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Genetics

Evidence for unequal crossing over within the mouse *T/t* complex

(*T* locus/two-dimensional gel electrophoresis/recombination/partial peptide mapping/major histocompatibility complex)

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ABSTRACT **The *Tcp-1* gene located within the *T/t* complex on chromosome 17 of the mouse codes for a major cell surface-associated protein p63/6.9. Previously, we identified two structural alleles of this gene which specify alternate forms of the p63/6.9 protein. The *Tcp-1*^{b} allele is associated with all wild-type chromosome 17; the *Tcp-1*^{a} allele is found only with chromosome 17 carrying a complete *t* haplotype. Normal recombination along a major length of chromosome 17 is suppressed in mice that are heterozygous for any complete *t* haplotype. Suppression is not complete, however, and rare crossing over between wild-type and *t* haplotype chromatin does occur.**

In this report, 15 rare recombinant chromosomes have been analyzed for *Tcp-1* alleles. The results indicate that in four independent events the *Tcp-1*^{b} and *Tcp-1*^{a} alleles have become associated in *cis* position in a single DNA molecule. Further genetic analysis provides support for the hypothesis that a significant nonhomology exists between the arrangement of DNA sequences on wild-type and *t*-carrying chromosome 17. This could account for both the suppression of normal recombination along the stretch of *t* chromatin and the frequent unequal crossing over when rare recombinational events do take place.

t haplotypes are found at a high frequency in wild mouse populations. These *t* haplotypes represent a variant form of an extensive region of chromosome 17, called the *T/t* complex, which has major effects on embryonic development and the differentiation of spermatozoa (for reviews see refs. 1–5). Recently, we identified a gene (*Tcp-1*) within the *T/t* complex that specifies a major cell surface-associated protein called p63/6.9 (6). *Tcp-1* was previously called *p63*, but has now been renamed to conform with rules for genetic nomenclature in the mouse (7). Three alleles of the *Tcp-1* gene have been defined. All wild-type chromosomes have the *Tcp-1*^{b} allele that specifies a basic form of the p63/6.9 protein (p63/6.9b). All complete *t* haplotypes have the *Tcp-1*^{a} allele that specifies an acidic form of the p63/6.9 protein (p63/6.9a). A deletion within the *T/t* complex known as T^{hp} is associated with a null allele, *Tcp-1*^{n}, which does not express any discernible form of the p63/6.9 protein. In a cell-free translation analysis of isolated testicular cell RNA, it has been found that the difference between p63/6.9b and p63/6.9a is encoded within the mRNA for these proteins (unpublished data). Therefore, *Tcp-1* appears to be the structural gene for the p63/6.9 protein.

An unusual property of the *T/t* complex is that, in mice of either sex heterozygous for a *t* haplotype, the entire chromosomal region from the centromere to the *H-2* complex is excluded from the normal process of recombination, but recombination along all other regions of the genome does not appear to be affected (8). Suppression of recombination within the *T/t* complex is not complete, however, and infrequent crossing over has been noted with the use of the genetic markers *T* and tufted (*tf*) (Fig. 1). The normal map distance between these markers is 7 cM; in the presence of a *t* haplotype, this distance may be reduced to 0.1–0.3 cM (only 1–3 gametes of every 1000 scored contain a recombinant chromosome). Rare recombinational events produce reciprocal forms of chromosome 17 that include only a portion of the original *t* haplotype. Chromosomes carrying the proximal portion of the original *t* haplotype will be called proximal *t* haplotypes; more than 100 of these haplotypes have been recovered during the last 50 years (9–11). All proximal *t* haplotypes remain associated with a t^T factor that interacts in *trans* with *T* to cause taillessness in T/t^T animals. Chromosomes carrying the distal portion of the original *t* haplotype will be called distal *t* haplotypes. The few distal *t* haplotypes identified thus far are lethal when homozygous and do not interact with the t^T factor (12).

Both proximal and distal partial *t* haplotypes continue to exclude normal recombination, in +/*t* heterozygotes, along the chromatin derived from the parental *t* haplotype; recombination is permitted in those regions of the genome that are separate from *t* chromatin (12, 13). These data led Lyon *et al.* (1) to postulate that *t* haplotypes have an altered form of "intercalary DNA" (defined as the DNA between structural genes) along their entire length and that this altered DNA prevents normal recombination.

