CHARACTERIZATION OF A RECOMBINANT MOUSE *T* HAPLOTYPE THAT EXPRESSES A DOMINANT LETHAL MATERNAL EFFECT

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ABSTRACT

The t^{wLub2} chromosome was generated by rare recombination between a complete *t* haplotype and a wild-type form of mouse chromosome *17*. This recombinant chromosome expresses a dominant lethal effect in all embryos that inherit the mutant chromosome from their mothers. The phenotype of this maternal effect is indistinguishable from that expressed by the previously described T^{hp} deletion chromosome. It appears likely that the crossing over event that gave rise to t^{wLub2} was unequal and resulted in the alteration or deletion of a gene (which is named the T-associated maternal effect locus, *Tme*) that must be inherited from the mother in order for normal development to proceed through late stages of gestation. The results presented here allow a mapping of the *Tme* locus between the quaking and tufted loci which are 3 cM apart within the proximal region of chromosome *17*.

FEMALE mice that are heterozygous for the dominant Hairpin (T^{hp}) mutation cannot normally transmit this mutation to any of their viable live-born offspring. All heterozygous $T^{hp}/+$ embryos that have received T^{hp} from their mothers develop in an apparently normal fashion until late in gestation when death inevitably occurs and the fetuses are resorbed (JOHNSON 1974). Transmission of this mutation on the paternal chromosome is normal, and in matings between $T^{hp}/+$ males and $T^{hp}/+$ females, heterozygous individuals receiving the paternal T^{hp} chromosome live, whereas those receiving the maternal T^{hp} chromosome die. The T^{hp} chromosome can be recovered from the mother if both T^{hp} and wild-type forms of chromosome *17* are maternally inherited simultaneously through complementary nondisjunction in both parents (WINKING 1981). These results indicate that the lethality is determined by the embryonic genome rather than by the maternal environment. The T^{hp} maternal effect is the only one of its kind to be reported in mammals.

The physiological basis for this maternal effect is not understood. However, cells from a maternal $T^{hp}/+$ embryo can be rescued into somatic and germ line tissues of a normal adult animal by chimerization with a wild-type embryo

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(BENNETT 1978). This result suggests that the T^{hp} maternal effect is caused by the absence of a diffusable factor or a particular cell surface component.

Recent nuclear transplantation experiments performed by MCGRATH and SOLTER (1984) have demonstrated a nuclear (as opposed to a cytoplasmic) origin of the T^{hp} lesion within the fertilized egg. This result suggests the possibility of a differential functioning of maternal and paternal forms of chromosome *17* during embryogensis. However, normal mouse embryos can express both maternal and paternal alleles of *Glo-1* and *H-2* on chromosome *17* as well as *Tcp-1* (ALTON 1982) which is located within the region deleted by T^{hp} (SILVER, ARTZT and BENNETT 1979).

The T^{hp} mutation has been characterized genetically as a 3- to 5-cM deletion spanning the loci of Bachyury (*T*), quaking (*qk*) (BENNETT *et al.* 1975) and t complex protein-1 (*Tcp-1*), within the proximal region of chromosome *17* known as the t complex (see Figure 1A). Another deletion in this region, Tt^{Or1}, also spans *T* and *qk* but does not extend to *Tcp-1* (ALTON *et al.* 1980) and does not express the maternal effect. These data indicate that the maternal effect is a consequence of a locus situated between the centromere and *T* or between *qk* and tufted (*tf*).

We report here the discovery of another mutant form of mouse chromosome *17* that expresses a maternal effect very similar to that associated with the T^{hp} deletion. This novel chromosome (t^{wLub2}) was generated in a rare recombination event between a complete *t* haplotype and a wild-type form of chromosome *17*. Genetic and embryological analyses suggest that the maternal effects associated with T^{hp} and t^{wLub2} result from the same primary lesion at a *T*-associated maternal effect locus (*Tme*) mapping between *qk* and *tf*.

MATERIALS AND METHODS

The t^{wLub2} chromosome originated in the laboratory of H. WINKING in Lübeck. Genetic studies were carried out in Lübeck and Cold Spring Harbor. Embryological studies were carried out in Lübeck.

