Recombination between two mouse t-haplotypes ($t^{w12}tf$ and t^{Lub-1}): segregation of lethal factors relative to centromere and tufted (tf) locus

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SUMMARY

Recombination between two mouse t-haplotypes, $t^{w12}tf$ and t^{Lub-1}, was investigated by screening the tailless progeny of the cross ♀ $t^{w12}tf/t^{Lub-1}$ + × ♂$Ttf/+tf$ for the segregation of tufted phenotype, t^{w12} and t^{Lub-1} lethal factors, and metacentric chromosome (since t^{Lub-1} haplotype is associated with a Robertsonian fusion involving chromosomes 4 and 17). The results give a 17 % estimate of the recombination frequency between centromere and tf, with t^{Lub-1} lethal factor mapping about two-thirds of the distance from centromere to tf and the t^{w12} lethal factor behaving as if closely linked to tf. This further extends the findings of Silver & Artzt (1981) and of Artzt, McCormick & Bennett (1982), and shows that two t-haplotypes with quite independent laboratory histories recombine at a normal level, supporting the notion that all t-haplotypes basically share the same structure.

1. INTRODUCTION

Mouse t-haplotypes are chromosome 17 segments obtained from wild animals, extending from the centromere to the *tufted* (tf) locus region and possibly even further. They comprise genes which interfere with tail development, embryonic viability, sperm differentiation and segregation, and the frequency of crossing over (Bennett, 1975; Klein & Hammerberg, 1977). Although the latter feature has made a genetical dissection by conventional crosses a difficult task, evidence could be obtained, clearly demonstrating that these various effects are indeed controlled by at least three independent regions (reviewed in Lyon *et al.* 1979 and Silver, 1981; see also Styrna & Klein, 1981).

Some time ago, the important observation was made that recombination in fact occurs at a high rate between two t-haplotypes (Silver & Artzt, 1981). While this strongly supported the contention that recombination suppression between t and normal chromosomes is due to some lack of homology resulting in chromatin mismatching (Lyon *et al.* 1979; Forejt, 1972; Forejt & Gregorová, 1977), it also made it possible for the first time to study the segregation of markers from two

different t chromosomes. Artzt, McCormick & Bennett (1982) were thus able to prove that the lethal genes from four distinct t-haplotypes are non-allelic. A further result from these experiments was that the H-2 locus had a segregation pattern implying its being located on the centromere proximal side of the tf region in t-haplotypes (Artzt, Shin & Bennett, 1982). This was unexpected, since the tf distal location of H-2 in wild-type chromosomes has been known for a long time (Green & Stimpfling, 1966; for a recent work confirming this location see Forejt, Čapková & Gregorová, 1980).

The four t-haplotypes (t^{w5}, t^{w12}, t^{w18} and t^{w32}) used in the study of Artzt & Bennett and coworkers all appear to have been introduced into laboratory stocks rather a long time ago. There is evidence that they are related to each other and may all have one or a few closely related ancestors (Shin *et al.* 1982; Silver, 1982). However, new t-haplotypes, sometimes carrying hitherto unknown lethal factors, are still being extracted from feral mice of wide geographical distribution (Winking & Guénet, 1978; Guénet *et al.* 1980; Sturm, Figueroa & Klein, 1982). It thus seemed important to check that the Artzt *et al.* experiment would still yield similar results when carried out with t-haplotypes of strongly different origins, as far as both time and place of origin into the laboratory are concerned.

The results of such an experiment are presented here. Two t-haplotypes with an independent laboratory history were chosen: $t^{w12}tf$ and t^{Lub-1}.

$t^{w12}tf$ is a tufted derivative (probably due to a mutational event, see Materials and Methods) of the t^{w12} haplotype, extracted from Californian wild mice at the beginning of the sixties (Dunn, Bennett & Beasley, 1962). It carries a lethal factor (l^{w12}) acting at the beginning of the second half of gestation and not complemented by the members of the so-called t^{w1} complementation group (Bennett, 1975).

t^{Lub-1} was extracted in the late seventies from an Italian wild mouse by Heinz Winking. Its main characteristics known so far are as follows. It is carried by a metacentric chromosome resulting from a Robertsonian translocation between chromosomes 4 and 17 (Winking & Guénet, 1978). It strongly suppresses recombination with a normal chromosome 17 in the region extending from the centromere to tf and H-2 (Winking, 1979). It carries a T interacting factor (T/t^{Lub-1} individuals are tailless) and a lethal factor (t^{Lub-1}), apparently complemented by all other known t lethal factors, but whose precise time of action during embryogenesis has not yet been determined (Winking, 1979; Guénet & Winking, 1979). It induces a strong segregation distortion in heterozygous males (see below) which favours the transmission of t^{Lub-1}-carrying gametes.

