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NONHOMOLOGOUS PAIRING IN MICE HETEROZYGOUS FOR A *T* HAPLOTYPE CAN PRODUCE RECOMBINANT CHROMOSOMES WITH DUPLICATIONS AND DELETIONS

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ABSTRACT

We have investigated the structure and properties of a chromosomal product recovered from a rare recombination event between a *t* haplotype and a wild-type form of mouse chromosome *17*. Our embryological and molecular studies indicate that this chromosome (t^{wLub2}) is characterized by both a deletion and duplication of adjacent genetic material. The deletion appears to be responsible for a dominant lethal maternal effect and a recessive embryonic lethality. The duplication provides an explanation for the t^{wLub2} suppression of the dominant *T* locus phenotype. A reanalysis of previously described results with another chromosome *17* variant called Tt^{Orl} indicates a structure for this chromosome that is reciprocal to that observed for t^{wLub2}. We have postulated the existence of an inversion over the proximal portion of all complete *t* haplotypes in order to explain the generation of the partial *t* haplotypes t^{wLub2} and Tt^{Orl}. This proximal inversion and the previously described distal inversion are sufficient to account for all of the recombination properties that are characteristic of complete *t* haplotypes. The structures determined for t^{wLub2} and Tt^{Orl} indicate that rare recombination can occur between nonequivalent genomic sequences within the inverted proximal *t* region when wild-type and *t* chromosomes are paired in a linear, nonhomologous configuration.

THE proximal region of mouse chromosome *17* occurs naturally in variant forms known as *t* haplotypes, which express a number of effects on early embryonic development and germ cell differentiation (for reviews, see GLUECKSOHN-WAELSCH and ERICKSON 1970; BENNETT 1975; SHERMAN and WUDL 1977; SILVER 1985). *t* haplotypes are propagated through wild populations of mice by a male-specific transmission ratio distortion, and the chromosomal region defined by *t* DNA is maintained as an intact unit by suppression of recombination with wild-type homologues (see SILVER 1985). Insight toward the genetic basis for recombination suppression has been obtained by ARTZT and co-workers, who have demonstrated the existence of an inversion across

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the distal portion of *t* haplotypes (ARTZT, MCCORMICK and BENNETT 1982). This inversion is sufficient to account for recombination suppression along the distal portion of complete *t* haplotypes; however, the basis for recombination suppression along the proximal portion of *t* haplotypes has not been determined.

Although recombination is suppressed, rare crossing over between *t* haplotypes and wild-type chromosome *17* does occur in one of every 500–1000 viable gametes (BENNETT, DUNN and ARTZT 1976). Recently, the product of one such rare recombination event (named t^{wLub2}) was characterized and found to express two unusual phenotypes not associated with either of the parental chromosomes from which it was derived (WINKING and SILVER 1984). First, the t^{wLub2} chromosome suppresses the dominant short-tail phenotype expressed by mutations at the Brachyury (*T*) locus, so that animals which are doubly heterozygous for *T* and t^{wLub2} are born with tails of normal length. Second, the t^{wLub2} chromosome expresses a dominant lethal effect in all embryos that inherit the mutant chromosome from their mother, so that heterozygous females cannot transmit t^{wLub2} to their offspring.

Embryological studies have shown that the t^{wLub2} dominant lethal maternal effect is indistinguishable from that expressed by another chromosome *17* variant called T^{hp} (JOHNSON 1974, 1975; WINKING and SILVER 1984). Embryos that receive either mutant chromosome from their mother develop in an apparently normal fashion until late in gestation, when death inevitably occurs. There are no other published examples of lethal maternal effects in mammals. The expression of this effect by t^{wLub2} and T^{hp} is almost certainly a result of two independent mutations at the same locus [called *T*-associated maternal effect (*Tme*)] mapping between the *quaking* (*qk*) and *tufted* (*tf*) loci on chromosome *17* (WINKING and SILVER 1984). The T^{hp} chromosome expresses several independent phenotypes that are indicative of a large deletion over a 3–5 cM region of chromosome *17* encompassing the loci of *T* (JOHNSON 1975; BENNETT *et al.* 1975), *qk* (BENNETT *et al* 1975), *t* complex protein 1 (*Tcp-1*; SILVER, ARTZT and BENNETT 1979), *t* complex lethality-*w73* (tcl^{w73}; BABIARZ, GARRISI and BENNETT 1982) and *Rp-17* (E. MANN and R. W. ELLIOTT, unpublished results). Hence, it would appear that the expression of the maternal effect phenotype by T^{hp}-carrying embryos is a consequence of the deletion of the *Tme* locus.