RESULTS AND DISCUSSION

A proximal region of mouse chromosome *17* occurs in variant forms known as *t* haplotypes which have been recovered from commensal and feral mouse populations from around the world (BENNETT 1975; NIZETIC, FIGUEROA and KLEIN 1984). All of these naturally occurring *t* haplotypes are closely related to each other but are structurally distinct from "wild-type" forms of the chromosome (see SILVER 1981 for review). *t* haplotypes are propagated through wild populations by a male-specific transmission ratio distortion, and the chromosomal region defined by *t* chromatin is maintained as an intact unit by suppression of recombination with a wild-type homologue. The t^{wLub1} haplotype was recovered from a population near Ancarano, Italy (WINKING 1978). The original mutant haplotype was present on a metacentric chromosome that resulted from a Robertsonian translocation of mouse chromosomes 4 and 17 [designated Rb(4.17)13Lub].

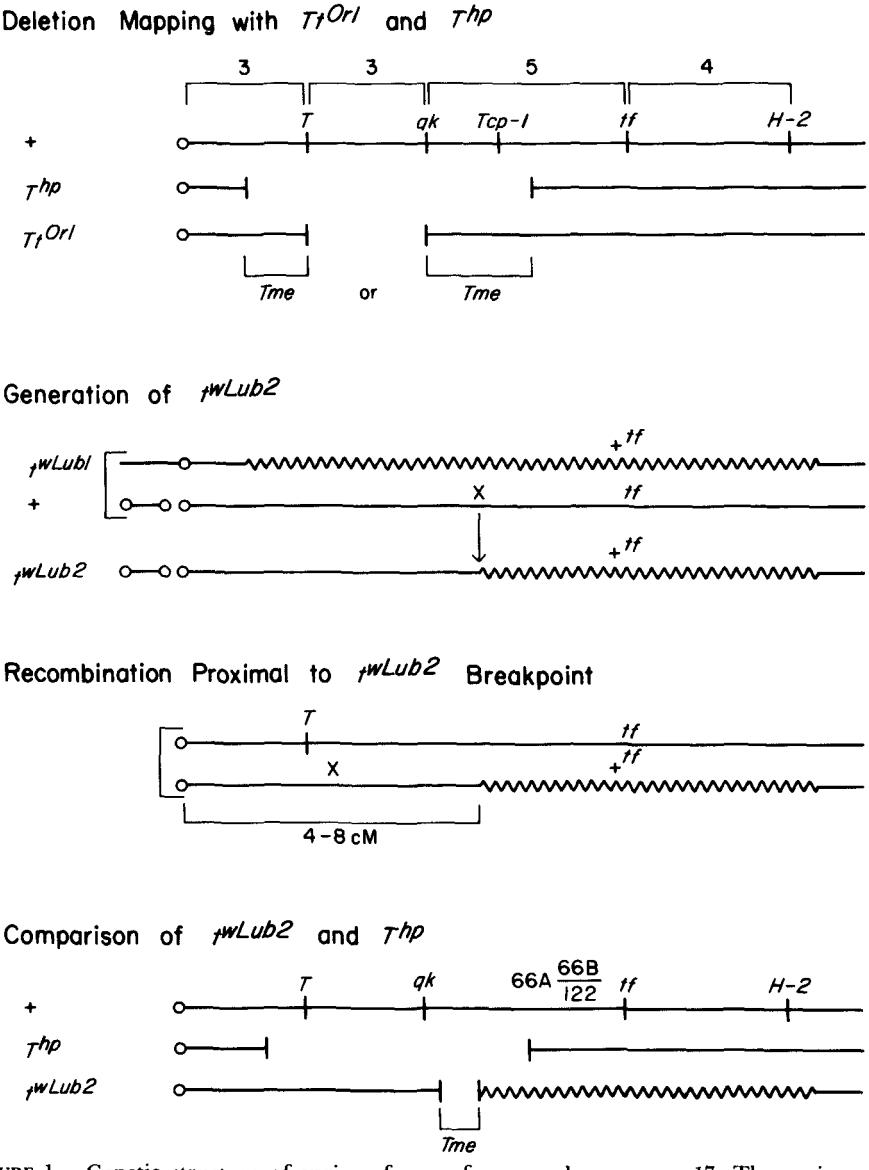

FIGURE 1.—Genetic structure of various forms of mouse chromosome *17*. The region of *t* chromatin present on each chromosome is indicated by the zigzag line. A, Deletion mapping of the *Tme* locus. B, Generation of t^{wLub2}. C, Region of free recombination between the centromere and the t^{wLub2} chromatin. D, Comparison of t^{wLub2} and T^{hp}.

The t^{wLub2} chromosome is a partial *t* haplotype recovered, in the laboratory, as a rare recombinant between t^{wLub1} and a wild-type chromosome. As shown in Figure 1B, recombination occurred in an animal that carried a centromerically marked t^{wLub1} haplotype with a second acrocentric chromosome *17* homologue marked at the *tf* locus; crossing over occurred between the centromere and *tf*.

Whereas complete *t* haplotypes can be transmitted from males at very high ratios, partial *t* haplotypes are generally transmitted at normal or even low ratios (LYON and MASON 1977). To determine the male transmission ratio of the t^{wLub2} haplotype, T/t^{wLub2} males were mated to wild-type (+/+) females; progeny that receive the t^{wLub2} chromosome have a normal tail, whereas those receiving the *T*-marked wild-type chromosome have a short tail. Of 115 offspring scored, 38% carried t^{wLub2}. This slightly (but significantly) lowered male transmission of t^{wLub2} could be a consequence of *t* haplotype sperm distortion factors or of a reduction in the viability of t^{wLub2}-carrying embryos. It is interesting that the T^{hp} deletion chromosome is also transmitted from males at a ratio significantly less than 50% (of 207 offspring obtained from $T^{hp}/+$ males in our colony at Cold Spring Harbor, 38% carried the T^{hp} chromosome).

