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# Persistence of a lethal *t* haplotype in a laboratory stock of outbred mice

BY LEE M. SILVER, DEBORAH LUKRALLE, JAMES I. GARRELS, AND KEITH R. WILLISON[1]

*Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724*

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SUMMARY

A cryptic, lethal *t* haplotype (named $t^{Lmb}$) has been discovered in the non-inbred MF1 laboratory stock of mice. Detailed genetic and molecular studies demonstrate a close relationship between $t^{Lmb}$ and a well-studied *t* haplotype, $t^6$, previously extracted also from a laboratory stock. It appears likely that $t^6$ is an aberrant *t* haplotype derived by recombination from a $t^{Lmb}$-like ancestral chromosome. The persistence of $t^{Lmb}$ through 40 years of random breeding in a laboratory stock indicates that high transmission ratio distortion is sufficient to overcome the deleterious effect of a recessive lethal gene in a mouse population.

## 1. INTRODUCTION

*t* haplotypes are variant forms of an extensive region of chromosome 17 present in *Mus musculus* populations throughout the world. All naturally occurring *t* haplotypes are closely related and easily distinguisable from wild-type forms of this chromosome owing to a series of *t*-specific effects on development and sperm function (see Lyon, 1981; Silver, 1981; Klein & Hammerberg, 1977; and Sherman & Wudl, 1977, for reviews). All naturally occurring *t* haplotypes are lethal or semi-lethal when homozygous and would not therefore be expected to occur at high frequencies in wild populations. It appears that the persistence of *t* haplotypes is a consequence of transmission ratio distortion through males.

Animals heterozygous for *t* haplotypes are indistinguishable in gross morphology from wild-type (+/+) animals. The discovery of *t* haplotypes in wild mice depended upon the recovery in the laboratory of the Brachyury (*T*) mutation located close to the centromere on chromosome 17 (Dobrovalskaia-Zavadskaia, 1927). While *T*/+ animals have short tails, *T*/*t* animals are tailless. This obvious phenotype allows easy identification of cryptic *t* haplotypes in wild animals by progeny testing with *T*/+ mates.

In 1950, Carter & Phillips reported the discovery of a *t* haplotype (named $t^6$) in a laboratory stock of 'genetically heterogeneous material'. This observation indicated that *t* haplotypes could persist in non-inbred laboratory stocks without

[1] Current address: Institute for Cancer Research, Chester Beatty Laboratory, London SW3 6JB, England.

any form of a directed selection protocol. We report here the continued persistence of a cryptic $t^6$-like haplotype (called $t^{Lmb}$) within a non-inbred stock that has been maintained in the laboratory for at least 40 years. A detailed characterization of $t^{Lmb}$ suggests that $t^6$ was actually derived from $t^{Lmb}$ through a recombination event.

## 2. METHODS

All breeding studies were performed at the Cold Spring Harbor Laboratory. Analysis of *H-2* restriction patterns was performed as described previously (Silver, 1982). Two-dimensional gel analysis was also performed as described previously (Silver *et al.* 1983).

## 3. RESULTS

### (i) *History of* $t^{Lmb}$

In 1937, a Swiss Webster stock was obtained from the Rockefeller Institute by Carworth. It was originally called CFW, but was later changed to MF1 by the Laboratory Animals Centre. Animals of this stock were purchased from OLAC Ltd, Shaws Farm, Blackthorn, Bicester, Oxon, in 1977. These mice were shipped from the Laboratory of Molecular Biology (*Lmb*), Cambridge, England to Cold Spring Harbor, New York in 1979.

The suspected existence of a *t* haplotype in this stock of mice was confirmed by two independent observations of different individual animals. First, the mating of a female from this stock with a $T/+$ male resulted in tailless offspring that must have a $T/t$ genotype. Second, a two-dimensional gel analysis of testicular cell proteins synthesized by a male of this stock indicated expression of the acidic (p63/6.9a) form of the p63/6.9 protein. Expression of p63/6.9a is specifically correlated with mutant *t* haplotypes (Silver *et al.* 1980).