In order to gain a better understanding of the genetic basis for the various phenotypes expressed by the t^{wLub2} chromosome, we have used several molecular probes to analyze the structure of this unusual chromosome. These studies have proved successful in correlating particular chromosomal aberrations with the *T* locus suppressor and lethal maternal effect phenotypes. Furthermore, the accumulated data provide information on the structure of all complete *t* haplotypes and the mechanism by which rare recombination occurs across inverted regions in *t* haplotype heterozygotes.

MATERIALS AND METHODS

Animals: All breeding experiments were performed at the Cold Spring Harbor Laboratory. The mutant forms of mouse chromosome *17* used in these experiments have

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been described previously and are referenced where they are first discussed. Gestational age of embryos was determined after detection of a vaginal plug in naturally ovulating females, and the previous midnight was taken to be time zero.

DNA isolation and analysis: High molecular weight DNA was isolated from the organs of mice according to previously described procedures (SILVER 1982). High molecular weight DNA was isolated from embryos as follows. Individual embryos were stored at −20° in 10 μl of phosphate-buffered saline. Two hundred microliters of 50 mM Tris-HCl, pH 8.0, 50 mM EDTA, 100 mM NaCl, and 0.2% Triton X-100 were added, and the sample was vortexed briefly. Twenty-five microliters of 20% SDS were added, followed by 25 μl of Proteinase K (10 mg/ml). Proteolytic digestion was allowed to continue for 6 hr at 56°. Samples were extracted sequentially with equal volumes of phenol, phenol/chloroform (1:1) and chloroform. Five micrograms of carrier tRNA was added, ammonium acetate was added to 2 M and the nucleic acids were precipitated in 2 volumes of ethanol at −20° for 8 hr. Nucleic acids were recovered by centrifugation, washed in 70% ethanol, allowed to dry briefly and resuspended in 200 μl of 5 mM Tris-HCl, pH 7.6, 0.1 mM EDTA by gentle rocking at 4°.

Restriction enzyme digestion, gel electrophoresis, Southern blot hybridization, and washing were performed as previously described (SILVER 1982; ELLIOTT and BERGER 1983). For the embryonic samples, miniaturized gel system was used, in which 4 μl samples (out of a final sample volume of 5–8 μl) were loaded in 1-mm wide wells. Hybridization signals could be obtained from as little as 100 ng of genomic DNA with this protocol.

Protein radiolabeling and analysis: Partially purified populations of spermatogenic cells or splenocytes were prepared, radiolabeled and analyzed by two-dimensional gel electrophoresis as described previously (SILVER, ARTZT and BENNETT 1979; SILVER *et al.* 1983). The first-dimension isoelectric focusing gels contained pH 6–8 ampholytes, and the second-dimension SDS gels contained 12.5% polyacrylamide.

RESULTS

The t^{wLub2} chromosome expresses a new recessive embryonic lethal mutation: The t^{wLub2} chromosome was generated by crossing over between a *t* haplotype and a wild-type homologue. Previous studies have suggested that such rare recombination events are often unequal, resulting in the duplication or deletion of genetic material (LYON and MEREDITH 1964; BENNETT, DUNN and ARTZT 1976; SILVER, WHITE and ARTZT 1980; SILVER 1983). There are many examples of deletions that cause recessive lethal phenotypes as a consequence of the absence of a vital gene product within the homozygous mutant embryo (GLUECKSOHN-WAELSCH *et al.* 1974; RUSSELL 1971). If t^{wLub2} is associated with a lethal mutation not present in the haplotype from which it was derived (t^{wLub1}), this might imply that a deletion of genetic material accompanied the formation of the t^{wLub2} chromosome.