In no previous case has a *t* haplotype been found to affect transmission ratios in female mice. However, when T/t^{wLub2} females were mated to +/+ males, 177 of 188 progeny scored at birth were found to carry the *T* mutation. Further analysis was performed on four of the 11 progeny obtained with normal tails—in all four cases, a recombination event had occurred between *T* and t^{wLub2}, resulting in a chromosome that carried neither *T* nor t^{wLub2} (see Figure 1C). Hence, transmission of t^{wLub2} has not been observed from t^{wLub2}-carrying females. There are two possible explanations for this result—either t^{wLub2} causes segregation distortion during oogenesis in females or t^{wLub2}-carrying embryos are dying prenatally.

A comparison of litter sizes from t^{wLub2}-carrying males or females mated to wild-type animals was possible since our closed colony conditions provide a generally similar genetic background in each mating configuration. Whereas the average litter size obtained in 18 litters from +/+ females mated to T/t^{wLub2} males was 6.3 ± 1.8, the average litter size obtained in 43 litters from T/t^{wLub2} females mated to +/+ males was only 2.8 ± 1.4. This reduction in litter size from t^{wLub2}-carrying females suggested that the missing progeny class was present *in utero* but was resorbed prior to the birth of normal littermates.

To test this hypothesis, matings were set up in which the maternal t^{wLub2} chromosome was uniquely marked with a Robertsoninan translocation that is easily identifiable as the only metacentric chromosome in either the maternal or paternal karyotypes. Fetuses obtained from 16 days postfertilization up until the time of birth were scored for the presence or absence of the metacentric chromosome (Table 1). At 16 days, 47% of the fetuses were metacentric positive; at 18 days, 36% were positive; at 19 days 26% were positive and at birth 4% were positive (this frequency of live-born animals carrying the metacentric chromosome is expected from recombination between the centromere and the actual t^{wLub2} haplotype as described before and shown in Figure 1C). Hence, it would appear that t^{wLub2}-carrying eggs are fertilized normally and that all, or nearly all, t^{wLub2}-carrying fetuses are alive up until 16 days after conception. The t^{wLub2} fetuses die during the last 4 or 5 days prior to the birth of their normal littermates.

