Title
The structure and organization of the mouse t complex

Author
Fox, Howard S.

Publication Date
1985

Peer reviewed|Thesis/dissertation

The Structure and Organization of the Mouse t Complex

by

Howard S. Fox

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

Anatomy

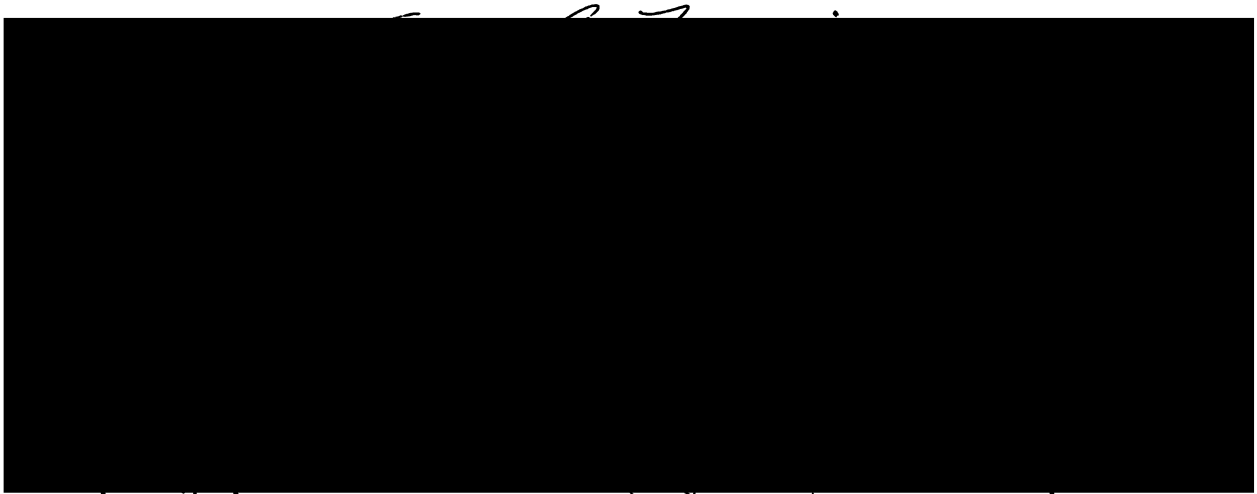
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



Date University Librarian

Degree Conferred: MAR 31 1985

For my parents and Nora

ACKNOWLEDGEMENTS

I could never thank enough everyone who helped throughout the course of this work. Jeanne Bahrenberg, Vicki Bautch, Lois Cisek, Tom Cooper, David Cox, Carolyn Doyle, Greg Evans, Marianne Gallup, Doug Hanahan, Steve Hughes, Aya Jakobovitz, Alex Joyner, Mike Krangel, Leslie Lock, Paul Mains, Charles Ordahl, Joe Sambrook, Nora Sarvetnick, and Jane Uman have all contributed in many different ways to this work, and I appreciate their support. I would also like to thank Hans Lehrach for supplying the microdissection-derived clones and Mary Lyon for making available many of the partial t haplotypes. Special thanks go to Lee Silver, who has given his constant intellectual interest, considerable effort and support to this work, and who desirves my deepest gratitude for all his help. Most of all, I would like to thank my advisor, Gail Martin, for her guidance, encouragement, support, help, and understanding both in this work and through my graduate training, without which none of this would have been possible.

I would also like to both apologize to and thank my family and friends, who have both suffered with me at times and rejoiced with me at others. John Meisner and Frank Pardo will never know how they have helped through the years. Nora, what can I say here? Love goes a long way.

Portions of this dissertation have been previously published in the following papers:

Fox, H.S., Silver, L.M., and Martin, G.R. (1984). An alpha globin pseudogene is located within the mouse t complex. Immunogenetics 19: 129-135.

Fox, H.S., Martin, G.R., Lyon, M.F., Herrmann, B., Frishauf, A.-M., Lehrach, H., and Silver, L.M. (1985). Molecular probes define different regions of the mouse t complex. Cell 40: 63-69.

Rohme, D., Fox, H., Herrman, B., Frishauf, A.-M., Edstrom, J.-E., Mains, P., Silver, L.M., and Lehrach, H. (1984). Molecular clones of the mouse t complex derived from microdissected metaphase chromosomes. Cell 36: 969-976.

ABSTRACT

The Structure and Organization of the Mouse t Complex

Howard S. Fox

Variant forms of the proximal region of mouse chromosome 17, known as t haplotypes, are found at high frequencies in wild mice. t haplotypes differ from wild-type in their effects on reproduction, embryonic development, and meiotic recombination. Partial t haplotypes, laboratory derived portions of complete t haplotypes, express only a subset of the t-specific phenotypes. Genetic and molecular studies performed on t haplotypes have led to theories concerning the nature of the t-specific phenotypes. However many of these theories are in conflict with one another, in part because of the relative difficulty of comparing t haplotypes with each other and with wild-type chromosomes.

In order to obtain a better understanding of t haplotypes, DNA probes recognizing t complex sequences have been sought. Novel DNA clones as well as previously described clones have been used as probes of genomic DNA from t haplotype-containing and wild-type mice in blot-hybridization experiments. These experiments mapped

sequences homologous to these clones to the t complex, and have allowed the comparison of wild-type and t DNA.