With the use of partial *t* haplotypes and the T^{hp} deletion, we have mapped the *Tcp-1* gene to the region of chromosome 17 bounded by the centromere and *tf* (6), a distance of approximately 9 cM (Fig. 1). *Tcp-1* has been definitively separated from other genes identified in this region, including *T* (unpublished data), *qk*, *tf*, and *Low*.

In this report, we describe a further analysis of the *Tcp-1* alleles associated with partial *t* haplotypes. The results imply that rare crossing over between wild-type and *t* haplotype chromosomes may give rise to a new chromosome 17 which has a partial *t* haplotype and both structural alleles of the *Tcp-1* gene. Possible mechanisms by which alternate alleles of *Tcp-1* frequently become associated with a single chromosome are discussed.

MATERIALS AND METHODS

Animals. All mice were obtained from our colony at the Sloan–Kettering Institute. References for the characterization of each *t* haplotype used are as follows: t^{AE5} (14); t^{h2} (10); t^{h17} (15); t^{wPA} (16); t^0–t^{12} and t^{w1}–t^{w32} (9); t^{w71} and t^{w73} (17); t^{w75} (18); t^{46} and t^{w84} (11). The following proximal *t* haplotypes have not been previously described (the parental *t* haplotype from which each of these proximal *t* haplotypes was derived is listed in parentheses): t^{52}(t^{12}); t^{w86}(t^{w12}); t^{w88}(t^{w8}); t^{w92}(t^{w18}); t^{w95}(t^{w1}); t^{w100}(t^{w18}); t^{w108}(t^{w12}); t^{w109}(t^{w93}); t^{w110}(t^{w71}); t^{w111}(t^{w32}). t^{w93}, t^{w105}, and t^{w106} are complete *t* haplotypes that have been recovered from feral mice and assigned to the

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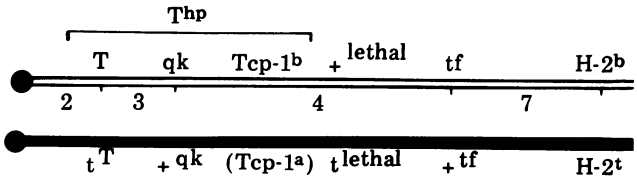

FIG. 1. Map of the *T/t* complex region of mouse chromosome 17. The bottom chromosome represents a complete *t* haplotype. The top chromosome includes markers that are normally present on the wild-type chromosome of laboratory animals. Map distances are marked in centimorgans; centromere is at the left. The locations of *t* haplotype factors (t^T and t^{lethal}) can only be estimated. The extent of the T^{hp} deletion is shown above the wild-type chromosome.

complementation groups listed in Table 1 (unpublished data).

Identification of Specific *T/t* Complex Genotypes. Complete *t* haplotypes have been identified by their interaction with the dominant mutation *T* to produce taillessness in *T/t* animals. A *t* haplotype in combination with a wild-type chromosome 17 produces an adult mouse that is morphologically normal. The *T* mutation in combination with a wild-type allele produces a mouse with a short tail; in combination with a *t* haplotype, a distinctive tailless mouse is formed. Chromosomes that contain only a proximal portion of the original *t* haplotype interact with *T* to produce taillessness; animals homozygous for proximal *t* haplotypes are viable and fertile and have tails of normal length.

Characterization of *Tcp-1* Alleles. Testicular cells or splenocytes were obtained and labeled in culture with [^{35}S]methionine as described (6). Two-dimensional gel analysis was performed on proteins soluble with the nonionic detergent Nonidet P-40 (19). All complete *t* chromosomes were analyzed in combinations with a chromosome carrying $Tcp\text{-}1^b$ in a $+/t$ genotype. All proximal *t* haplotypes were analyzed initially as homozygotes. Whenever $Tcp\text{-}1^a$ and $Tcp\text{-}1^b$ were identified on one chromosome, this was confirmed in at least three nonsibling animals carrying the same haplotype. In an analysis of >100 different animals carrying *t* haplotypes, the determination of *Tcp-1* types has been completely reproducible.

