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SHORT PAPER

A new spontaneous mutation at the tufted locus within a mouse *t* haplotype

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SUMMARY

We report here the occurrence of a new spontaneous mutation at the tufted locus present within the crossover-suppressed region of the complete *t* haplotype t^{wLub1}. This new tufted allele shows complete expressivity of the tufted phenotype without any apparent effect on viability or reproduction. The presence of a tufted mutation within a *t* haplotype that is cytologically marked by a Robertsonian translocation is particularly useful in genetic experiments aimed at understanding the structure and function of *t* haplotypes.

1. INTRODUCTION

Mutations at the tufted locus on mouse chromosome 17 have played a critical role in numerous experiments that have shed light on the genomic organization and phenotypic properties of *t* haplotypes. The tufted locus was first defined by a recessive mutation (*tf*) that appears to have occurred spontaneously in a wild-type form of chromosome 17 present within a non-inbred stock of mice maintained at the MRC Radiobiology Unit in Harwell, England (Lyon, 1956). The *tf* mutation causes waves of hair loss with 100% penetrance in homozygous animals. The tufted locus maps to a position 7 cM distal to the Brachyury (*T*) locus.

Lyon & Phillips (1959) demonstrated that the lethal *t* haplotype t^6 causes a suppression of recombination over the region of chromosome 17 encompassed by the loci of *T* and *tf*. However, rare recombination between these loci was observed at a frequency (0·4%) comparable with that at which lethal *t* haplotypes appeared to mutate to new forms (Dunn & Gluecksohn-Waelsch, 1953). The results obtained led Lyon & Phillips (1959) and Lyon (1960) to propose what is essentially our current view of the structure of *t* haplotypes – that they represent a variant genomic region with multiple mutant loci, and that rare recombination between wild-type and *t* chromosomes generates novel *t* haplotypes with only a portion of the original variant *t* DNA (see Fig. 1). These new *t* chromosomes (now called partial *t* haplotypes) express only a subset of the original *t* haplotype effects (Lyon & Meredith, 1964; Lyon & Mason, 1977).

Only two additional spontaneous mutations at the tufted locus have been reported since the original discovery of *tf* by Lyon. Bennett (1975) reported a spontaneous

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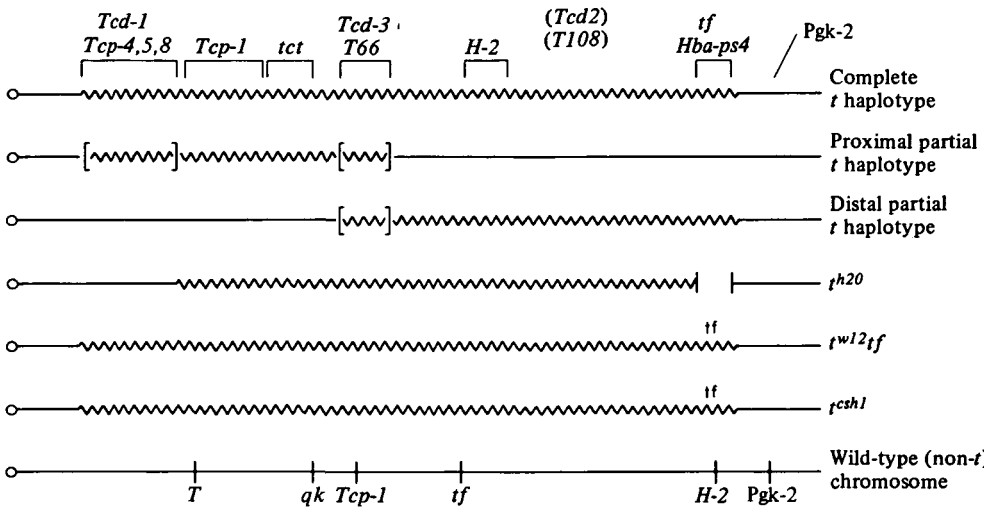