The segregation of centromere, tf, l^{w12} and l^{Lub-1} was followed in the progeny of $t^{w12}tf/t^{Lub-1}+$ females. The results of this investigation show that recombination occurs freely between these two t-haplotypes in the region which extends from centromere to tf. Both l^{Lub-1} and l^{w12} behave as single independent Mendelian units which can be mapped relative to centromere and tf. This experiment further exemplifies the close structural similarity which any two t-haplotypes seem to share.

The results concerning the segregation of H-2 markers in this experiment will be presented elsewhere (Pla and Condamine, manuscript in preparation).

2. MATERIALS AND METHODS

(i) *Mice and crosses*

A few breeding pairs of $T\,tf/t^{w12}tf$ and $T\,tf/t^{Lub-1}+$ animals were used to start the experiment. The $t^{w12}tf$ stock was initially obtained from D. Bennett in New York. It has been inbred for numerous generations and can be considered as a homozygous stock except for the chromosome 17 region (centromere $-tf-H$-2) where recombination with t chromatin is impaired. It is admitted that the tf mutation carried by the t^{w12} chromosome was probably not picked up from the Ttf chromosome as a result of some cross-over, since the H-2 specificities of the original t^{w12} haplotype are still present on the $t^{w12}tf$ chromosome (Styrna & Klein, 1981). It is thus assumed to have appeared as a spontaneous mutation, the precise nature of which is unknown (Silver & Artzt, 1981). The t^{Lub-1} stock originally came from H. Winking (Lübeck). As mentioned in the Introduction, this stock is of recent origin and had been inbred in Paris for a few generations only at the time this experiment was undertaken. Consequently, the t^{Lub-1} founder animals used were almost certainly heterozygous at some loci in their genome.

t^{w12}/t^{Lub-1} females were produced by intercrossing $T\,tf/t^{w12}\,tf$ and $T\,tf/t^{Lub-1}+$ animals and collecting the normal tailed progeny. Fifteen such females were chosen for carrying the experiment. Males were discarded since t^x/t^y males are characteristically sterile (Bennett, 1975) and $t^{w12}tf/t^{Lub-1}+$ males were in fact found to follow this rule.

It was checked that the females were non-tufted and that their karyotype did carry one metacentric chromosome, an indication that no recombination had occurred between chromosomes 17 of the T/t^{Lub-1} parent, at least in the region extending from centromere to tf. Absence of recombination in the $t^{w12}tf$ parent is more difficult to ascertain (and in fact was not checked in this case) but it is admitted that recombinational events in this region are very rare in this stock ($< 0.5\%$, Silver & Artzt, 1981).

$t^{w12}tf/t^{Lub-1}+$ females were mated with a BTBR $T\,tf/+tf$ male, yielding tailless and normal-tailed progeny. Only the tailless progeny were analysed further individually (males and females 'X', see Fig. 1). Mice were given a code number and ear-punched accordingly. (The segregation of two coat colour markers (A and a^t) among them helped in recognizing them unambiguously.) The animals were then checked for their tf phenotype, tested for the presence of the l^{w12} and/or l^{Lub-1} lethal factors and analysed for their karyotype. The overall scheme of the experiment is presented in Fig. 1.