The maternal t^{wLub2} lethal period corresponds with that observed for maternally derived T^{hp} fetuses. At 15 days postfertilization, these fetuses are alive

TABLE 1

Analysis of the t^{wLub2} *lethal period and phenotype*

Day of development	Total analyzed	No. (%) with metacentric	Fetal weight	
			With metacentric	Without metacentric
16	49	23 (47)	0.48 ± 0.09	0.33 ± 0.04
18	28	10 (36)	1.1 ± 0.2	0.78 ± 0.07
19	38	10 (26)	1.8 ± 0.3	1.2 ± 0.2
Newborn	73	3 (4)		

Wild-type males were mated with female heterozygous for a t^{wLub2} chromosome marked karyotypically by a Robertsonian translocation of chromosomes *2* to *17*. [The complete chromosome is called Rb(2.17)11Rma t^{wLub2}.] The first day of development was determined by the presence of a vaginal plug. All fetuses and newborn animals were analyzed independently for the presence of a metacentric chromosome. The fetal weight (of alcohol preserved specimens after Bouin's fixation) is indicated in grams for each genotype. The three newborn animals recovered with a metacentric chromosome were all very healthy and are presumed to have undergone recombination between the centromere and the mutant t^{wLub2} chromatin (see Figure 1C).

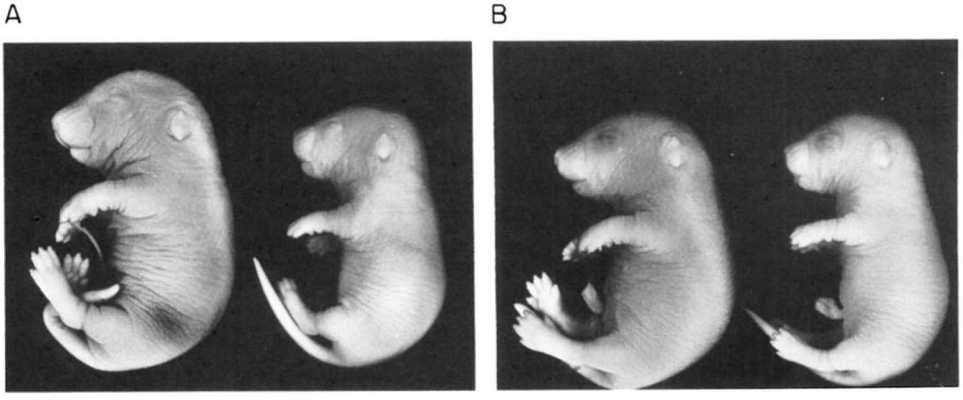

FIGURE 2.—Comparison of maternally derived t^{wLub2} and T^{hp} fetuses at day 19 of gestation. A, t^{wLub2} littermates. The mutant fetus was identified by the presence of a metacentric chromosome and is on the left (see Table 1). B, T^{hp} littermates. The mutant fetus was identified by a short tail phenotype and is on the left.

and apparently healthy (JOHNSON 1974; ALTON 1982); by 19 days, all T^{hp} fetuses have been or are in the process of being resorbed. The only morphological abnormalities found in maternal T^{hp} fetuses during the lethal period are severe edema and extra toes preferentially on the forefeet (JOHNSON 1974). The maternal t^{wLub2} fetuses were examined directly to determine whether they were phenotypically similar to the maternal T^{hp} fetuses. At the same time, a reexamination of T^{hp} fetuses was performed. Extra toes were observed in both types of fetuses. As shown in Figure 2 and as indicated in Table 1, t^{wLub2} fetuses are significantly heavier than their normal littermates because of severe edema. Hence, the maternal effects associated with T^{hp} and t^{wLub2} are phenotypically indistinguishable.