Fig. 1. Schematic representation of the extents of *t* DNA associated with a variety of *t* haplotypes. The region of *t* DNA is represented by a zig-zag line, and its extent has been defined by the coincidence of recombination suppression as well as by the presence or absence of molecular and phenotypic markers including t complex *T* locus interaction factor (*tct*), t complex (transmission ratio) distortion loci (*Tcd-1*, *Tcd-2*, *Tcd-3*; see Lyon (1984) for further description), t complex protein-specifying loci (*Tcp-1*, *Tcp-4,5,8*) and t complex DNA restriction fragments defined by the clones Tu66 and Tu108 (see Roehme *et al.* 1984), pH2IIa (for *H-2*; see Silver, 1982), and alpha globin pseudogene-4 (for *Hba-ps4*; see Fox *et al.* 1984). Representative proximal and distal partial *t* haplotypes are diagrammed; the *t* DNA regions in brackets are present in some but not all partial haplotypes of a particular class. The $t^{h\,20}$ haplotype is deleted for *Hba-ps4* and *tf*. The $t^{w\,12}$ *tf* and $t^{csh\,1}$ haplotypes are mutated at the *tf* locus but there is no evidence for deletions in these chromosomes. The wild-type loci within the t region include Brachyury (*T*), quaking (*qk*), *Tcp-1*, *tf*, *H-2* and Phosphoglycerate kinase-2 (*Pgk*-2).

mutation to *tf* within a $t^{w\,12}$ haplotype (this mutated chromosome actually appeared in 1962 and is referred to as $t^{w\,12}$ *tf*). The $t^{w\,12}$ *tf tf* allele is distinguishable from the original mutation in the expression of a more subtle phenotype which is often very difficult to score in compound $t^{w\,12}$ *tf*/*tf* animals (unpublished observations). Lyon & Bechtol (1977) reported a mutation to *tf* within a t^{6} haplotype – the mutated chromosome is called $t^{h\,20}$ and is deleted for a genomic region encompassing the *tf* locus as well as the nearby loci of *Ki* and *Hba-ps4* (Fox *et al.* 1984). The $t^{h\,20}$ *tf* allows complete expressivity of the tufted phenotype when opposite the original mutation, however, animals carrying the $t^{h\,20}$ chromosome are difficult to breed, presumably because of the extensive deletion which is present.

The *tf*-marked $t^{w\,12}$ *tf* chromosome was of crucial importance to the demonstration that free recombination can occur between different *t* haplotypes (Silver & Artzt, 1981). This mutation has been used in further breeding experiments to provide important insight toward a structural picture of *t* haplotypes with evidence for an inversion of the *H-2* complex and the *tf* locus relative to the positions of these loci on normal forms of chromosome 17 (Artzt, Shin & Bennett, 1982). However, because of the different phenotype expressed by the $t^{w\,12}$ *tf tf* allele, there is a formal (albeit unlikely) possibility that this *tf*-like mutation is actually present at a locus other than that defined by the original *tf* mutation. To rule out this formal possibility, and to confirm the existence of an inverted region within *t* haplotypes, it will be necessary to perform breeding studies

with an independently derived *t* haplotype-associated *tf* mutation. We report here the discovery and characterization of a new spontaneous mutation to tufted that occurred within the complete *t* haplotype t^{wLub1}.

2. MATERIALS AND METHODS

Mice carrying the t^{wLub1} haplotype were provided by H. Winking (Lubeck, FRG) in the fall of 1980 (Winking & Silver, 1984). All breeding experiments were conducted at the Cold Spring Harbor Laboratory. DNA analysis was performed as described previously (Silver, 1982; Fox, Silver & Martin, 1984; Rohme *et al.* 1984). Two-dimensional gel analysis was performed as described previously (Silver *et al.* 1983).

3. RESULTS

(i) *Derivation of* t^{csh1}

A tailless tufted female (mouse number 4488) was born on 15 September, 1982 from a balanced lethal cross between two animals of the genotype $T\ tf/t^{wLub1}+^{tf}$. Twelve other direct siblings of female 4488 were tailless but non-tufted. To date, a total of 173 progeny from this balanced lethal t^{wLub1} line have been scored as non-tufted. Female 4488 was mated to a $T\ tf/+^{T}tf$ male and transmitted her *t* haplotype to five progeny that survived to weaning when the tufted phenotype can be scored; all five animals were tufted. This result indicates the spontaneous occurrence of a new *t* haplotype in female 4488 with a tufted mutation in association with t^{wLub1}-derived chromatin. This new *t* haplotype is called t^{csh1}.