We set out to determine if the t^{wLub2} chromosome carries a new lethal mutation that blocks development at an earlier stage than the lethal period characteristic of the homozygous t^{wLub1} phenotype [between 11 and 12 days after conception (WINKING 1979)]. Males which transmit the t^{wLub2} chromosome at a ratio of 95% or greater were mated to females carrying t^{wLub2}, and DNA was isolated from 22 7½- to 8½-day postfertilization embryos. The *t* genotype of each embryo was determined by genomic blot analysis with a DNA probe (for the *Hba-4ps* locus) that detects a restriction fragment length polymorphism between the t^{wLub2} chromosome and the wild-type homologue present in each

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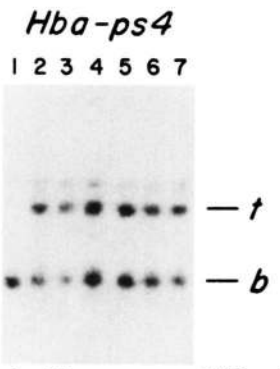

FIGURE 1.—Lethal period analysis of homozygous t^{wLub2} embryos. Embryos were obtained at 7½–8½ days after conception from the t^{wLub2} mating described in the RESULTS section. DNA was prepared, digested with *Taq*I, subjected to electrophoresis and analyzed by Southern blot hybridization with the α-globin pseudogene-4 DNA probe. Lane 1 represents control DNA from an adult wild-type animal; only the wild-type allele of *Hba-4ps* is observed. Lanes 2 through 7 are representative of the results obtained with all embryos; both wild-type and *t*-specific alleles at the *Hba-4ps* locus are observed, indicating a heterozygous ($+/t^{wLub2}$) genotype. If homozygous t^{wLub2} embryos survived to this stage, only the *t*-specific restriction fragment would be present in the pattern.

parent (FOX, SILVER and MARTIN 1984). If the t^{wLub2} does not carry an early lethal mutation, we would expect to find 10.5 homozygous mutant embryos, 11 heterozygous embryos, and 0.5 homozygous wild-type embryos in a group of 22; however, no homozygous t^{wLub2} embryos were detected at this stage of development (see Figure 1). A χ^2 goodness-of-fit test with Yate's correction for small samples was performed to compare the data obtained with that expected, and a value of 19 was obtained. This result provides evidence ($P < 0.001$) for the expression by the t^{wLub2} chromosome of a new recessive lethal mutation before day 7½–8½ of development.

The t^{wLub2} chromosome fails to complement the t^{w73} lethal mutation: The t^{w73} haplotype contains a lethal factor that maps within the T^{hp} deletion (BABIARZ, GARRISI and BENNETT 1982), and T^{hp}/t^{w73} embryos express the same lethal phenotype as homozygous t^{w73} embryos, with both dying at 6½–7½ days after conception. Since the t^{wLub2} and T^{hp} chromosomes may have lesions in the same genomic region, it was possible that the t^{w73}-defined lethal locus might also be deleted or otherwise mutated within the t^{wLub2} chromosome. We set up a complementation experiment to test this hypothesis [previous complementation studies have shown that the parental t^{wLub1} haplotype can complement the t^{w73} haplotype lethality (KLEIN, SIPOS and FIGUEROA 1984)]. Heterozygous males which transmit t^{wLub2} to greater than 95% of their offspring were mated to T/t^{w73} females. Progeny with normal tails may have either a t^{wLub2}/t^{w73} or t^{wLub2}/T genotype; an equal number of progeny with each genotype would be expected if t^{w73} and t^{wLub2} were fully complementing. The *Hba-4ps* clone was used as a probe of genomic restriction digests to distinguish between these two genotypes. Of the 47 progeny with normal tails analyzed, no homozygous *t*-specific (t^{wLub2}/t^{w73}) patterns were observed. This indicates that the t^{w73}/t^{wLub2} progeny class is not viable (or has an extremely low viability). Therefore, the

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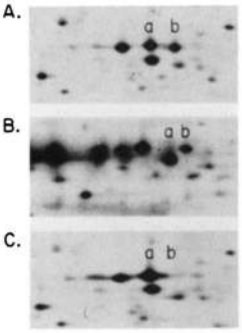

FIGURE 2.—Two-dimensional gel analysis of *Tcp-1* expression by animals with different *t* complex genotypes. A, Control pattern of expression of both wild-type (b) and *t*-specific (a) products of the *Tcp-1* gene by an animal heterozygous for a complete *t* haplotype (t^{wLub1}) and a wild-type form of chromosome *17*. B, Pattern of *Tcp-1* expression observed with an animal heterozygous for t^{wLub2} and a wild-type form of chromosome *17*. Only the wild-type product (b) of *Tcp-1* is observed. C, Pattern of *Tcp-1* expression observed with an animal heterozygous for t^{wLub2} and a proximal partial *t* haplotype. Only the *t*-specific product (a) is observed. Patterns in A and C are from testicular cells; the pattern in B is from splenocytes. We have shown previously that the *Tcp-1* gene products are identical in testis and spleen (SILVER, ARTZT and BENNETT 1979). The animal used for pattern B was a female.

event that gave rise to t^{wLub2} most likely also inactivated the locus defined by the t^{w73}-associated lethal mutation. Because homozygous t^{w73} embryos die 6½ to 7½ days postconception, the lethality associated with t^{wLub2} could be equivalent to the t^{w73} lethality.

