MEREDITH (1964b), t^{wLub2} (WINKING and SILVER 1984) and t^{Tu3} (STYRNA and KLEIN 1981) that suppress that tail shortening effects of *T* mutations, and t' (present study) and t^{AE5} (VOJTISKOVA *et al.* 1976) that produce short-tailed mice in homozygotes.

LYON and MEREDITH (1964b) accounted for all interactions between *T*, wild-type and *tct* loci with two postulates. The first was that the wild-type allele provides normal gene activity, that *T* mutations are amorphs, and that *tct* within the *t* complex provides less than normal activity but more than half normal activity. They hypothesized that a duplication of these loci in the t^{h7} haplotype would account for this haplotype's ability to suppress the tail-shortening effect of *T* mutations and its high reversion frequency. The second postulate was that the combined activity of wild-type and *tct* genes in *+/t* mice results in a tail of normal length, that the activity of the *tct* gene associated with the *t* haplotype in *T/t* mice results in absence of a tail, and that the activity of the wild-type allele associated with the wild-type haplotype in *T/+* mice results in a short tail. This model as presented does not account for the short-tailed phenotype associated with the t^{AE5} and t' haplotypes: how can t'/t' mice, which are presumably *tct/tct*, have a short tail when t^x/t^y mice which are also *tct/tct*, have a normal tail.

The t^{AE5} and t' haplotypes can be readily incorporated into the Lyon-Meredith model by postulating first that the "*tct* locus" consists of at least two genetically separable genes, *tct-1* and *tct-2*, and second that these two genes differ considerably in activity. The first postulate is necessary to account for the appearance among progeny of phenotypes not present in either parent. These anomalous phenotypes result either from *cis-trans* effects, or more likely from genetic separation through recombination of two or more loci within *t* haplotypes that interact with *T* mutations. The unique and parallel locations of recombination breakpoints that led to the t' and t^{AE5} haplotypes supports this argument (Figure 2). The second postulate is necessary for the following reason: if the alternative argument is made that these two components have equal activity, *i.e.*, *tct-1* = *tct-2*, then both *T/t* (null/*tct-1*, *tct-2*) and t'/t' or t^{AE5}/t^{AE5} (*tct-1/tct-1*) mice should have indistinguishable phenotypes, assuming that *cis-trans* effects are not involved. Both mice would have two *tct* loci, in *cis* in the complete *t* haplotype in *T/t* mice but in *trans* in t'/t' homozygotes. These mice of course have very different phenotypes; *T/t* mice lack a tail, whereas t'/t' (and t^{AE5}/t^{AE5}) mice have a short tail on certain genetic backgrounds. If the t' and t^{AE5} haplotypes retain the locus that provides greater activity, t'/t' and t^{AE5}/t^{AE5} homozygotes would have greater cumulative activity than *T/t* heterozygotes. The argument that these two loci have unequal activity therefore accounts for the different phenotype in these mice.

Comparison of the $t'$and t^{AE5} haplotypes provides insight into the likely location of the *tct-1* and *tct-2* genes. One should be located proximal to the two recombination breakpoints in the t' and t^{AE5} haplotypes and the other should be located distal to the two breakpoints. One locus is therefore proximal, the other distal to *D17Leh66aa* (Figure 2). The proximal *tct-1* locus must be the locus with greater activity since it would be the locus retained by both the t' and t^{AE5} haplotypes. The t^{h7} haplotype could have a duplication of either or both of these two loci. Because tail length

associated with many of the partial *t* haplotypes resulting from recombination in the proximal portion of the *t* complex has not been rigorously examined, it is difficult to place outside bounds on the likely location of the two *tct* loci. One would predict, for example, that *tct-2* should be located proximal to the recombination breakpoint resulting in the t^{h2} haplotype, since t^{h2}/t^{h2} mice have a normal tail (LYON and MEREDITH 1964a). If, however, tail length in t^{h2}/t^{h2} mice was examined on a genetic background that suppressed the short-tail phenotype, then the test was uninformative. Failure to find the short-tail phenotype in homozygotes for this partial *t* haplotype could result either from retention of both *tct* loci or from genetic background effects.