(ii) t^{csh1} *retains the* t^{wLub1} *lethal mutation*

When T/t^{csh1} animals are mated *inter se*, only tailless offspring (a total of 71 to date) have been obtained, implying that homozygous t^{csh1}/t^{csh1} embryos die *in utero*. This result indicates the presence of a recessive lethal mutation within the t^{csh1} chromosome. To determine if the t^{wLub1} lethal mutation was still present within the t^{csh1} haplotype, T/t^{csh1} females were mated to T/t^{wLub1} males. The male transmission ratio of t^{wLub1} in our colony averages 95%. Hence, if t^{csh1} does not carry the t^{wLub1} lethal mutation, one would expect close to 50% of the offspring to be double heterozygous t^{wLub1}/t^{csh1} animals with tails of normal length. The observed result was that all 25 offspring born were tailless. In summary, the breeding data indicate that the t^{csh1} chromosome carries the lethal mutation characteristic of the t^{wLub1} haplotype.

(iii) t^{csh1} *is transmitted at a high ratio from males*

T/t^{csh1} males were mated to wild-type +/+ females in order to determine transmission ratio. Of 68 offspring from 5 different males, 66 carried the t^{csh1} chromosome for an average transmission ratio of 97%. This ratio is not significantly different from the current value in our colony for t^{wLub1} (95%).

(iv) *Two-dimensional gel analysis of* t^{csh1}

High-resolution two-dimensional gel analysis of proteins expressed by testicular cells has led to the identification of a family of polypeptides that are specified uniquely by *t* haplotypes (Silver *et al.* 1983). When outbred mice are analysed, it is possible to score for the expression of the *t* complex proteins TCP-1, TCP-3, TCP-4, TCP-5 and TCP-8 which are specified by genes that map along the entire length of the *t* complex (see Fig. 1 for map locations). Gel studies of testicular cell proteins from t^{csh1}-carrying animals demonstrates the expression of all five scorable TCP proteins (data not shown).

(v) *The* H-2 *region associated with* t^{csh1}

The *H-2* complex is an integral component of all complete *t* haplotypes, and a cross-hybridizing *H-2* class I cDNA probe has been used to identify a series of restriction fragment length polymorphisms (RFLPs) that are indicative of the *t* haplotype form of the *H-2* complex (Shin *et al.* 1982; Silver, 1982). Genomic analysis with this *H-2* cDNA probe demonstrates that the $t^{csh\ 1}$ chromosome retains the *H-2* complex region of the $t^{wLub\ 1}$ chromosome (Fig. 2A).

(vi) *Genomic analysis with random t complex region DNA clones*

Random DNA fragments from the t complex region have been cloned from micro-dissected pieces of mouse chromosome 17 (Roehme *et al.* 1984). These clones have been used to identify RFLPs that distinguish *t* haplotype DNA, from wild-type DNA, and the relative location of particular restriction fragments has been determined by analysis of partial *t* haplotypes (Fox *et al.* 1985). Two such clones – Tu66 and Tu108 – were used in an analysis of $t^{csh\ 1}$. All t-specific RFLPs detected by these clones were present within the $t^{csh\ 1}$ chromosome (Fig. 2B, C).

(vii) *Genomic analysis with an alpha globin pseudogene clone*

An alpha globin pseugogene DNA clone has been used in genomic RFLP studies to map the pseudogene locus (*Hba-ps4*) to the t complex region of chromosome 17 (Fox *et al.* 1984; D'Estachio *et al.* 1984). Complete *t* haplotypes are associated with an *Hba-ps4* restriction fragment that is distinguishable from that associated with most wild-type forms of chromosome 17. The $t^{h\ 20}$ chromosome is deleted for a region encompassing both the *Hba-ps4* locus as well as the tufted locus (Fox *et al.* 1984). This result indicates the close linkage of the alpha globin pseudogene to the tufted locus. Genomic analysis of the $t^{csh\ 1}$ chromosome demonstrated the presence of the *t* haplotype form of *Hba-ps4* (Fig. 2D).