The t^{wLub2} chromosome does not express either known form of the TCP-1 protein: The *Tcp-1* structural gene maps to the approximate region within which the t^{wLub2} recombination event occurred. Since *Tcp-1* appears to be deleted from the T^{hp} chromosome, it was possible that *Tcp-1* might also be functionally deleted from the t^{wLub2} chromosome. The *Tcp-1* gene codes for a protein (named TCP-1 or p63/6.9) expressed at high levels in male germ cells (SILVER, ARTZT and BENNETT 1979). Two allelic forms of the TCP-1 protein are distinguishable by two-dimensional gel electrophoresis. The basic form of the protein (TCP-1B) is a product of the $Tcp\text{-}1^b$ allele carried by all wild-type (non-*t*) examples of chromosome *17*, whereas the acidic form of the protein (TCP-1A) is a product of the $Tcp\text{-}1^a$ allele associated with all complete *t* haplotypes. T^{hp} has been the only known example of chromosome *17* that expressed neither TCP-1B nor TCP-1A (SILVER, WHITE and ARTZT 1980).

To determine the *Tcp-1* allele carried by the t^{wLub2} chromosome, we performed a comparative two-dimensional gel analysis of [^{35}S]methionine labeled proteins from animals heterozygous for t^{wLub2} with either a wild-type chromosome or with another *t* haplotype. As shown in Figure 2, heterozygous $+/t^{wLub2}$ mice express only TCP-1B as previously described (SILVER *et al.* 1983), whereas heterozygous t^{h2}/t^{wLub2} mice express only TCP-1A. This result indicates that the t^{wLub2} chromosome is not expressing either known form of the TCP-1 protein.

Thus, three new linked mutations arose in t^{wLub2}. A deletion would be the

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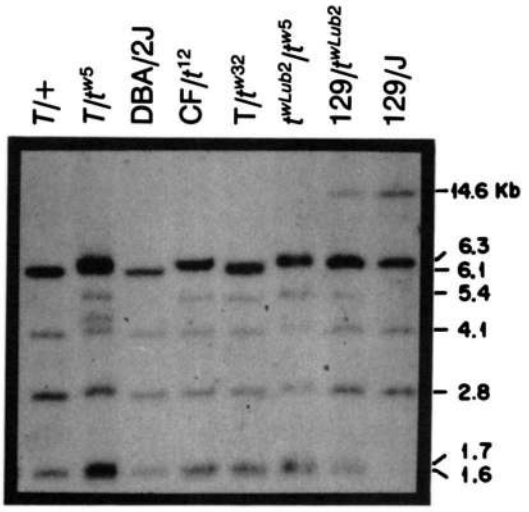

FIGURE 3.—Genomic blot analysis of *Rp-17* DNA alleles associated with different chromosome *17* genotypes. Total liver DNA was digested with *Bam*HI, subjected to electrophoresis and analyzed by Southern blot hybridization with the pMK174 probe. The first five lanes illustrate the presence of two *t*-specific restriction fragments at 5.4 kb and 1.6 kb in all the DNAs from mice carrying a *t* chromosome. These appear to be allelic to the 4.1- and 2.8-kb wild-type restriction fragments. The restriction fragment directly above the 4.1-kb restriction fragment in the T/t^{w5} lane is due to plasmid contamination of this genomic DNA preparation.

simplest explanation for multiple, genetically linked mutations. The maternal effects exhibited in both t^{wLub2} and T^{hp} females are probably a consequence of deletions over the same *Tme* locus, which must be closely linked to *Tcp-1* and the t^{w73}-associated lethality.