(ii) *Karyotypes*

A small fragment was cut from one ear, shaved as much as possible, minced with scissors and put in culture in Dulbecco modified Eagle's medium added with 15 % fetal calf serum. Clumps attached within 4–5 days and cells (mostly fibroblasts) began to spread on the bottom of the dish. Cells were reseeded following trypsinization and karyotypes were determined on such secondary cultures
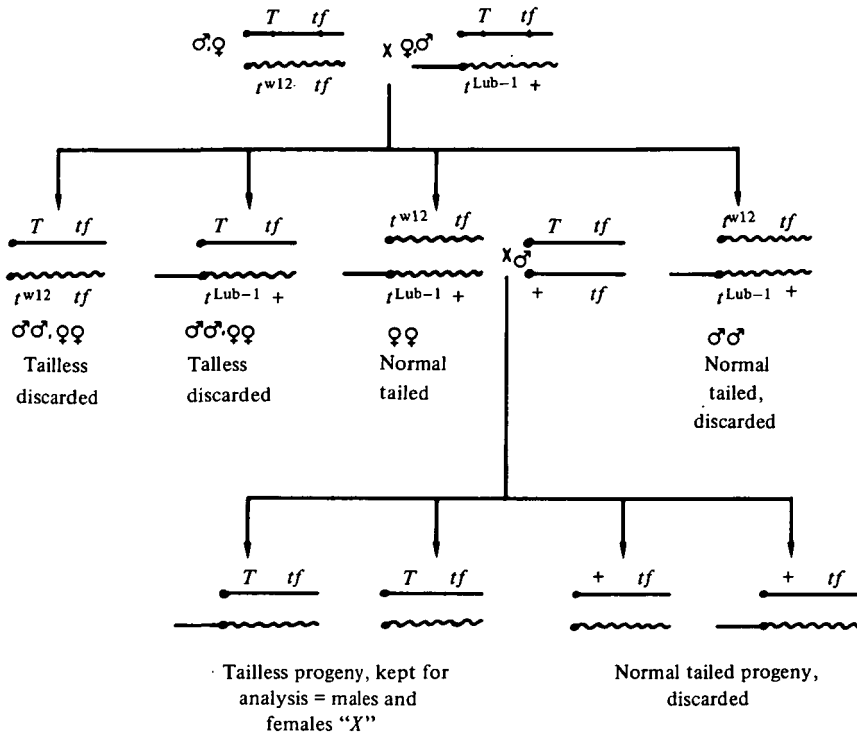
Fig. 1. General outline of the $t^{w12}tf/t^{Lub-1}+$ recombination experiment. Chromosomes 17 of the various mice involved have been represented. Wavy lines stand for t chromosomes. The t^{Lub-1} haplotype is carried by a metacentric chromosome and has been represented accordingly. For details, see text (Materials and Methods).

following the method in Jakob *et al.* (1973). Four or 5 fully spread metaphases with a diploid complement were recorded for each individual.

(iii) *Checking for the presence of lethal factors*

So-called 'complementation tests' (see Bennett, 1975) were based on the assumption that crosses of the type $♀T/t^{w12} × ♂T/t^{w12}$ or $♀T/t^{Lub} × ♂T/t^{Lub-1}$ yield tailless animals exclusively, while both tailless and normal-tailed babies are produced in $T/t^{w12} × T/t^{Lub-1}$ crosses or in crosses where at least one parent carries a t-haplotype devoid of any lethal factor (a situation which may result from recombination between normal and t chromosomes).

Female 'X' (see Fig. 1) were circulated among reference $T\ tf/t^{w12}\ tf$ and $T\ tf/t^{Lub-1}+$ males. When a mating produced tailless babies exclusively, the test cross was maintained until at least 15 tailless individuals were obtained. Since $t^{w12}tf$ males have a very high transmission ratio ($> 90\%$) favouring the transmission of the t^{w12} haplotype, the probability that a T/t^{Lub-1} female should give rise to 15 tailless babies when crossed with such a male is very low. Reference t^{Lub-1} males appeared to have a lower and somewhat more variable transmission ratio of their t-haplotype from 50 to 75 %, a variation reflecting perhaps the variability of the

genetic background (see Bennett, Alton & Artzt, 1983). The lowest transmitters (50 %) were expected to give on the average $\frac{1}{3}$ normal-tailed and $\frac{2}{3}$ tailless individuals when crossed with T/t^{w12} females. Even in this case, the probability of obtaining a progeny of 15 tailless mice is still very low ($= (\frac{2}{3})^{15} < 10^{-2}$).

Table 1. *Segregation of various* t *chromosome markers in the tailless progeny of* ♀♀ $t^{Lub-1}+/t^{w12}tf \times$ ♂ T $tf/+tf$ (cf. *Fig. 1*)

Markers of parental and Recombinant t chromosomes	Class of t chromosome		Number of 'X' individuals (cf. Fig. 1) in each class
	Parental types	Recombinant classes	
Rb, $+^{l^{Lub-1}}$, $+^{l^{w12}}$, $+tf$	t^{Lub-1} haplotype	—	45
O, $+^{l^{Lub-1}}$, l^{w12}, tf	$t^{w12}tf$ haplotype	—	82
			127
Rb, $+^{l^{Lub-1}}$, l^{w12}, tf	—	Ia	5
O, l^{Lub-1}, $+^{l^{w12}}$, $+tf$	—	Ib	12
			17
Rb, l^{Lub-1}, l^{w12}, tf	—	IIa	4
O, $+^{l^{Lub-1}}$, $+^{l^{w12}}$, $+tf$	—	IIb	5
			9
Rb, l^{Lub-1}, l^{w12}, tf	—	IIIa	Not found
O, $+^{l^{Lub-1}}$, l^{w12}, $+tf$	—	IIIb	1
			1
		Total number	154