The t^{wLub2} maternal effect mutation appears to have been generated as a

direct result of the recombination event that occurred between the complete *t* haplotype t^{wLub1} and a wild-type chromosome. Previously accumulated data indicate that rare recombination events between + and *t* may often be unequal, resulting in the duplication or deletion of genetic material at the crossover point between wild-type and *t* haplotype DNA (LYON and MEREDITH 1964a; SILVER, WHITE and ARTZT 1980; SILVER 1983). Since the T^{hp} maternal effect is caused by a deletion, it would appear most likely that the t^{wLub2} maternal effect is also the result of a deletion or an inactivation of a gene normally present on both wild-type and t^{wLub1} forms of chromosome *17*. The data are most readily interpreted by postulating the deletion or alteration of the T-associated maternal effect locus (*Tme*) at the breakpoint between wild-type and *t* chromatin within the t^{wLub2} chromosome. The location of this breakpoint can be circumscribed through an analysis of genetic markers in this region.

The Tt^{Or1} chromosome is deleted for the region extending from the *T* locus to the *qk* locus on chromosome *17* but does not express the maternal effect (BENNETT *et al.* 1975; ERICKSON, LEWIS and SLUSSER 1978). When Tt^{Or1} is placed *in trans* to a chromosome carrying the recessive *qk* allele, a quaking phenotype is observed. We have found that Tt^{Or1}/t^{wLub2} animals do not express this phenotype. Therefore, the wild-type allele of *qk* must be present on the t^{wLub2} chromosome. Since recombination occurs freely between *T* and t^{wLub2} (and, by inference, *Tme*), the *Tme* locus must map distal to *qk*. The DNA markers T66A, T66B and T122 represent restriction fragment length polymorphisms recognized by low copy number DNA clones obtained from the *t* complex (ROEHME *et al.* 1984). These markers have been mapped between the *qk* and *tf* loci, and the t^{wLub2} chromosome is found to be associated with *t*-specific alleles at each of these loci (H. FOX, A. FRISCHAUF, H. LEHRACH, M. F. LYON, G. MARTIN and L. M. SILVER, unpublished observations). The accumulated data map the t^{wLub2} breakpoint and, by inference, the *Tme* locus to the chromosomal region bounded proximally by *qk* and distally by T66A/T66B/T122, as shown in Figure 1D.

The t^{wLub2} chromosome expresses a second phenotype—suppression of dominant *T* locus mutations—not expressed by either of the parental chromosomes from which it was derived. Animals heterozygous for dominant mutations at the *T* locus (*T*/+) are born with short tails. Naturally occurring *t* haplotypes interact *in trans* with *T* mutations to produce *T*/*t* animals that are tailless. Generally, proximally located partial *t* haplotypes continue to express this *T* interaction effect, whereas distally located partial *t* haplotypes act as wild type with respect to the *T* mutations. Two exceptions to this axiom have been reported. The original exception was the t^{h7} haplotype (recovered from an X-irradiation experiment) which interacts with *T* in a suppressive manner to produce T/t^{h7} animals with normal tails (LYON and MEREDITH 1964b). The structure of this haplotype is not clear, but it appears to have a duplication of the "*T* interaction factor" since three revertants to the original *t* allele at this locus have occurred (presumably by unequal sister chromatid exchange). The second exception to express this same phenotype is the proximal t^{Tu3} haplotype (T/t^{Tu3} animals have normal tails) recovered from a rare recombination event

between a complete *t* haplotype and wild-type chromatin (STYRNA and KLEIN 1981). Further characterization of the t^{Tu3} haplotype has not been reported. Neither t^{Tu3} nor t^{h7} expresses a maternal effect.

The t^{wLub2} haplotype is the third example of a chromosome that suppresses the expression of the dominant *t* mutation. Both T/t^{wLub2} animals and Tt^{Or1}/t^{wLub2} animals have tails of normal length. Although a unified picture of this *T* suppressor effect is not yet possible, the accumulated data indicate that this effect on tail length is independent of the maternal effect locus. Furthermore, it would appear that the *T* suppressor factor can not be an allele of the *T* locus since recombination occurs freely between t^{wLub2} and *T* (Figure 1C).