Evidence that t^{wLub2} also carries a duplication: In order to further investigate the structure of the t^{wLub2} chromosome, we performed a genetic analysis with a cDNA clone (pMK174) that hybridizes to sequences located in the proximal portion of chromosome *17*. These sequences define the *Rp-17* locus (MANN, ELLIOTT and HOHMAN 1984). As shown in Figure 3, pMK174 hybridizes with four *Bam*HI restriction fragments in wild-type DNA. Three of these are located on chromosome *17*. All complete *t* haplotypes contain 5.4- and 1.6-kb fragments that are replaced by 4.1- and 2.8-kb fragments in wild-type chromosomes (E. MANN and R. W. ELLIOTT, unpublished results). Other studies have clearly demonstrated the allelic relationship of the 5.4- and 1.6-kb pair of bands with the 2.8- and 4.1-kb pair of bands (L. M. SILVER and R. W. ELLIOTT, unpublished results). The 6.1-kb fragment is allelic to the 6.3-kb fragment, one of them being present in each of the mouse strains studied. They have been mapped to chromosome *17* using two sets of recombinant inbred strains (MANN, ELLIOTT and HOHMAN 1984). The fourth fragment has four alleles, two of which, 1.7 kb and 14.6 kb, are shown in Figure 3. These fragments are not located on chromosome *17* (E. MANN and R. W. ELLIOTT, unpublished results).

Our genetic analysis with the pMK174 clone was based on the same principle

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used for the analysis of *Tcp-1*. Genomic DNA obtained from animals heterozygous for t^{wLub2} with either another *t* haplotype (t^{w5}) or a wild-type (strain 129/SvJ) chromosome was digested with *Bam*HI and was analyzed by Southern blot hybridization to pMK174. As shown in Figure 3, heterozygous t^{wLub2}/t^{w5} mice carry both the *t*-specific and the wild-type chromosome *17* restriction fragments identified by pMK174. Since the t^{w5} chromosome is known not to carry the wild-type 2.8- and 4.1-kb fragments, the t^{wLub2} chromosome must be responsible for their presence in the genome. However, animals that carry t^{wLub2} and a wild-type (strain 129) homologue of chromosome *17* have both *t*-specific and wild-type pMK174 restriction fragments. This result indicates that the t^{wLub2} chromosome must also be responsible for the *t*-specific 1.6- and 5.4-kb fragments. These data demonstrate that the t^{wLub2} chromosome carries a duplication of genetic material, with both wild-type and *t*-specific forms of pMK174 sequences at the *Rp-17* locus.

The t^{wLub2} chromosome has been analyzed in a similar fashion with two other molecular probes [Tu48 and Tu122 described by FOX *et al.* (1985)] that detect restriction fragment length polymorphisms within the proximal portion of the *t* complex. The Southern blot results demonstrate that t^{wLub2} carries only the wild-type allele of T48, and only the *t*-allele of T122 (FOX *et al.* 1985 and data not shown).

The structure of the Tt^{Orl} chromosome appears reciprocal to that of t^{wLub2}: Previous studies of the Tt^{Orl} chromosome have demonstrated a duplication as well as a deletion of genetic material from this partial *t* haplotype (SILVER, LUKRALLE and GARRELLS 1983). The *Tcp-1* gene is duplicated with both wild-type and *t*-specific alleles present (ALTON *et al.* 1980), and sequences identified by pMK174 are deleted from the Tt^{Orl} chromosome (E. MANN and R. W. ELLIOTT, unpublished results). This pattern of duplication and deletion is the reciprocal of that found with t^{wLub2}. Furthermore, the pattern of T48 and T122 DNA alleles found in association with Tt^{Orl} [Tt^{Orl} carries a *t*-specific allele of T48 and a wild-type allele of T122; FOX *et al.* (1985) and data not shown] also provides support for the reciprocal relationship between the Tt^{Orl} and t^{wLub2} chromosomes. The simplest explanation for this relationship is that t^{wLub2} and Tt^{Orl} represent reciprocal products of the same type of recombination event.

DISCUSSION

A t^{wLub2} duplication can explain the *T* locus suppressor effect: Many independent mutations at the *T* locus have been recovered, and the phenotypes expressed by nearly all of these mutations are the same: heterozygous *T*/+ animals express a dominant short-tail phenotype, whereas homozygous *T*/*T* embryos die before day 12 of development (BENNETT 1975). Several *T* locus alleles (including T^{hp} and Tt^{Orl} have been characterized as deletions (SILVER, ARTZT and BENNETT 1979; SILVER, LUKRALLE and GARRELLS 1983). This result implies that a dosage effect is responsible for the short-tail phenotype. Wild-type mice carry two doses of a functional *T* gene and develop a normal tail, but mice that carry a single dose of a functional *T* gene are born with a short tail.