Partial Peptide Analysis. Two-dimensional gel electrophoresis was performed on approximately 50 μg of [^{35}S]methionine-labeled proteins from homozygous $Tcp\text{-}1^b$ and $Tcp\text{-}1^a$ testicular cells. Proteins were stained with Coomassie brilliant blue R, and stained gels were dried directly onto paper. Stained p63/6.9b, p63/6.9a, and p60/6.9 spots were excised from the dried gel and rehydrated with minimal quantities of sample buffer [0.125 M Tris·HCl, pH 6.8/1 mM EDTA/0.1% NaDodSO$_4$/10% (wt/vol) glycerol]. Rehydrated gel spots were used for partial peptide analysis with *Staphylococcus aureus* V8 protease as described by Cleveland *et al.* (20). Peptide gels were stained and fluorographed (21).

Table 1. *Tcp-1* alleles associated with *t* haplotypes

Complementation group	Complete *t* haplotypes: $Tcp\text{-}1^a$ allele	Proximal partial *t* haplotypes: $Tcp\text{-}1^a$ allele	Proximal partial *t* haplotypes: $Tcp\text{-}1^{ab}$ allele
t^0	t^0, t^1, t^6	t^{42}, t^{h2}	t^{AE5}
t^9	t^{w18}	t^{w92}, t^{w100}	—
t^{12}	t^{12}, t^{w32}	t^{52}, t^{w82}	t^{w111}
t^{w1}	$t^{w1}, t^{w12}, t^{w71}, t^{w75}$	$t^{w95}, t^{w110}, t^{w84}$	t^{w86}, t^{w108}
t^{w2}	t^{w2}, t^{w106}, t^{w8}	t^{w88}	
t^{w5}	$t^{w5}, t^{w93}, t^{w105}$	t^{w109}	
t^{w73}	t^{w73}		
t^{wPA}	t^{wPA}		

All lethal *t* haplotypes are assigned to a complementation group on the basis of the phenotype of homozygotes dying as embryos and by classical genetic complementation tests. The t^{w2} group is semilethal. The complete *t* haplotypes listed have all been analyzed for *Tcp-1* expression and have been shown to have a $Tcp\text{-}1^a$ allele. Proximal partial *t* haplotypes are listed according to the lethal complementation group from which they were derived.

RESULTS

Two Structural Alleles of the *Tcp-1* Gene. In our previous study (6), complete *t* haplotypes representing each of the known *t* complementation groups were analyzed for the expression of p63/6.9 by the technique of two-dimensional gel electrophoresis. The results demonstrated an association of the p63/6.9a form of the protein with all complete *t* haplotypes ($Tcp\text{-}1^a$ allele) and an association of the p63/6.9b form of the protein with all wild-type chromosomes 17 ($Tcp\text{-}1^b$ allele). In a continuation of this analysis, we have confirmed the presence of $Tcp\text{-}1^a$ in the 18 complete *t* haplotypes listed in Table 1. At least one member of each *t* complementation group has been analyzed within congeneic animals (greater than 14th backcross generation) having C3H or 129/Sv/TER backgrounds (Fig. 2).

***Tcp-1* Alleles of Partial *t* Haplotypes.** In our previous study (6), the *Tcp-1* alleles associated with a single proximal *t* haplotype (t^{w82}) and a single distal *t* haplotype (t^{h17}) were determined. The data demonstrated that only the proximal *t* haplotype was associated with $Tcp\text{-}1^a$. These results mapped the *Tcp-1* gene to the proximal region of complete *t* haplotypes. We have now conducted a survey of the *Tcp-1* alleles associated with 15 different proximal *t* haplotypes. Eleven of these haplotypes have a $Tcp\text{-}1^a$ allele (Fig. 2 *C* and *D*; Table 1). The other four proximal *t* haplotypes appear to express products of both the $Tcp\text{-}1^b$ and $Tcp\text{-}1^a$ alleles (Fig. 2*E*; Table 1).

Demonstration of Two Alleles on One Chromosome. The association of both *Tcp-1* alleles with a particular proximal *t* haplotype was demonstrated in animals assumed to be homozygous for this haplotype. One trivial interpretation of the data might be that these animals were actually not homozygous but in fact carried two different proximal *t* haplotypes, each of them associated with a single alternate *Tcp-1* allele. This interpretation is unlikely because the association of both alleles with t^{AE5} has been confirmed in five animals and with t^{w108} in four animals. Nevertheless, it is possible to rule out this interpretation through an analysis of *Tcp-1* expression in a genotype set up with a known deletion (T^{hp}) including both *T* and *Tcp-1* on one chromosome 17 and a t^{AE5} haplotype on the opposite chromosome 17. The cross $T^{hp}/+ \times t^{AE5}/t^{AE5}$ makes this possible by the production of tailless T^{hp}/t^{AE5} animals. All forms of the p63/6.9 protein synthesized by T^{hp}/t^{AE5} animals must be coded for by genes on the undeleted t^{AE5} chromosome present in this genotype. As shown in Fig. 2*E*, both p63/6.9b and p63/6.9a were expressed, indicating that both forms of the *Tcp-1* gene are carried by the same chromosome. This gene complex on a single chromosome will be referred to as $Tcp\text{-}1^{ab}$.

