

Brief Communication

Reevaluation of the Evidence for the Generation of New Lethal *t* Haplotypes by Mutation

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Complete *t* haplotypes are found at a high frequency in wild mouse populations from around the world (Klein 1975). These complete *t* haplotypes represent a variant form of a region of chromosome 17, extending from the locus of *T* to beyond the *H-2* complex (see Fig. 1 and Klein and Hammerberg 1977, Lyon et al. 1979, and Silver 1981 for reviews). All complete *t* haplotypes are either lethal or semi-lethal when homozygous and each complete lethal *t* haplotype studied to date can be placed into one of seven different complementation groups. Heterozygous (+/*t*) males will transmit a complete *t* haplotype to their offspring at ratios greater than 95%. It is this property of transmission ratio distortion that allows the maintenance of these deleterious chromosomes in natural populations of mice.

Recent genetic and molecular studies have resulted in a much better understanding of the genomic organization of *t* haplotypes, and have served to focus attention on the origin of *t* haplotypes in general, and the relationship between different lethal groups in particular (Silver and Artzt 1981, Artzt et al. 1982a, Silver 1982, Shin et al. 1982). All complete *t* haplotypes share a high level of homology as determined by a variety of studies: (1) all carry a family of genes responsible for transmission ratio distortion in +/*t* heterozygous males and sterility in compound t^x/t^y males (Lyon and Mason 1977, Hammerberg 1981, Styrna and Klein 1981); (2) all carry another gene that interacts with the dominant *T* mutation to produce tailless *T/t* animals; (3) all carry a set of nine mutant genes that specify altered forms of a diversified family of testicular cell proteins (unpublished data and Silver et al. 1980); (4) all demonstrate a high level of homology at DNA restriction sites within the *H-2* complex region (Silver 1982, Shin et al. 1982); (5) while recombination between wild type and *t* haplotype DNA is suppressed, normal levels of recombination will occur between overlapping regions from any two haplotypes (Silver and Artzt 1981, Artzt et al. 1982a). The accumulated data indicate that all naturally occurring *t* haplotypes are closely related forms of a region of mouse chromosome 17 easily distinguishable from wild-type at many different loci.

It has been proposed that a single ancestral chromosome is responsible for all *t*

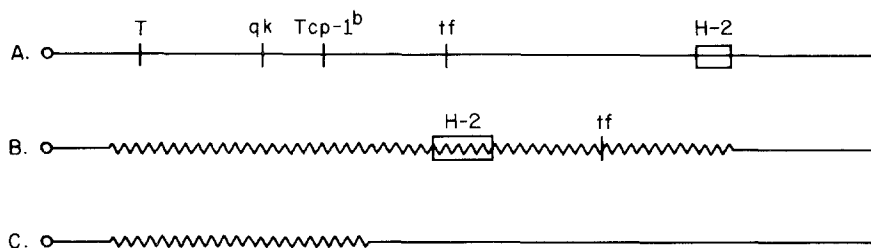


Fig. 1 A–C. The *t* complex region of mouse chromosome 17. **A** A wild-type chromosome with well-defined genetic markers: *T-Brachyury*, *qk-quaking*, *Tcp-1^b* – the *b* allele of *t complex protein 1*, *tf-tufting*, *H-2* – the *H-2* complex region. **B** A complete *t* haplotype occupies the region of chromosome 17 indicated by the zigzag line (see Silver 1981). The relative positions of the *tf* locus and the *H-2* complex have been reversed (see Artzt et al. 1982b). **C** Partial haplotypes of the *t⁹* complementation group extend only over the proximal portion of the *t* complex, as indicated by the zigzag line.

haplotypes and that different lethal *t* haplotype genes have been generated by mutation of this chromosome (Shin et al. 1982). Support for this viewpoint comes from the contention that the direct generation of one lethal *t* haplotype from another has been observed in the laboratory on eight independent occasions (listed in Fig. 1 of Bennett et al. 1976). The purpose of this report is to reevaluate the original data upon which this contention is based.

All eight cases in which a new lethal *t* mutation has been reported to be generated can be placed into one of two categories. The first category, accounting for five of the eight cases, is the generation of lethal *t* haplotypes in the *t⁹* complementation group from several different complete *t* haplotype groups. The five *t⁹* group haplotypes that have been generated are *t⁴* and *t⁹* (Dunn and Gluecksohn-Waelsch 1953), *t^{w18}* (Bennett and Dunn 1960), *t^{w30}* (Dunn et al. 1962) and *t^{w52}* (Bennett et al. 1976). The *t⁹* group of haplotypes has several unique features that distinguish it from all other lethal *t* groups. Most important, each *t⁹* group haplotype is associated with variant *t* chromatin that extends only over a proximal portion of the region defined by complete *t* haplotypes (see Fig. 1). This fact has become clear through the realization that recombination suppression, in +/*t* heterozygotes, is coincident with the extent of *t* chromatin (Lyon et al. 1979). It has been shown that each of the five members of the *t⁹* group will allow normal recombination in a genomic region proximal to the marker *tufted* (*tf*; Dunn et al. 1962, Bennett et al. 1976). Further studies of the *t^{w18}* haplotypes in particular indicate that the region of *t* chromatin present extends from a proximal point between the centromere and *T* to a distal point between *quaking* (*qk*) and *tf* (Artzt et al. 1982a).