4. DISCUSSION

In many laboratories complete *t* haplotypes are routinely maintained in balanced lethal stocks that are also heterozygous for the *tf* mutation. In such stocks, all matings are set up between two tailless $T\ tf/t^{x}+^{tf}$ animals, and nearly all progeny obtained from such a mating scheme are tailless and phenotypically non-tufted. However, at a rate of 1 in 250 (Lyon & Phillips, 1959) to 1 in 500 (Bennett, Dunn & Artzt, 1976), a rare tailless tufted animal or one with a tail of normal length will be observed. Over 100 such exceptional animals have been characterized, and in nearly all cases these animals have been found to carry a new proximal partial *t* haplotype that was generated by recombination between the parental *t* haplotype and the wild-type homologue of chromosome 17 (see Fig. 1; Lyon & Phillips, 1959; Bennett *et al.* 1976). Partial *t* haplotypes are clearly distinguishable from complete *t* haplotypes with the use of molecular or phenotypic criteria. All proximal partial *t* haplotypes express a wild-type form of the TCP-3 protein, and are associated with wild-type DNA restriction fragments detected by the Tu108, Hba-ps4, and H-2 probes (see Fig. 1). Distal partial *t* haplotypes express wild-type forms of TCP-1, TCP-4 and TCP-8 and do not interact with dominant mutations at the T locus to cause taillessness. Neither proximal nor distal haplotypes express a high male transmission ratio, because this effect is controlled by interacting genes that map in both the proximal and distal regions (Lyon, 1984).

The $t^{csh\ 1}$ chromosome has all of the properties expected of a complete *t* haplotype, including high transmission ratio and *t* forms of all available molecular markers. In fact, with the exception of the *tf* mutation, $t^{csh\ 1}$ is indistinguishable from the $t^{wLub\ 1}$ haplotype. Only two previous examples of this type of chromosome have been reported – $t^{h\ 20}$ and