***Tcp-1*^{ab} Is Haplotype-Independent.** It is possible that the particular complete *t* chromosome involved in a recombinational event determines whether the resulting proximal *t* haplotype has a $Tcp\text{-}1^a$ allele or a $Tcp\text{-}1^{ab}$ complex. That this is not the case is clear from an analysis of two proximal *t* haplotypes (t^{w82} and t^{w111}) that were derived from the same complete chromosome (t^{w32}) in two independent recominational events; t^{w82} has a $Tcp\text{-}1^a$ allele, and t^{w111} has a $Tcp\text{-}1^{ab}$ complex. Hence, the acquisition of both of the parental *Tcp-1* alleles by a single recombinant chromosome appears to be a random event occurring with a probability of approximately 0.27 (4/15) coincident with rare recombination between a wild-type chromosome and a chromosome carrying any complete *t* haplotype.

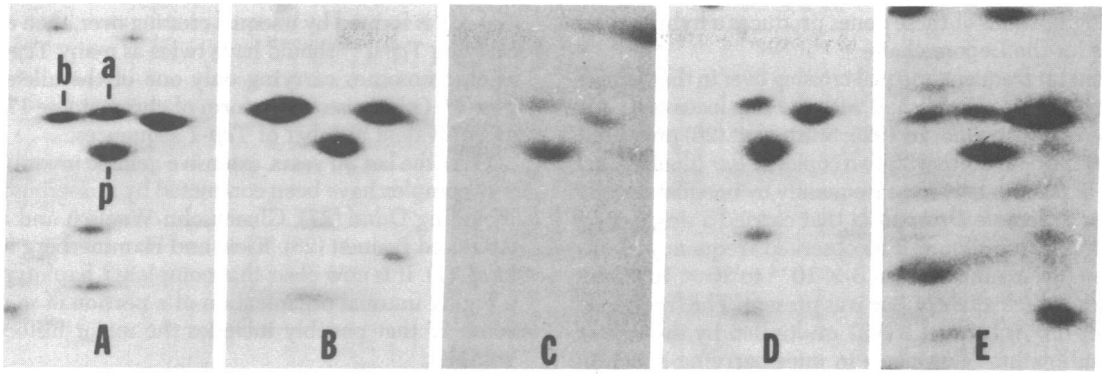

FIG. 2. Expression of the p63/6.9 proteins. A segment of the Coomassie blue-stained (*C*) gel or fluorograph (*A*, *B*, *D*, and *E*), encompassing the p63/6.9 region, is shown for the following genotypes: (*A*) $+/t^{w1}$ on a C3H background; a, p63/6.9a; b, p63/6.9b; p, p60/6.9. (*B*) +/+ C3H. (*C*) t^{h2}/t^{6}. (*D*) T^{hp}/t^{46}. (*E*) T^{hp}/t^{AE5}. (*A–D*) Testicular cells. (*E*) Female spleen.

Partial Peptide Analysis of p63/6.9. If p63/6.9b and p63/6.9a are actually coded for by two different closely linked genes, *Tcp-1*^{ab} could result from a recombinational event between the two genes. This explanation of the data is unlikely because it necessitates the presence of a silent allele on all complete *t* haplotypes opposite the position of *Tcp-1*^{b} and a silent allele on all wild-type chromosomes opposite the position of *Tcp-1*^{a}.

Furthermore, if p63/6.9b and p63/6.9a were products of different genes, one might expect to observe a difference in the peptide maps of these proteins. The partial peptide mapping procedure of Cleveland *et al.* (20) provides a means for estimating the relative homologies of proteins separated in analytical polyacrylamide gels. This procedure has been used to identify multiple differences in true allelic forms of major histocompatibility gene products (22). Partial peptide analysis of p63/6.9b and p63/6.9a provided no discernible difference (Fig. 3). Hence the amino acid difference(s) that must exist between p63/6.9b and p63/6.9a is subtle because it is not resolved by this technique.

