

Genetic Organization of the Mouse *t* Complex

Minireviews

Lee M. Silver

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York 11724

The mouse *t* complex (also known as the T locus or T/*t* complex) is a large region of chromosome 17 that has intrigued geneticists for more than 50 years with its striking and unorthodox features. These include suppression of recombination, disturbances of embryonic development and alterations of sperm differentiation and function. Much of our knowledge concerning the properties of this genetic complex is based on the early work performed by S. Gluecksohn Waelsch and L. C. Dunn with mutant forms of the *t* complex called *t* haplotypes (previously known as *t* alleles or *t* mutations). Many *t* haplotypes are characterized by lethal actions at particular stages during early embryogenesis. Lethal *t* haplotypes can be placed into a number of groups where members of each group do not complement each other. Complementation between *t* haplotypes of different groups is not complete, and prenatal death occurs in many, but not all, doubly heterozygous (*t*^x/*t*^y) embryos. Furthermore, all males doubly heterozygous for two lethal haplotypes are completely sterile. Other genes defined separately from *t* haplotypes but mapping within the *t* complex include *Brachyury* (*T*), *Kinky* (*Fu*^{ki}) and *Knobbly* (*Fu*^{kb}), which are recessive embryonic lethals; *Hybrid sterility-1* (*Hst-1*) and *Quaking* (*qk*), which cause male-specific sterility; *Hye*, which affects the level of male-specific H-Y antigen expression; and *Gt1*, which determines acceptance or rejection specifically of embryonal carcinoma cell grafts. All observations to date imply a crucial role for the *t* complex in mechanisms of development and differentiation. At the present time, however, the molecular events responsible for these *t* complex effects remain elusive. This review will therefore focus on recent advances in our understanding of the structure, rather than the function, of this region.

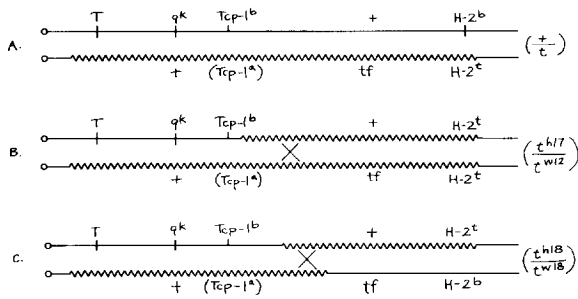
All known *t* haplotypes originated from wild populations, and almost all wild mouse populations analyzed from around the world have been found to be polymorphic for lethal or semi-lethal *t* haplotypes. Maintenance of a deleterious gene in a wild population is unexpected, but can be accounted for in this case by a further property of *t* haplotypes called transmission-ratio distortion (also known as segregation distortion). A wild heterozygous (+/*t*) male can transmit his *t* haplotype to more than 95% of his offspring, and it appears that the advantage of the *t* haplotype at fertilization can overcome the disadvantage of a certain frequency of homozygous lethality in the population as a whole. Genetic systems that involve the maintenance of deleterious gene complexes in wild populations by transmission-ratio distortion have been identified in other well studied species, and include the *SD* complex of *Drosophila melanogaster*, and the *Spore Killer* (*SK*) complex in *Neurospora* (Turner and

Perkins, *Genetics* 93, 587-606, 1979). A further property shared by the *t* complex and each of these other genetic systems is suppression of recombination over the localized region of chromatin encompassing the deleterious genes.

Recombination suppression along a region of chromosome 17 in +/*t* heterozygotes is the single property shared by all *t* haplotypes. Cytogenetic analyses provide evidence for a reduction in chiasma formation within the *t* complex region (Lyon et al., *Nature* 279, 38-42, 1979). Hence, the actual physical process of meiotic recombination within this region is suppressed. Breeding experiments carried out by Hammerberg and Klein (*Genet. Res.* 26, 203-211, 1975) have defined the region of recombination suppression as extending from and including the locus of *T* and the *H2* complex (wavy line in (A) of the figure). Recombinations in the regions distal to the *H2* complex and proximal to *T* occur at a normal level in +/*t* heterozygotes. As an operational definition, a complete *t* haplotype is considered to be one that suppresses recombination along the entire 12 cM region between *T* and *H2* in +/*t* heterozygotes. This region of chromosome 17, which represents approximately 1% of the mouse genome, is called the *t* complex.

Suppression of recombination is not absolute and a small number of partial *t* haplotypes (consisting of either proximal, central or distal portions of a complete *t* haplotype) have been recovered from rare (and possibly unequal) recombinatorial events observed in the laboratory. These partial *t* haplotypes have been used in attempts to map genetic factors responsible for each of the phenotypic effects associated with complete *t* haplotypes. The factors responsible for lethality are located in the middle-to-distal portion of the *t* complex, while the *T* interaction factor is located in the proximal region. At least three separable factors (one proximal, one central and one distal) interact to distort male transmission ratio (Lyon and Mason, *Genet. Res.* 29, 255-266, 1977; Hammerberg, *Genet. Res.* 37, 71-77, 1981). Other proximal and distal factors also interact to affect male fertility (Hammerberg, op. cit.). Only a complete *t* haplotype can be transmitted consistently at a very high ratio (greater than 95%) from +/*t* males, and it is this property that appears to allow the propagation of the *t*-*H2* complex through wild populations as an indivisible genetic unit, since partial *t* haplotypes have not been recovered from wild populations. Finally, breeding analyses with a variety of partial *t* haplotypes indicate that recombination suppression is a property of all *t* haplotypes, whether complete or partial (Lyon et al., op. cit.). It appears that the region of recombination suppression is coincident with, and thereby defines, the extent of *t* DNA/chromatin associated with any one particular *t* haplotype.