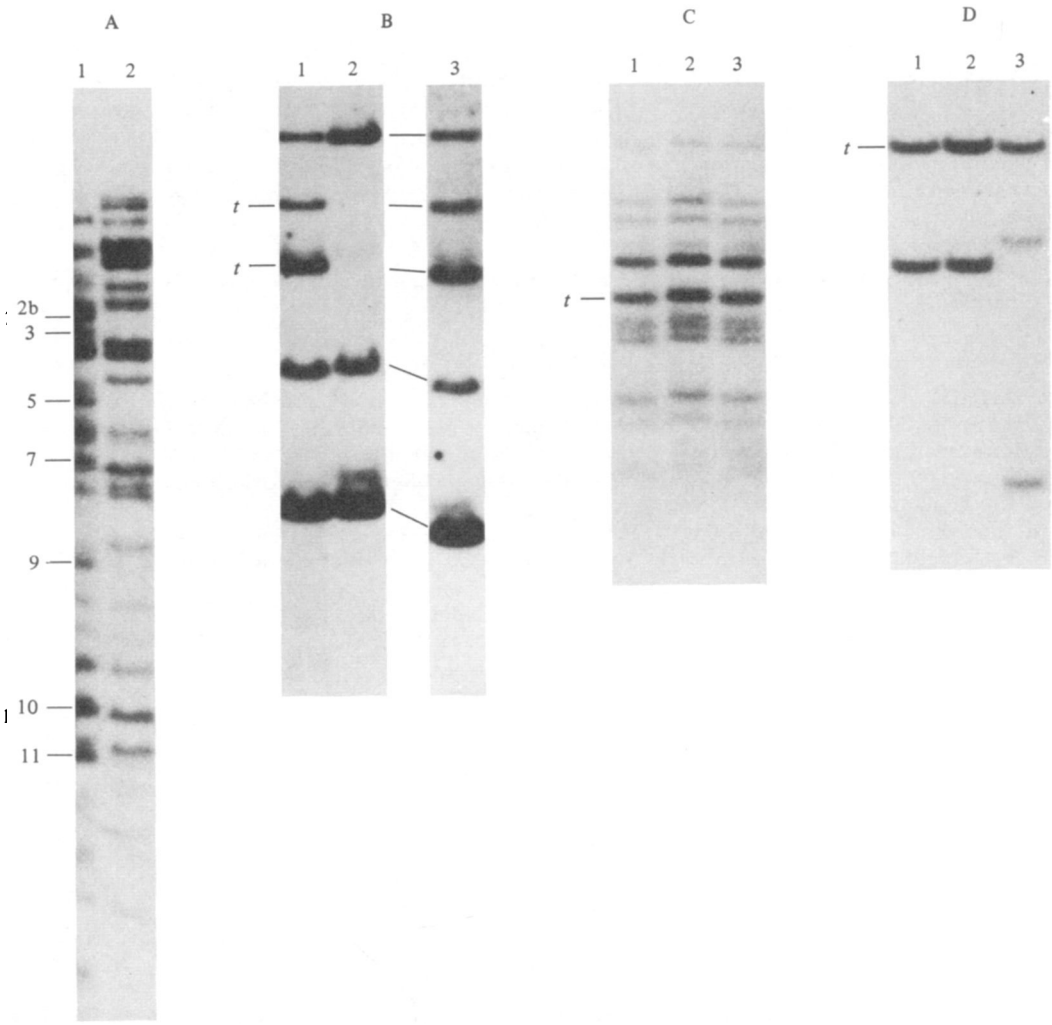

Fig. 2. Genomic analysis of *t* complex-associated restriction fragments. Five micrograms of total liver DNA from each mouse was digested to completion with the restriction enzyme listed below, electrophoresed on to agarose gels, and transferred to nitrocellulose. Cloned probes were obtained by nick-translation. (A) H-2. DNA aliquots from a heterozygous $+/t^{csh\,1}$ animal (lane 1) and an inbred 129/SvJ (+/+) animal (lane 2) were digested with Eco RI, and probed with pH2IIa. The numbers on the left indicate particular *t* haplotype-specific restriction fragments described in Silver (1982) and Silver *et al.* (1984). (B) Tu66. Lane 1, $+/t^{csh\,1}$; lane 2, 129(+/+); lane 3, $+/t^{wLub\,1}$. DNA was digested with TaqI and probed with Tu66. The two fragments labelled t are *t* haplotype-specific restriction fragments described in Roehme *et al.* (1984). Lanes 1 and 2 and lane 3 are from separate experiments. (C) Tu108. Lane 1, $+/t^{csh\,1}$; lane 2, $+/t^{wLub\,1}$; lane 3, $+/t^{csh\,1}$ (second animal). The band labelled t is the most prominent t haplotype-specific restriction fragment described in Roehme *et al.* (1984). (D) Hba-ps4. Lane 1, $+/t^{csh\,1}$ (129 background); lane 2, $+/t^{wLub\,1}$ (129 background); lane 3, $+/t^{csh\,1}$ (outbred background). The band labelled t is the *t* haplotype-specific fragment described by Fox *et al.* (1984). The other restriction fragments are polymorphic among non-*t* chromosomes.

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$t^{w\,12}$ *tf*. It would appear that all three of these chromosomes were generated by direct mutation of the parental *t* haplotype rather than by a recombinational event. However, *t-t* recombinational studies have mapped the tufted locus to the distal edge of *t* haplotypes, even though this locus maps to the central t complex region of wild-type chromosomes (Artzt *et al.* 1982; Condamine *et al.* 1984). It is possible that recombination could occur near the distal edge of the *t* haplotype and result in the deletion of the tufted locus. With the molecular probes currently available, only $t^{h\,20}$ has been found to have a deletion in this region, however, further studies are necessary before any model can be built to explain the derivation of these chromosomes.

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