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We have shown that DNA sequences mapping near the *T* locus are duplicated within the t^{wLub2} chromosome and that these sequences are also deleted from the reciprocal Tt^{Orl} chromosome. Furthermore, the *T* locus itself appears deleted from Tt^{Orl}. These facts alone provide support for the duplication of a normally functioning form of the *T* locus, with one wild-type allele and one *t* allele, on a single t^{wLub2} chromosome. Since heterozygous +/*t* animals have tails of normal length, one would expect T/t^{wLub2} animals to also have normal tails, as is observed. Therefore, we propose that the suppression of the *T* phenotype by the t^{wLub2} chromosome is almost certainly accounted for by a duplication of the normal allele at the *T* locus.

The t^{wLub2} lethal maternal effect is caused by a deletion of the *Tme* locus: The T^{hp} maternal effect results from a deletion of genetic material defining the *Tme* locus between the loci of *qk* and *tf* on chromosome *17*. A major goal of the studies described in this report was to determine whether the t^{wLub2} maternal effect was also caused by a deletion of the *Tme* locus. We have obtained evidence for the deletion of genetic material from the vicinity of the *Tme* locus within the t^{wLub2} chromosome. This deletion includes the *Tcp-1* locus and at least one locus necessary for early embryonic development. Further evidence for the deletion of the *Tme* locus from t^{wLub2} has been derived from embryological studies that demonstrate a duplication of the *Tme* locus within the Tt^{Orl} chromosome (N. SARVETNICK and L. M. SILVER, unpublished results). Together, these results provide strong support for a deletion of the *Tme* locus from the t^{wLub2} chromosome.

Evidence for a proximal inversion in complete *t* haplotypes: The accumulated data indicate that a recombination event between a *t* haplotype and a wild-type chromosome caused the duplication of certain genetic loci (*T* and *Rp-17*) as well as the deletion of other loci (*Tme*, *Tcp-1* and the t^{w73} lethal locus) in the generation of the t^{wLub2} chromosome. However, a simple unequal recombination event between homologous chromosomes would cause either a duplication or a deletion, but not both. In order to explain the generation of t^{wLub2}, we postulate the existence of an inversion over the proximal region of *t* haplotypes, extending from the *T* locus and *Rp-17* to the loci of *Tcp-1* and *Tme*. A correctly oriented recombination event between these two sets of inverted loci would produce a chromosome with all of the genetic properties of t^{wLub2} (see Figure 4).

The reciprocal chromosomal product generated by such a recombination event would have a set of genetic properties indistinguishable from that actually observed for the Tt^{Orl} chromosome, with a duplication of *Tcp-1* and *Tme*, and a deletion of the *T* locus and *Rp-17*. Therefore, the simplest explanation for the structures of both t^{wLub2} and Tt^{Orl} is that both were generated by the same type of nonhomologous crossing over between nonequivalent genomic sequences within an inversion, as diagrammed in Figure 4. The proximal and distal limits of such an inversion are defined by flanking sequences detected by the genomic clones Tu48 and Tu122. These sequences were neither duplicated nor deleted from t^{wLub2} or Tt^{Orl}.

Cytological examinations of paracentric inversion heterozygotes often show

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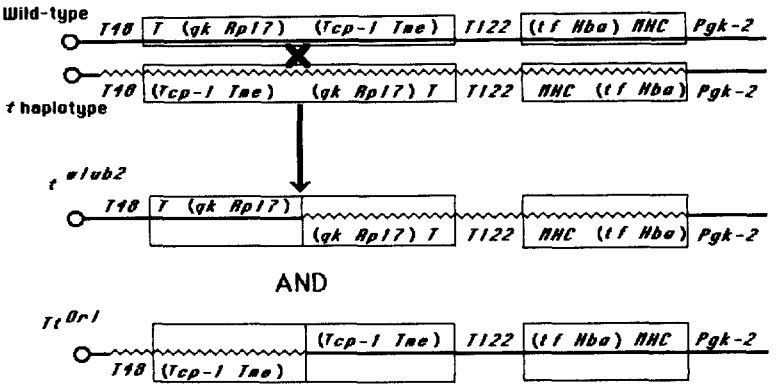