What molecular feature of *t* haplotypes could be responsible for recombination suppression? Until recently, the answer to this question remained enig-



matic. Possible explanations fall into two general categories. First, as suggested by Lyon, *t*-haplotype chromatin might be structurally different from wild-type chromatin. This structural difference could take the form of *t*-specific constitutive heterochromatin that is inherently defective in the process of recombination. Second, the DNA sequence organization of *t* haplotypes might be sufficiently different from its wild-type counterpart that recombination is suppressed through a lack of homology between the two forms of chromosome 17 in a $+/t$ heterozygote. The *t*-specific DNA sequence organization could be a result of rearrangements, multiple inversions, actual differences in primary sequence or a combination of these features.

Recent work has provided evidence for the latter explanation. In the reported study, mice were obtained that were heterozygous for two *t* haplotypes (t^{w12} and t^{h17}) with long overlapping regions of *t* chromatin (see (B) of the figure) (Silver and Artzt, *Nature* 290, 68–70, 1981). Recombination was observed in females between the markers *T* and *tf* at a level of 18%. Further analysis of recombinant chromosomes for alleles at *Tcp1* indicated that crossing-over was occurring only in the region of overlapping *t* DNA/chromatin distal to the *Tcp1* locus. This result clearly indicates that *t* chromatin is not inherently defective in the process of recombination. Instead, it would appear that a significant lack of homology exists between the DNA sequence organization of wild-type and *t*-haplotype regions of chromosome 17.

Studies with other *t* haplotypes indicate that normal recombination within regions of overlapping *t* DNA/chromatin is not unique to either t^{w12} or t^{h17} . Representative complete *t* haplotypes from two other lethal groups (t^{w5} and t^{12}) have been tested by the same experimental set-up described above and shown in (B) of the figure. Each of these *t* haplotypes recombines freely with *t* DNA from a distal portion of t^{w12} (Silver and Artzt, *op. cit.*). Lyon (*Mouse News Lett.* 64, 57, 1981) has examined recombination in a genotype with overlapping proximal (t^{w18}) and distal (t^{h18}) partial *t* haplotypes (see (C) of the figure). Free recombination was observed in the short region of overlapping *t* chromatin. These data imply the existence of a basic homology among all wild *t* haplotypes.

During early work on the *t* complex system, Lyon suggested that the rare recombination between wild-type DNA and *t* DNA might be unequal. In a direct test

of this hypothesis, a series of proximal partial *t* haplotypes was analyzed for alleles of the *Tcp1* gene, which maps to the central region of the *t* complex. All wild-type forms of mouse chromosome 17 carry a *Tcp1^b* allele, which codes for a basic form of the p63/6.9 protein, while all complete *t* haplotypes carry a *Tcp1^a* allele, which codes for an acidic form of the p63/6.9 protein. Surprisingly, Silver et al. (*PNAS* 77, 6077–6080, 1980) found that 25% of the proximal partial *t* haplotypes analyzed carried both alleles of *Tcp1* in cis position. If only a single *Tcp1* locus is present on each parental chromosome 17, the acquisition of two alleles by a single recombinant chromosome must involve unequal crossing-over relative to *Tcp1*. Those partial *t* haplotypes associated with only a single allele of *Tcp1* may have been derived by unequal crossing-over in a region outside of *Tcp1*, so that 25% is only the lower limit for the frequency of unequal crossing-over associated with rare recombinatorial events.

All the data concerning recombination within the *t* complex can be accounted for by a model in which the DNA sequence organization of *t* haplotypes is rearranged relative to the wild-type. Crossing-over would be infrequent in $+/t$ heterozygotes, because homologous regions of DNA would not be located at the same position on each homolog of chromosome 17. However, rare crossing-over that did occur between rearranged homologous sequences could result, because of shifts in pairing, in the duplication or deletion of particular genetic loci such as *Tcp1*. Finally, the general DNA sequence organization of the same region of all *t* haplotypes would be the same, allowing for normal crossing-over in regions of overlapping *t* DNA/chromatin.

Why is an altered genetic organization an inextricable part of all mouse *t* haplotypes? Perhaps this property is a necessary consequence of the mechanism by which *t* haplotypes originated. It is known from studies of *Drosophila* that relatively rapid changes can occur in the genomic organization of middle repetitive sequences, while structural loci remain unaffected. In fact, when *D. melanogaster* flies from certain populations that have evolved away from each other in this manner are crossed, offspring are afflicted with a phenomenon called hybrid dysgenesis (Thompson and Woodruff, *Nature* 274, 317–321, 1979). It is intriguing to speculate that all *t* haplotypes originated in a common ancestral pool of mice that were genetically isolated from the main population of *Mus musculus*. This could explain both structural and functional properties of *t* haplotypes as a partial mouse analog to *Drosophila* hybrid dysgenesis.

The power of classical genetic techniques to decipher the intricacies of a complex biological system has been amply demonstrated by studies of the mouse *t* complex. A further understanding of this system, however, will most certainly require use of the contemporary molecular technology.