Rb, presence of a Robertsonian chromosome (Rb (4-17) 13 Lub); O, acrocentric chromosome 17; l^{Lub-1}, t^{Lub-1} lethal factor: $+^{l^{Lub-1}}$, viable counterpart of l^{Lub-1}; l^{w12}, t^{w12} lethal factor; $+^{l^{w12}}$, viable counterpart of l^{w12}; tf, tufted.

Males 'X' were test-crossed with one or several reference T tf/t^{w12} tf and T $tf/t^{Lub-1}+$ females and their progeny was scored for the segregation of tailless and normal-tailed individuals in the same way. In addition, 25 of them were directly tested for their transmission ratio by crossing them with 129, C3H or STR $+^t/+^t$ females and examining the segregation of short-tailed versus normal-tailed phenotypes. All males appeared to transmit their t-haplotypes at a high rate (usually about 80–85 % in the case of males carrying a t^{Lub-1} haplotype and up to more than 90 % in the case of t^{w12} males). Again, this makes very unlikely the possibility that individuals not carrying the same lethal factor would yield up to 15 tailless descendants in such a test-cross.

3. RESULTS

Some 154 tailless individuals obtained from the cross ♀$t^{w12}tf/t^{Lub-1}+ \times$ ♂T $tf/+tf$ ('X' animals, see Fig. 1) were scored for the presence or absence of a metacentric chromosome, tf phenotype, and lethal factors initially carried by t^{Lub-1} and t^{w12} tf haplotypes (Table 1). Of these, 127 individuals were classified as having a t-haplotype indistinguishable from one of the two parental types as far as the four markers under study were concerned (Table 1, lines 1 and 2). Eighty-two of them appeared to have a t^{w12} tf parental haplotype, as opposed to only 45 carrying a

t^{Lub-1} haplotype. Although t^{w12}/t^{Lub-1} females appeared to transmit their acrocentric ($t^{w12}tf$) chromosomes at a slightly higher rate (58%, i.e. 107 out of 185 randomly chosen 'X' individuals whose analysis was undertaken but not completed in all cases), this discrepancy primarily reflects the fact that complete analysis of t^{Lub-1} haplotypes was more difficult to perform, due to a significantly lower fertility of $T/t^{Lub-1} \times T/t^{Lub-1}$ crosses (data not shown).
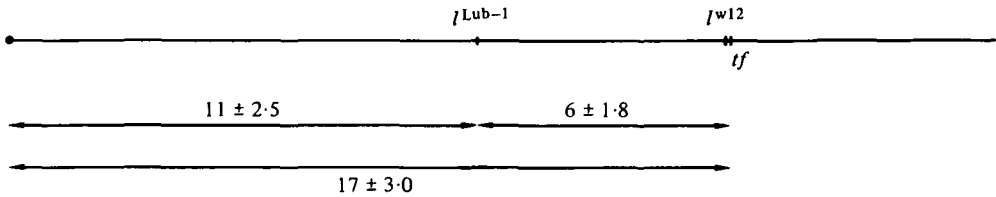

Fig. 2. Mapping of t^{Lub-1} and t^{w12} lethal factors (l^{Lub-1} and l^{w12}) relative to centromere and tf. Distances in centimorgans $\pm$ S.E.

Twenty-seven haplotypes were recovered in which the parental combination of markers had been somehow modified (Table 1, lines 3–8). In all instances, however, the recombination event appeared to occur between centromere and the tf locus. This gives an estimate of about 17% for the frequency of recombination in this region between the two t-haplotypes, a value somewhat higher than the genetic distance from centromere to tf as measured in 'normal' laboratory chromosomes 17 ($c.$ 11%, see review in Klein, 1975) but slightly lower than expected on the basis of recombination frequency from T to tf between other t-haplotypes (c 16%, Silver & Artzt, 1981; Artzt, McCormick & Bennett, 1982), added to the recombination frequency between centromere and T (about 2·5%, Klein, 1975).