FIGURE 4.—Genetic organization of the wild-type and *t* haplotype forms of the mouse *t* complex and the derivation of t^{wLub2} and Tt^{Ori}. The top chromosome diagram represents the wild-type organization and the second chromosome diagram represents the *t* haplotype organization of the proximal portion of the *t* complex on chromosome *17*. The zig-zag line indicates *t* DNA. The region present within the brackets has been inverted in *t* haplotypes relative to wild type. The recombination events that gave rise to both t^{wLub2} and Tt^{Ori} occurred in the region indicated by the X. The reciprocal products of crossing over in this region are indicated as the third and fourth chromosome diagrams, which represent the structures of t^{wLub2} and Tt^{Ori}, respectively.

the formation of an inversion loop during the early pachytene stage of meiosis. Recombination within these inversion loops produces anaphase bridges and chromosome fragments representing dicentric and acentric chromosomes (STURTEVANT and BEADLE 1936). Although crossing over occurs at near-normal frequencies, these products will result in unbalanced gametes and inviable zygotes. Therefore, although recombination appears to be suppressed across such inversions, it is the observation in viable progeny and not the process that is mainly affected.

Cytological analysis of *t* haplotype heterozygotes has never revealed inversion loops, anaphase bridges or chromosome fragments (WOMACK and RODERICK 1974; TRES and ERICKSON 1982). The lack of inversion loops in +/*t* heterozygotes could be attributed to topological limitations imposed by the relatively small size of the inversions. Alternatively, the close proximity of the adjacent proximal and distal inversions might cause steric hindrance and render the resulting double-loop structures highly unstable. Two independent laboratories have demonstrated a suppression of the actual physical process of crossing over in the *t* region (FOREJT and GREGOROVA 1977; FOREJT, CAPKOVA and GREGOROVA 1980; LYON *et al.* 1979). Hence, recombination suppression in *t* heterozygotes is probably not a consequence of the inviability of recombination products.

Evidence has been obtained by TRES and ERICKSON (1982) for the linear, nonhomologous pairing of + and *t* regions in at least one experiment, with sufficient resolution to detect inversions of the size predicted from the *t* genetic data. The failure to form inversion loops has been previously reported in other systems (MCCLINTOCK 1931; MCCLINTOCK 1933; COLEMAN 1948; WHITE 1962; ASHLEY, MOSES and SOLARI 1981). In these systems, linear pairing is observed

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in many nuclei, with straight synaptonemal complexes joining genetically inverted segments. These observations have led to the conclusion that chromosome pairing initiates at a terminus and proceeds by a zipping mechanism that is not dependent on DNA homology (reviewed in COMINGS and OKADA 1972).

Recent cytological analysis of mouse inversion heterozygotes actually suggests that chromosome pairing is a biphasic process with inevitable nonhomologous associations (MOSES *et al.* 1982). During the early pachytene stage of meiosis, chromosomes are directed to pair homologously with the formation of inversion loops. At the late pachytene stage, they become subject to steric parameters and pair as closely as possible. Thus, at late pachytene, all inversion loops resolve into linear, nonhomologously paired structures.

Our model for the generation of both t^{wLub2} and Tt^{Orl} predicts that the crossing-over event occurred while the two chromosomes were paired in this type of linear nonhomologous fashion. The demonstration of nonhomologous chromosomal pairing in *t* heterozygotes makes it possible to explain the mechanism by which rare recombination occurs in this region. The accumulated molecular data suggest that other rare recombination events have occurred within the well-characterized inverted regions with the production of partial *t* haplotypes that have both duplicated and deleted loci (HERRMANN *et al.* 1986). These events can be explained by the occasional chance pairing and recombination of nonallelic members of a repetitive DNA family. It is important to emphasize that such events will occur only rarely, and only a fraction will produce viable recombinant products.

The site at which each illegitimate crossing-over event has occurred will define the new limit of the inverted region that remains on each partial *t* haplotype product (see Figure 4). As a consequence, a continued suppression of recombination should be observed in coincidence with the region of *t* DNA that remains in all descendants of the original recombinant animal. Breeding experiments have confirmed the correlation between the extent of *t* DNA and recombination suppression (FOX *et al.* 1985). Furthermore, all genetic and cytological data are consistent with the model presented here for the genetic and physical structure of *t* chromosomes.

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Note added in proof: WILLISON, DUDLEY AND POTTER (1986) have recently obtained and characterized clones of the *Tcp-1* gene studied in this report. Their data provide conclusive evidence that the *Thp* chromosome is deleted for the structural gene coding for the TCP-1 protein. Their data are also consistent with the deletion of this same gene by the *twlub2* haplotype.

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