Two other studies should be considered to determine whether their results are compatible with the model proposed here. JUSTICE and BODE (1986) reported several ENU-induced *T* mutations within the t^{w5} haplotype. These mutations produce a short tail in *T* t^{w5}/++ mice and absence of a tail in *T* t^{w5}/t mice suggesting that these are true brachyury mutations. To reconcile these observations with the model proposed here, these *T* mutations would have to result in loss of activity of the proximal *tct* locus. Similarly, MACMURRAY and SHIN (1988) argue that the T^c mutation is an antimorph because T^c/+ mice are tailless rather than short-tailed. Their argument does not compromise the model presented here since T^c could be a gain-of-function rather than a loss-of-function mutation.

None of the numerous alternative models that we evaluated were satisfactory. For example, one could postulate that the short-tail phenotype results from presence of a *tct* locus and its wild-type homolog on a single chromosome. Although it is true that the *t'* haplotype has acquired part of the inverted duplication found in wild-type chromosomes (Figure 2), this hypothesis does not explain why a *t'*/*t'* homozygote, which according to this argument is presumably *tct*+/*tct*+ has a short tail rather than a tail of normal length, unless genetic background effects compromised the experiments. If this duplication hypothesis is correct, then t^{h45}/t^{h45} homozygotes should have a comparable duplication leading to short-tailed mice on certain genetic backgrounds. Similar difficulties were encountered with all other alternative models that were considered.

Comparison of maps of the proximal portion of chromosome *17* shows that gene order varies within the inverted duplication and suggests that an unusual kind of chromosome rearrangement may be responsible for the variable order. HERRMANN, BARLOW and LEHRACH (1987) and SCHIMENTI *et al.* (1987) showed that the *D17Leh119* and *D17Leh66e* loci mark an inverted duplication on wild-type chromosomes.

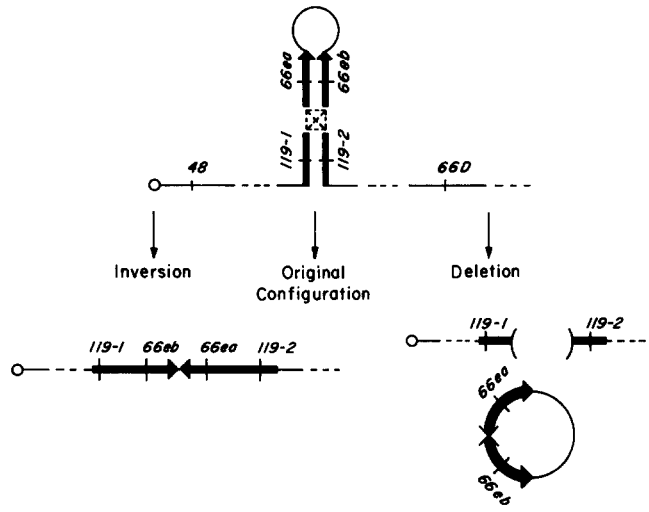

FIGURE 4.—Alternative consequences of recombination within the inverted duplication on the proximal portion of wild-type homologs of chromosome *17*. *D17Leh* was deleted from the relevant gene symbols. Rejoining of ends along the vertical axis restores the original configuration, rejoining along the diagonal results in inversion, and rejoining along the horizontal axis results in deletion.

These loci occur in one orientation in some chromosomes and in the reversed orientation in others. The order of these loci the wild-type chromosome with which the t^{w71} haplotype recombined to produce the partial *t'* haplotype must be *centromere-D17Leh66ea-D17Leh66eb,* otherwise complex crossovers must be postulated to account for the genetic constitution of the *t'* haplotype (Figure 2). This order is like that reported by HERRMANN, BARLOW and LEHRACH *et al.* (1987), rather than like the order reported by SCHIMENTI *et al.* (1987). An interesting explanation for the variable order of these loci involves intrachromosomal recombination between genes within the inverted duplication. If these duplicated elements pair homologously with themselves, rather than with homologous elements in their meiotic partner, recombination could produce one of three results: original configuration, inversion or deletion (Figure 4). A comparable argument has been proposed to account for certain rearrangements in immunoglobulin genes (JACK *et al.* 1988). The occurrence of comparable intrachromosomal rearrangements involving loci on chromosome *17* suggests that these events, however novel, are not restricted to immunoglobulin genes.

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