In one case (*t^{w30}*), the presence of the *tf* marker allowed the clear demonstration that a *t⁹* haplotype could be generated by a recombinant event between a complete *t* haplotype and a wild-type chromosome (page 298 in Dunn et al. 1962). It seems likely that all members of the *t⁹* group were generated in an analogous manner. The lethal genes present in the parental, complete *t* haplotypes are located distal to the site of recombination and would not be carried in the newly generated *t⁹* haplotypes. If the recombinant event were unequal, a region of genome would be either deleted or duplicated; this deleted or duplicated gene(s) would be located at

the distal end of the t^9 chromatin and could be equivalent to the t^9 lethal gene itself (Lyon and Meredith 1964, Lyon and Bechtol 1977). Molecular evidence indicates that rare recombination between t chromatin and wild-type chromatin is often unequal relative to a particular structural locus called *Tcp-1* (Silver et al. 1980).

At least three separate t haplotype genes are required for a high transmission ratio distortion (Lyon and Mason 1977, Styrna and Klein 1981). The t^9 haplotypes do not carry the distal distortion gene and are not transmitted at a high ratio (Dunn and Gluecksohn-Waelsch 1953, Bennett et al. 1976). This fact explains why examples of the t^9 haplotype have never been found in natural populations. Hence, the laboratory observation of lethal t^9 haplotype generation by unequal crossing-over is not relevant to an understanding of the stable generation of different lethal t haplotypes in wild mice.

The second category of lethal t haplotype generation in the laboratory includes only three reported cases in which it is contended that one complete lethal t haplotype mutated to a second complete t haplotype of a different complementation group. The three newly generated complete t haplotypes are t^{w20} , t^{w21} , and t^{w32} (Bennett et al. 1976). It is reported that t^{w20} was generated from t^{w15} (Dunn and Bennett 1960), t^{w21} was generated from t^{w17} (Bennett and Dunn 1964), and t^{w32} was generated from t^{w10} (Bennett and Dunn 1964). However, a careful examination of the original records of L. C. Dunn (maintained by D. Bennett at the Sloan-Kettering Institute, New York, New York) reveals unequivocally that t^{w15} and t^{w20} were both transmitted directly from the same wild mouse (Dunn alludes to this observation on page 303 of Dunn 1957), and that t^{w17} and t^{w21} were also transmitted directly from a single wild mouse.

The progenitor of t^{w15} and t^{w20} was a wild male (number 1209) trapped in Kansas during November, 1955 (p. 303 in Dunn 1957). When this wild animal was mated to a $T/+$ female, only tailless (T/t) and normal tailed ($+/t$) offspring were born. It would be convenient to explain the origin of both t^{w15} and t^{w20} by suggesting that male 1209 was a compound heterozygote carrying both a complete t^{w5} group haplotype (t^{w15}) and a complete t^{w1} group haplotype (t^{w20}). Both t^{w1} and t^{w5} group haplotypes are common in North American populations of mice. However, males carrying two complete t haplotypes are known to be sterile. The progenitor of t^{w17} and t^{w21} was a wild female (number 1881) trapped in Arizona during February, 1956 (p. 304 in Dunn 1957). In this case, it would still be convenient to postulate that female 1881 was a compound heterozygote (t^{w17}/t^{w21}), since compound heterozygous females are fertile. However, according to Dunn (1957), female 1881 produced Brachy (short tailed) offspring when mated to a $T/+$ male. This result indicates that mouse 1881 carried a wild-type ($+$) allele at the t tail interaction factor within the t complex.

Both haplotypes t^{w20} and t^{w21} were shown to be transmitted through males at very high ratios (0.99 and 0.98, respectively, according to Bennett and Dunn 1964). Therefore, it is almost certain that both represent complete t haplotypes and were not derived by simple recombination between a wild-type chromosome and t^{w15} or t^{w17} . Rather, each postulated, direct generation — t^{w20} from t^{w15} and t^{w21} from t^{w17} — requires two separate but simultaneous genetic alterations. The t^{w5} group lethal gene had to revert to wild-type in each case at the same time as a new mutation occurred at another gene on the same chromosome. [Artzt et al. (1982a) have shown

that different, complementing lethal genes map to different loci spread throughout a 10cM region of the *t* complex.] This postulated mechanism of generation seems most unlikely, since no case of lethal gene reversion to wild-type has ever been observed for any *t* haplotype.