### (ii) $t^{Lmb}$ *is a member of the* $t^0$ *complementation group*

The $t^{Lmb}$ chromosome has been maintained in our colony over a period of two years through the mating of $T/t^{Lmb}$ females with $T/t^{Lmb}$ males. Only tailless ($T/t^{Lmb}$) offspring have resulted from this mating scheme, indicating that $t^{Lmb}$ is associated with a recessive lethal mutation.

Because $t^{Lmb}$ was discovered in a non-inbred stock of mice maintained in the UK, it seemed likely that $t^{Lmb}$ was in fact a re-isolate of $t^6$. The $t^6$ haplotype is a member of the $t^0$ complementation group. To determine if $t^{Lmb}$ was also a member of this complementation group, $T/t^{Lmb}$ animals were crossed to $T/t^0$ animals. A total of 37 offspring were obtained from three separate pairs – all 37 were tailless. Hence, $t^{Lmb}$ fails to complement $t^0$, and therefore $t^{Lmb}$ has the same lethal factor as $t^6$.

### (iii) *Transmission ratio distortion of* $t^{Lmb}$

Males of the genotype $T/t^{Lmb}$ were mated to wild-type (+/+) females. Of 119 offspring obtained from three males, 117 (98 %) carried $t^{Lmb}$. This high transmission ratio distortion is a characteristic of complete *t* haplotypes from wild populations.

### (iv) t *complex proteins (TCPs) expressed by* $t^{Lmb}$

A recent high-resolution two-dimensional gel analysis of testicular cell proteins has allowed the identification of 9 *t*-specific proteins expressed by all complete *t* haplotypes irrespective of genetic background (Silver *et al.* 1983). With the use of partial *t* haplotypes (recovered as rare recombination events between *t* haplotypes and wild-type chromosomes), it has been possible to map the genes specifying each of these proteins to a subregion of the *t* complex. Two-dimensional gel analysis can now be used to determine the extent of *t* chromatin present within a a particular chromosome. Analysis of testicular cell proteins expressed by animals carrying the $t^{Lmb}$ haplotype indicates the presence of all 9 *t* complex proteins (Plate I). This result confirms the 'complete' *t* haplotype status of $t^{Lmb}$.

### (v) *The* H-2 *region of* $t^{Lmb}$

The *H-2* complex is an integral component of naturally occurring *t* haplotypes (Silver, 1982; Shin *et al.* 1982). Many *t* haplotypes have been analysed at the genomic DNA level with a cDNA probe that cross-hybridizes to all 30–36 genes and pseudogenes of the *H-2* class I family. These analyses indicated a high degree of homology among all *t* haplotypes, with a high level of shared *t*-specific *H-2*-associated restriction fragments. A small number of restriction fragment length polymorphisms did, however, distinguish among the different *t* haplotypes. While $t^6$ and $t^0$ are members of the same complementation group, their associated *H-2* regions are clearly distinguishable by serological analysis (Hammerberg *et al.* 1976) as well as through DNA analysis (Silver, 1982).

Genomic DNA from mice carrying the haplotypes $t^0$, $t^6$ or $t^{Lmb}$ was digested with the restriction enzyme Bg1 II, electrophoresed on an agarose gel, transferred to nitrocellulose, and hybridized to one of two different H-2 class I cDNA probes. The pH2IIa clone recognizes the 3′ end of each class I gene, while pH2III recognizes the 5′ end of each gene (Steinmetz *et al.* 1981). These two probes identify four restriction fragments (labelled, B, C, D and E in Plate II) present in the DNA of $t^0$ and most other complete *t* haplotypes but absent from both $t^6$ and $t^{Lmb}$. Hence the *H-2* region of the $t^{Lmb}$ haplotype appears similar to that of the $t^6$ haplotype.

## DISCUSSION

We have discovered a cryptic, lethal *t* haplotype in a laboratory stock of mice commonly used by embryologists for studies of normal developmental processes. This finding should induce embryologists to be cautious in interpreting 'unusual' results obtained with mice from any non-inbred stock. The fact that the $t^{Lmb}$

haplotype has persisted in the MF1 stock through 40 years of random breeding demonstrates clearly that transmission ratio distortion through males at the 98% level is sufficient to overcome the deleterious effect of a recessive lethal gene in a population of mice.