The results described here strongly suggest the existence of a discrete maternal effect locus (*Tme*) that is deleted or inactivated by both the t^{wLub2} and T^{hp} chromosomes. The available genetic data indicate that the t^{wLub2} lesion is significantly smaller than the T^{hp} deletion (Figure 1D). It might be possible to use the t^{wLub2} chromosome as a tool, with recombinant DNA techniques, as means for the cloning of the *Tme* locus. Analysis at the DNA level could lead to an understanding of the molecular basis for this unique effect on mammalian embryogenesis.

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LITERATURE CITED

ALTON, A. 1982 The histological, cellular and molecular correlates of the $T^{hairpin}$ maternal effect. Ph.D. Thesis, Cornell University Graduate School of Medical Sciences, New York.

ALTON, A., L. M. SILVER, K. ARTZT and D. BENNETT, 1980 Genetic relationship of trans interacting factors at the *T/t* complex: a molecular analysis. Nature **288:** 368–370.

BENNETT, D., 1975 The T-locus of the mouse. Cell **6:** 441–454.

BENNETT, D., 1978 "Rescue" of a lethal *T/t* locus genotype by chimerism with normal embryos. Nature **272:** 539.

BENNETT, D., L. C. DUNN, M. SPIEGELMAN, K. ARTZT, J. COOKINGHAM and E. SCHERMERHORN, 1975 Observations on a set of radiation-induced dominant T-like mutations in the mouse. Genet. Res. **26:** 95–108.

ERICKSON, R. P., S. E. LEWIS and K. S. SLUSSER, 1978 Deletion mapping of the t complex of chromosome 17 of the mouse. Nature **274:** 163–164.

JOHNSON, D. R., 1974 Hairpin-Tail: a case of post-reductional gene action in the mouse egg? Genetics **76:** 795–805.

LYON, M. F. and I. MASON, 1977 Information on the nature of t-haplotypes from the interaction of mutant haplotypes in male fertility and segregation ratio. Genet. Res. **29:** 255–266.

LYON, M. F. and R. MEREDITH, 1964a The nature of *t* alleles. III. Short tests of some further mutant alleles. Herdity **19:** 327–330.

LYON, M. F. and R. MEREDITH, 1964b The nature of *t* alleles in the mouse. II. Genetic analysis of an unusual mutant allele and its derivatives. Heredity **19:** 313–325.

MCGRATH, J. and D. SOLTER, 1984 Maternal T^{hp} lethality in the mouse is a nuclear, not a cytoplasmic, defect. Nature **308:** 550–551.

NIZETIC, D., F. FIGUEROA and J. KLEIN, 1984 Evolutionary relationships between the *t* and *H-2* haplotypes in the house mouse. Immunogenetics **19:** 311–320.

ROEHME, D., H. FOX, B. HERRMANN, A.-M. FRISCHAUF, J.-E. EDSTROM, P. MAINS, L. M. SILVER and H. LEHRACH, 1984 Molecular clones of the mouse t complex derived from microdissected metaphase chromosomes. Cell **36:** 783–788.

SILVER, L. M., 1981 Genetic organization of the mouse t complex. Cell **27:** 239–240.

SILVER, L. M., 1983 Reevaluation of the evidence for the generation of new lethal *t* hapotypes by mutation. Immunogenetics **18:** 91–96.

SILVER, L. M., K. ARTZT and D. BENNETT, 1979 A major testicular cell protein specified by a mouse *T/t* complex gene. Cell **17:** 275–284.

SILVER, L. M., M. WHITE and K. ARTZT, 1980 Evidence for unequal crossing over within the mouse *T/t* complex. Proc. Natl. Acad. Sci. USA **77:** 6077–6080.

STYRNA, J. and J. KLEIN, 1981 Evidence for two regions in the mouse t complex controlling transmission ratios. Genet. Res. **38:** 315–325.

WINKING, H., 1978 A presumable t-mutation on chromosome Rb(4.17)13Lub. Mouse News Lett. **59:** 33.

WINKING, H. 1981 Possible viability of mice with maternally inherited T^{hp} (Abstr.). Hereditas **94:** 19.

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