The t^{w32} haplotype was observed in an animal (number 16143) that was only two generations removed from a wild male (number 11093; Bennett and Dunn 1964). As Bennett and Dunn suggest, it is possible that the mother of 16143 already carried the t^{w32} haplotype. If this were true, it would imply that a single wild animal (11093) produced offspring carrying either t^{w10} (a member of the t^{w5} group) or t^{w32} (a member of the t^{12} group). The wild progenitor (11093) of t^{w32} was a male trapped in Montana and sent to L. C. Dunn during September, 1957. Though t^{w20} and t^{w21} are extinct, the t^{w32} haplotype is carried by many investigators and has been studied in great detail. Since t^{w32} is a complete *t* haplotype, it cannot have arisen by recombination between a wild-type chromosome and t^{w10} . In fact, the generation of t^{w32} directly from t^{w10} requires at least three separate but simultaneous genetic alterations. First is the unlikely reversion of the t^{w5} lethal gene associated with t^{w10} to wild type. At the same time, mutations at two independent loci must have occurred to produce the t^{w32} lethal gene set (Artzt et al. 1982a). Finally, it is interesting to note that the *H-2* haplotype carried within the t^{w32} haplotype is shared only by one other *t* haplotype, t^{12} , which is also the only other known member of the t^{w32} complementation group (Hammerberg et al. 1976).

While it is difficult to understand how t^{w20} , t^{w21} , and t^{w32} could have arisen independently of t^{w15} , t^{w17} , and t^{w10} , it is at least as difficult to assume that each arose by mutational events. One could postulate that (1) each wild progenitor carried one complete *t* haplotype opposite a short partial *t* haplotype with a different, independent lethal gene in an unknown nonsterile genomic configuration, and that (2) the generation of each new haplotype resulted from normal recombination between the complete and partial *t* haplotype. This model is far from satisfactory. Since the original wild animals involved can never be analyzed again, the relationship between each "newly generated" *t* haplotype and each progenitor must remain unknown.

With the demonstration that normal recombination can occur between different *t* haplotypes in a compound heterozygous female (Silver and Artzt 1981), the derivation of certain unusual *t* haplotypes can now be explained without resorting to mutational mechanisms. The t^{w75} haplotype carries both t^{w5} and t^{w1} lethal genes, in *cis*, but, as Shin and co-workers (1982) point out, "a seemingly unique haplotype, t^{w75} , is just a recombinatorial derivative of preexisting *t* chromosomes ...". Another set of *t* haplotypes— t^0 and t^6 —carry the same lethal gene but a different *H-2* haplotype (Hammerberg et al. 1976). Again, the data indicate that the t^6 haplotype could have been derived by a simple recombinant event between a t^0 chromosome and a t^{w1} chromosome. Further support for the generation of new *t* haplotype combinations by recombination comes from a recent, comparative survey of *H-2* antigenic determinants associated with many newly identified, wild *t* haplotypes (Sturm et al. 1982). All of the different *H-2* haplotypes observed could have been simply derived through recombination from a smaller set of ancestral *t* chromosomes.

Mouse *t* haplotypes have been studied for over 50 years, with a surge in

popularity among many investigators during the last decade. Nevertheless, only three cases of lethal gene switching in complete *t* haplotypes have been reported. In two of the three cases, the “new” *t* haplotypes were observed in the first generation of progeny obtained from wild animals. In the third case, the “new” *t* haplotype was observed in the second generation from the wild. The wild mice in all three cases were trapped between 1955 and 1957 from feral populations in the midwestern part of the United States. The interpretation that one lethal *t* haplotype generated a second lethal *t* haplotype is not fully supported in any of these three cases. Therefore, no unequivocal laboratory data exist to support the hypothesis that new lethal *t* haplotype genes are generated in wild populations by mutation of previously existing *t* haplotypes. It appears that *t* haplotype lethal genes are very stable and do not revert to wild-type or switch at a detectable frequency. A model of independent origin for different *t* haplotypes has been proposed and supported in a previous publication (Silver 1982). This model suggests that *t* haplotypes originated by introgression into *mus musculus* from an alien species. Each lethal gene could be explained by a different ancestral introgression event from the same alien species. According to this hypothesis, *t* haplotypes represent normal genomic regions (of the mouse chromosome 17 analogue) within the species from which they were derived and are mutant only in the context of the mouse genome. Many of the unusual structural and functional properties characteristic of *t* haplotypes can be explained by nonhomologies between wild-type and alien *t* haplotype forms of the *t* complex region and its gene products (see Silver 1982, for further discussion).

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