The $t^{Lmb}$ haplotype appears to carry a lethal gene indistinguishable from that associated with the $t^6$ haplotype. In addition, genomic DNA studies indicate a

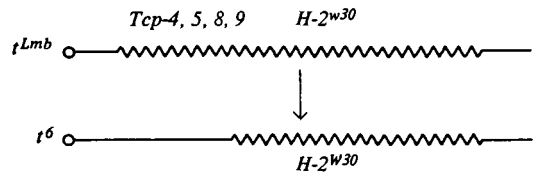


Fig. 1. Postulated relationship between $t^{Lmb}$ and $t^6$. *The* $t^6$ and $t^{Lmb}$ haplotypes were both discovered in non-inbred laboratory stocks of mice maintained in the UK. They are indistinguishable in lethal factor and *H-2* DNA sequences. However, the $t^6$ haplotype is missing a proximal region of *t* chromatin with the structural genes *Tcp-4*, *Tcp-5*, *Tcp-8* and *Tcp-9*. $t^6$ appears to be a truncated version of $t^{Lmb}$, derived through recombination with a wild-type chromosome.

complete identity in a comparison of the *H-2* regions associated with each of these haplotypes. These results would lead one to the suggestion that $t^{Lmb}$ is simply a recurrence of $t^6$ in a non-inbred laboratory stock. However, while $t^{Lmb}$ is a typical complete *t* haplotype, $t^6$ is unique in missing a small region of proximal *t* chromatin (Fig. 1). This proximal chromatin carries a gene affecting male fertility (Lyon, 1981), as well as a series of genes specifying four testicular cell proteins (Silver *et al.* 1983). While $t^{Lmb}$ and all other complete *t* haplotypes specify nine *t*-specific testicular cell proteins, $t^6$ specifies only five ($t^6$ is associated with wild-type alleles at *Tcp-4*, *Tcp-5*, *Tcp-8* and *Tcp-9*). It appears likely that $t^{Lmb}$ represents the normal form of the *t* haplotype present in this laboratory stock, and that $t^6$ is an aberrant *t* haplotype derived by recombination from $t^{Lmb}$ (see Fig. 1).

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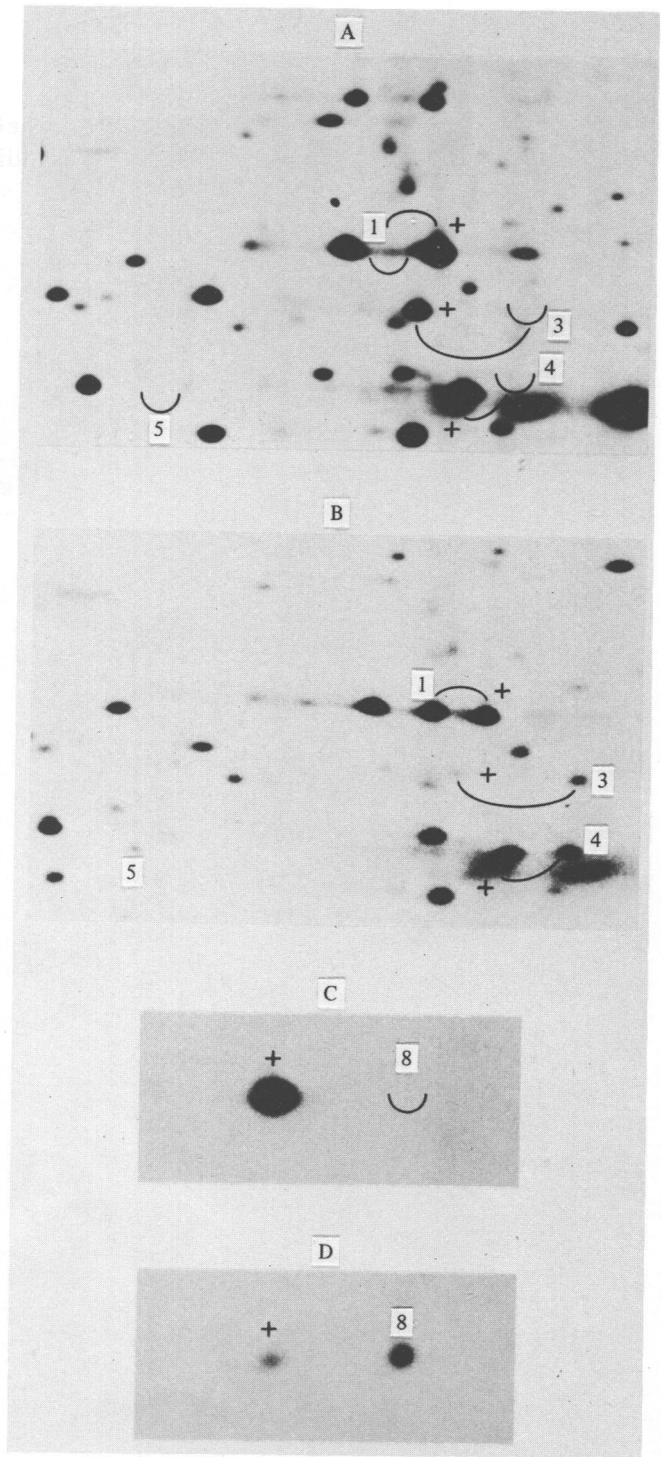