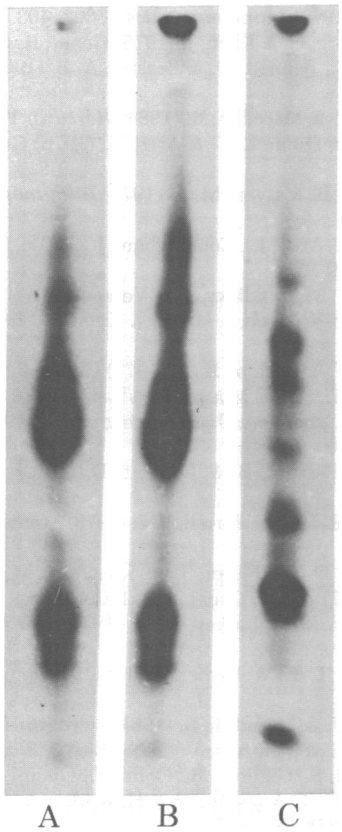

FIG. 3. Partial peptide patterns. (*A*) p63/6.9b. (*B*) p63/6.9a. (*C*) p60/6.9 (an unrelated protein identified in Fig. 2*A*). Each protein spot was overlayed with 100 ng of *S. aureus* V8 protease (Miles) and digested within the stacking gel for 30 min.

Map Location of the *Tcp-1*^{b} Gene. Our previous results clearly demonstrated that the *Tcp-1* gene was located proximal to *tf* on chromosome 17 (6). The data in this report allow a further localization of the *Tcp-1*^{b} allele. In two instances, t^{w108} and t^{w111}, a proximal *t* haplotype with *Tcp-1*^{ab} was formed by crossing-over within an animal having the genotype *T qk* $tf/t^{T} + ^{qk} + ^{tf}$. The proximal *t* haplotype derived in each case was $t^{T} + ^{qk}$ *tf*, indicating that breaks on both parental chromosomes occurred between the *qk* and *tf* loci. Because the recombinant chromosomes acquired the *Tcp-1*^{b} allele without acquiring *qk*, the *Tcp-1*^{b} allele must be located distal to *qk* on wild-type chromosomes. Hence, the *Tcp-1*^{b} allele has been mapped to the 4-cM region of chromosome 17 between the markers *qk* and *tf*, as indicated in Fig. 1.

DISCUSSION

By classical definition, alleles of a gene are mutually exclusive—any single chromosome can only be associated with a single allele of a particular gene. *Tcp-1*^{a} and *Tcp-1*^{b} appear to be alternate structural alleles of the *Tcp-1* gene, because all feral mouse chromosomes 17 analyzed to date carry either one allele or the other but not both. Furthermore, the proteins coded for by *Tcp-1*^{a} and *Tcp-1*^{b} are closely related and indistinguishable by a partial peptide analysis. This result implies an extensive amino acid sequence homology compatible with allelic gene products.

Normal meiotic recombination within the *T*/*t* complex region is suppressed in +/*t* heterozygotes. However, when rare crossing over is observed in the laboratory, we have found that it results frequently in the acquisition of both *Tcp-1*^{a} and *Tcp-1*^{b} by a single recombinant chromosome (*Tcp-1*^{ab}). These data indicate that *Tcp-1*^{a} and *Tcp-1*^{b} do not behave like classical alleles of a single gene. During early work on the nature of *t* chromosomes, it was suggested from indirect evidence that rare crossing over might be unequal (23). The data presented in this paper provide support for this hypothesis. If only a single copy of the *Tcp-1* gene is present on each parental chromosome 17, the acquisition of two alleles by a single recombinant chromosome must involve unequal crossing over relative to the *Tcp-1* gene.

Hemoglobin Lepore appears to be an example of a rare unequal crossing over event between two closely linked but nonallelic globin genes coding for the β chain and the δ chain (24). The two gene sequences are related enough to allow mispairing which results in the deletion of a portion of the β-chain gene and a portion of the δ-chain gene (25). Fusion of

the remaining portions of these genes produces a hybrid gene which codes for the Lepore chain.