Since both l^{Lub-1} and l^{w12} lethal factors segregate relative to centromere and tf among these recombinants, the implication is that both are located between these two markers, if every recombinant is to be interpreted as resulting from a single cross-over. t^{w12} lethal factor and tf are separated in only one recombinant (Table 1, line 8) and thus behave in this experiment as if closely linked. In contrast, t^{Lub-1} lethal factor is readily separable from both centromere and tf, with a cross-over between centromere and l^{Lub-1} in about 63% of total cross-over between centromere and tf (17/27, Table 1, lines 3 and 4). These results can be represented in the map shown in Fig. 2.

The validity of these results depends on the accuracy with which the four markers used were recorded. While karyotyping and checking for the tufted phenotype is straightforward, some misclassification concerning the presence of t^{Lub-1} and t^{w12} lethal factors is probably difficult to avoid. First, it is conceivable that complementation between the two factors, as revealed by the presence of normal-tailed progeny in an appropriate test-cross, depends to some extent on the genetic background of the parents. Since the t^{Lub-1} stock used most probably had a variable background (see Materials and Methods) it may be that some negative complementation tests reflected such an influence rather than the presence of a lethal factor. Estimating the degree of misclassification that such a phenomenon could contribute seems difficult, however.

On the other hand, positive complementation tests based on the presence of one single normal-tailed individual in a progeny test are somewhat ambiguous (see Artzt, McCormick & Bennett, 1982). Winking (1979) has obtained evidence that some degree of recombination (about 2 % frequency) occurs between t^{Lub-1} haplotype

Table 2. *Complementation data obtained with the individuals classified as recombinants. Each individual was test-crossed with* T/t^{Lub-1} *and* T/t^{w12} *reference animals*

Animal code number	Complementation test with t^{Lub-1}		Complementation test with t^{w12}		Genotype inferred from complementation data		Other markers		Recombinant class (cf. Table 1)
	o.t.	n.t.	o.t.	n.t.					
♀ 27	9	3	19	—	$+\,t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class I*a*
125	8	1	16	—	$+\,t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class I*a*
150	4	1	15	—	$+\,t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class I*a*
♂ 12	4	2	26	—	$+\,t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class I*a*
60	6	1	24	—	$+\,t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class I*a*
♀ 5	14	—	15	7	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
22	15	—	7	3	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
28	23	1	3	1	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
37	17	—	7	2	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
42	17	—	4	6	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
43	15	—	1	2	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
47	17	—	10	5	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
57	18	—	5	3	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
111	15	—	5	4	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
127	18	—	1	2	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
♂ 11	20	—	7	4	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
15	15	—	13	4	$t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class I*b*
♀ 2	27	—	11	—	$t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class II*a*
58	19	—	17	—	$t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class II*a*
115	15	—	11	—	$t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class II*a*
♂ 19	28	—	24	—	$t^{Lub\text{-}1}$	t^{w12}	Rb	tf	Class II*a*
♀ 14	12	5	3	4	$+\,t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class II*b*
38	9	7	9	5	$+\,t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class II*b*
72	3	2	5	3	$+\,t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class II*b*
145	4	1	13	6	$+\,t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class II*b*
♂ 5	3	3	2	7	$+\,t^{Lub\text{-}1}$	$+\,t^{w12}$	0	$+\,tf$	Class II*b*
♀ 82	15	2	27	—	$+\,t^{Lub\text{-}1}$	t^{w12}	0	$+\,tf$	Class III*b*

'o.t.' and 'n.t.' stand for the tailless and normal-tailed progeny produced in such test-crosses. Other symbols as in Table 1.

and normal chromosome 17 in the centromere-*tf* region. This could of course account for the presence of rare normal-tailed individuals in the progeny of two parents each carrying the t^{Lub-1} haplotype. (The same phenomenon is probably much less frequent with the t^{w12} haplotype due to the more stringent suppression of recombination in this case, see Materials and Methods.) Table 2 presents the actual data obtained with the animals which were classified as recombinants. It can be seen that five individuals yielded such ambiguous results (♀♀ 28, 125, 145 and 150, ♂ 60). The presence of one single normal-tailed individual in a small-sized

progeny ($<$ 10) was interpreted as an occurrence of true complementation. In contrast, one single normal-tailed individual in a large-sized progeny ($>$ 20) was considered as due to a recombination having taken place in one of the parents of the complementation test, resulting in the loss of the lethal factor. Thus ♀ 28 was considered as still carrying t^{Lub-1}, but not t^{w12} lethal factor (data obtained with descendants of this female have in fact confirmed that one lethal factor is present in this recombinant chromosome). Conversely, ♀♀ 125 and 150, and ♂ 60 were classified as carrying t^{w12}, but not t^{Lub-1} lethal factor. Similarly, the complementation pattern yielded by ♀ 145 was interpreted as absence of both l^{Lub-1} and l^{w12} factors.