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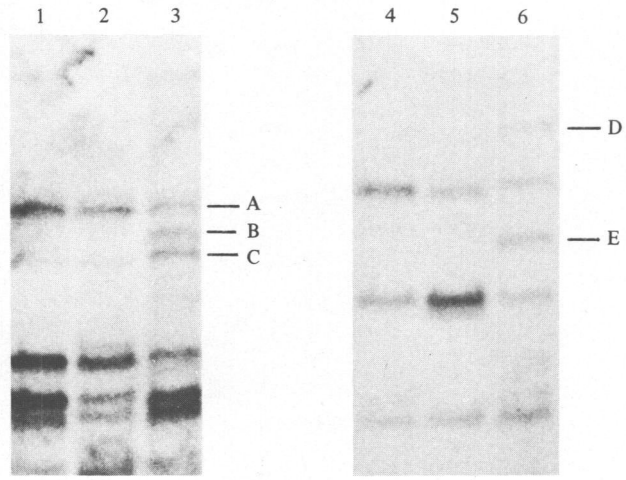




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## EXPLANATION OF PLATES

### PLATE 1

*t* complex proteins specified by the $t^{Lmb}$ haplotype. (A and B) The p63/6.9 region of the protein pattern. (A) The wild-type (+/+) pattern. (B) The heterozygous ($+/t^{Lmb}$) pattern. (C and D) The TCP-8 region of the protein pattern. (C) The wild-type (+/+) pattern. (D) The heterozygous ($+/t^{Lmb}$) pattern. The $t^{Lmb}$ haplotype specifies the expression of all nine *t*-specific proteins previously described (Silver *et al.* 1983). The proteins TCP-4, TCP-5 and TCP-8 (labelled 4, 5 and 8 on the plate), as well as TCP-9 (data not shown) are expressed by $t^{Lmb}$ but not by $t^6$.

### PLATE 2

Comparative DNA analysis of $t^6$, $t^{Lmb}$ and $t^0$. Liver DNA was prepared from $+/t^6$, $+/t^{Lmb}$ and $+/t^0$ animals, and was digested with the restriction enzyme Bg1 II. Digested DNA was electrophoresed on a 0·7% agarose gel, transferred to nitrocellulose, and hybridized with one of two H-2 class I cDNA probes. Lanes 1, 2 and 3 were probed with pH2 IIa. Lanes 4, 5 and 6 were probed with pH2 III. (Both cDNA clones were kindly provided by Dr Leroy Hood.) Lanes 1 and 4 are $+/t^6$, lanes 2 and 5 are $+/t^{Lmb}$, lanes 3 and 6 are $+/t^0$. Restriction fragments B, C (called bands 2 and 3 in figure 5 of Silver, 1982), D and E are associated with $t^0$ and most other *t* haplotypes but not with $t^6$ or $t^{Lmb}$. Restriction fragment A is a *t*-specific fragment (called band 1 in figure 5 of Silver, 1982) shared by all complete *t* haplotypes.