A precedent for frequent unequal crossing over in the vicinity of a specific gene was established with the *Bar* locus on the X chromosome in *Drosophila*. In 1925, Sturtevant (26) presented evidence that chromosomes with two copies of *Bar* (double-bar) or no copies of *Bar* (no-bar) arose frequently by unequal meiotic crossing over in female *Drosophila* that carried a single copy of *Bar* on each chromosome. The observed frequency of unequal crossing over ranged from 3×10^{-4} to 10×10^{-4}, depending upon which allele of *Bar* was present. The frequency at which *Tcp-1*^{ab} is formed is 0.27 multiplied by the rate at which recombination takes place in mice carrying a *t* chromosome (1×10^{-3} to 3×10^{-3}) (11). Therefore, *Tcp-1*^{ab} occurs at a frequency of 3×10^{-4} to 9×10^{-4}. The correspondence between the *Bar* and *Tcp-1* data is striking.

Knowledge of the structure of *t*-carrying chromosomes could provide an understanding of the molecular mechanisms responsible for the high frequency of apparent unequal crossover events in the vicinity of the *Tcp-1* gene. Lyon *et al.* (1) recently summarized the evidence which indicates that the actual physical process of meiotic recombination is suppressed along the length of *t* chromatin in +/*t* heterozygotes. The data imply that *t* chromatin is structurally different from its wild-type counterpart. According to Lyon *et al.*, this structural difference could take the form of *t*-specific constitutive heterochromatin inherently defective in the process of recombination. An alternative explanation might be that *t* chromatin is not inherently defective but rather is different enough from its wild-type chromatin counterpart to suppress recombination through nonhomology. To distinguish between these two possibilities, female mice were bred to have a genotype in which two *t* haplotypes (t^{w12} and t^{h17}) with long overlapping regions of *t* chromatin are present on opposite homologues of chromosome 17—i.e., *T* (t^{h17}) + /t^{w12} *tf*. Recombination was observed between the markers *T* and *tf* at a frequency of 0.18 (14 recombinational events were observed in 76 offspring scored; unpublished data). Similarly unpublished preliminary data with other *t* haplotypes indicate that the observation of normal recombination between two *t* haplotypes is not unique to either t^{w12} *tf* or t^{h17}. These results clearly indicate that *t* chromatin is not inherently defective in the process of recombination. Instead, it would appear that a significant nonhomology exists between the DNA sequence organization of wild-type and *t* chromosomes 17.

All of the data concerning recombination within the *T*/*t* complex can be accounted for by a model in which the DNA sequence organization of *t* haplotypes is rearranged relative to wild type. Crossing over would be infrequent in +/*t* heterozygotes because homologous regions of chromatin would not be located at the same position on each form of chromosome 17. However, rare recombination that did occur between displaced homologous sequences could result in the duplication of particular genetic loci such as *Tcp-1*.

It is possible to account for the acquisition of *Tcp-1*^{b} and *Tcp-1*^{a} by a single recombinant chromosome with a model that does not involve unequal crossing over. If each wild-type chromosome carried two or more copies of *Tcp-1*^{b} and each *t* chromosome carried two or more copies of *Tcp-1*^{a}, then equal crossing over within the *Tcp-1* gene complex would result in a *Tcp-1*^{ab} chromosome. However, the fact that at least one of every four recombinant chromosomes is associated with *Tcp-1*^{ab} is not compatible with an expected occasional crossover event between tandem repeats of the *Tcp-1* gene.

With a cloned cDNA probe for the *Tcp-1* gene, it will be possible to determine the relative number of *Tcp-1* sequences associated with various forms of mouse chromosome 17. If *Tcp-1*^{ab} is formed by unequal crossing over, then chromosomes carrying *Tcp-1*^{ab} should have twice as many *Tcp-1* sequences as chromosomes carrying only one of the alleles *Tcp-1*^{a} or *Tcp-1*^{b}. Otherwise, every form of chromosome 17 should have an equivalent number of *Tcp-1* sequences.

Over the last 50 years, extensive genetic investigations of the *T*/*t* complex have been conducted by numerous investigators including Dunn (27), Gluecksohn-Waelsch and Erickson (2), Artzt and Bennett (28), Klein and Hammerberg (4), and Lyon *et al.* (1). It is now clear that complete *t* haplotypes represent a highly unusual organization of a portion of mouse chromosome 17 that possibly includes the major histocompatibility complex.

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