Among the 127 non-recombinant individuals (Table 1, lines 1 and 2) 27 (20 females and 7 males) also gave similar complementation tests, with only one normal-tailed individual issued from one of the two test-crosses. Again, two types of situation were encountered. (*a*) Two females had one normal-tailed descendant, out of a total offspring of 29 and 34 individuals from t^{Lub-1} and t^{w12} tester males respectively. They were classified as having t^{Lub-1} and t^{w12} lethal factor respectively, with one recombination having presumably occurred in either of the two parents of the test-cross and eliminated this lethal factor. (*b*) The remaining 25 individuals all gave rise to a single normal-tailed descendant in small-sized progeny tests, namely 16 litters (totalling 85 individuals) in test-crosses with t^{Lub-1} reference animals, and 9 litters (totalling 51 individuals) in test-crosses with t^{w12} reference parents. Since the recombination rate in the centromere-*tf* region can be estimated as around 2 % in the T/t^{Lub-1} parent and below 1 % in the T/t^{w12} parent, it is likely that most if not all of these occurrences of a normal-tailed individual reflected true complementation, and the parents were classified accordingly.

Finally, it should be noted that all the recombinants presented in Table 2 display one exchange between centromere and *tf*, so that a misclassification concerning the presence or absence of lethal factors would only affect the map position of these, without changing the estimated frequency of recombination in this region. On the other hand, a similar misclassification among the non-recombinant individuals could mask the occurrence of rare double recombination events but should have little effect on recombination frequency, because of its happening only rarely.

4. DISCUSSION

The results presented here show that recombination takes place at a substantial rate between two *t*-haplotypes with quite independent laboratory histories, both in time and space. This further supports the recently emphasized view (Silver, 1982; Shin *et al.* 1982) that all *t*-haplotypes basically share a common structure which might in turn point to a common origin. What characterizes this structure, as opposed to the gene arrangement which prevails in 'normal' mouse chromosome 17, however, is almost entirely speculative at present. In a recent electron microscopy study, Tres & Erickson (1982) were unable to find any gross abnormality in the formation of synaptonemal complexes between *t* and non-*t* chromosomes at meiosis, suggesting that no inversion or rearrangement is involved in the formation of *t*-haplotypes. On the other hand, Artzt, Shin & Bennett (1982) have made the unexpected discovery that the H-2 complex of *t*-chromosomes is located between

T and *tf* rather than distal to *tf*; and a study, to be published elsewhere, of the segregation of *H-2* product expression in the experiment presented here (Pla and Condamine, manuscript in preparation) has confirmed this finding. Thus, an understanding of the actual differences between the structures of t and non-t chromosomes awaits further clarification.

Another clear-cut result obtained here is that the lethal factors of t^{Lub-1} and t^{w12} haplotypes each behave as single independent Mendelian units, located far apart on the chromosome. This is again a confirmation that t-lethal genes are non-allelic, and further exemplifies the notion that t chromosomes, due to their high transmission in males and impairment of recombination with normal chromosomes, can trap lethal recessive mutations in any gene of developmental importance located anywhere along the stretch of t chromatin.

Artzt, McCormick & Bennett (1982) have mapped the t^{w12} lethal factor some 6 cM distal to *tf*. This is somewhat at conflict with the results obtained here, which suggest a much tighter linkage between these two markers. While this might merely be due to a sampling effect, another possibility should perhaps be kept in mind, namely that different t-haplotypes extracted from wild mice might occasionally carry different lengths of t chromatin (t chromatin being defined as a stretch of chromosome 17 which recombines freely with another stretch of t chromatin, but has its recombination with 'normal' chromosome 17 almost entirely suppressed). If t^{Lub-1} chromatin stopped shortly after the tufted locus, then recombination with t^{w12} in a region distal to *tf* would be reduced, mimicking a tighter linkage between *tf* and any marker located in this region. However, the only recombinant found here between *tf* and t^{w12} lethal factor (♀ 82, Table 2) has also recombined between centromere and *tf*. To keep in line with this hypothesis, it would thus be necessary to assume that it is in fact a double recombinant.

New metacentric t chromosomes carrying a *tf* mutation and t^{w12} lethal factor or both t^{w12} and t^{Lub-1} lethal factors have been obtained in this experiment. They should prove useful in mapping the lethal factors of any other t-haplotypes relative to the centromere and the *tf* locus.

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