These studies also permitted the identification of t-specific polymorphic restriction fragments. Such restriction fragments are present in all complete t haplotypes and absent from wild-type mice. However, partial t haplotypes contain subsets of these restriction fragments, and each can be classified according to the t-specific fragments it contains. This is the first molecular evidence that independent partial t haplotypes contain different lengths of t haplotype DNA. The t-specific restriction fragments have been shown to map to and thus define different regions of the t haplotypes. These regions of t haplotype DNA could then be correlated with genetic loci proposed to control t-specific phenotypes, particularly the property of transmission ratio distortion.

The studies described here, in addition to recent studies performed by others, have allowed an evaluation of hypotheses about the evolution of t haplotypes. This has led to the proposal of a theory concerning the origin and maintainence of t haplotypes. A molecular and genetic examination of a novel wild-derived t haplotype is described and discussed in relation to this theory.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A. Discovery of t haplotypes

The t complex of mice has puzzled investigators since its discovery over 50 years ago. The study of the t complex was initiated by the finding of a short tailed mouse in a laboratory mouse stock (Dobrovolskaia-Zavadskaia, 1927). Genetic analysis of the inheritance of this short tail revealed that it was caused by a dominant mutation, which was given the name Brachyury (T). Heterozygotes (+/T) had a short-tailed phenotype, whereas homozygotes (T/T) could not be produced due to embryonic lethality (Chesley, 1935). It was then found, also in laboratory stocks, that there existed what were thought to be recessive alleles of T, denoted t alleles, which interacted with T (in T/t mice) to yield mice with no tail, and which were themselves homozygous embryonic lethals (Dobrovolskaia-Zavadskaia and Kobozieff, 1932; Chesley and Dunn, 1936; Gluecksohn-Schoenheimer, 1940). No recombination could be found between T and t alleles (Dunn and Gluecksohn-Schoenheimer, 1943). Some t alleles of independent origin were found to complement each other and were thus thought to be different mutations (Dunn, 1937; Dunn 1956). Since these "t alleles" or "t mutations" are now known to cause many different effects mediated by

distinct, separable genes, investigators now refer to them as *t* haplotypes.

An examination of wild mice revealed that *t* haplotypes could be found at relatively high frequencies in most mouse populations. Approximately 25% of wild mice carry *t* haplotypes, and *t* haplotypes have been found in all geographic areas in which they have been sought except one, an island in Long Island Sound, New York (Dunn, 1964; Klein, 1975; Klein et al., 1984). Thus *t* haplotypes can be thought of as a natural polymorphism in the mouse genome. The *t* complex was mapped to the IXth linkage group in the mouse, which was later located on chromosome 17 (Klein, 1971).

B. Properties of *t* haplotypes

t haplotypes have been found to contain a number of loci that function differently than their wild-type counterparts. The first of these is the property by which *t* haplotypes have been traditionally identified, interaction with the *T* mutation to cause taillessness. This locus in *t* haplotypes has been called the *t* complex tail interaction locus (*tct* or *t*^{int}). This gene causes the tailless phenotype only when it is heterozygous with *T*; when it is present with a wild-type Brachyury locus or when it is homozygous there is no effect (Dunn, 1937). A mutation with similar properties of interaction with *T* has been created in

a wild-type chromosome 17 by ENU mutagenesis. The fact that there is recombination between this locus and *T* demonstrates that it is not allelic to *T* (Bode, 1984).

The second property of *t* haplotypes is their association with embryonic lethalities. A mating between a tailless mouse, T/t^x, with a tailless mouse carrying the same *t* haplotype, T/t^x, yields only tailless offspring, T/t^x, since both T/T and t^x/t^x are lethal. As new *t* haplotypes were identified, they were tested to determine if they carried the same embryonic lethal as those carried by previously identified *t* haplotypes. If T/t^x crossed to T/t^y yielded only tailless offspring, t^x/t^y was considered lethal and t^x and t^y thus belong to the same complementation group. If normal tailed (t^x/t^y) offspring were produced, t^x and t^y must belong to different complementation groups.

Thus far, sixteen different complementation groups have been described, fifteen in wild mice and and one (t^9) derived only in the laboratory (Chesley and Dunn, 1936; Dunn, 1937, Dunn and Gluecksohn-Waelsch, 1953a; Dunn and Suckling, 1956; Dunn, 1957; Bennett and Dunn, 1958; Bennett et al., 1959a; Bennett and Dunn, 1960; Dunn and Bennett, 1971a; Dunn et al., 1973; Winking, 1979; Guenet et al., 1980; Klein et al., 1984). Six of these lethal haplotypes have been examined to determine the developmental abnormalities they cause. The *t* lethalities studied have

been found to act at different, distinct stages of embryogenesis and have characteristic patterns of defects.

The $\underline{t^{12}}$ complementation group, which includes $\underline{t^{w32}}$, is distinguished by preimplantation lethality (Smith, 1956; Hillman et al., 1970). The defect prevents the morula from progressing to the blastocyst stage. It has been reported (Smith, 1956; Mintz, 1964; Klein and Radska, 1968), and refuted (Hillman and Tasca, 1973; Erickson et al., 1974), that the defect is due to deficiencies in ribosomal RNA synthesis. Similarly, contradictory data exist regarding differences in ATP metabolism in $\underline{t^{12}}$ or $\underline{t^{w32}}$ homozygous embryos as compared to heterozygous or wild-type embryos (Ginsberg and Hillman, 1975; Erickson et al., 1974). The defect that is present may be cell lethal, since homozygous embryos could not be rescued in chimeras (Mintz, 1964).

$\underline{t^{wPa-1}}$ and $\underline{t^{w73}}$ appear to act soon after implantation. $\underline{t^{wPa-1}}$ leads to a general disorganization within the embryo, with no tissue being the obvious target for the mutation (Guenet et al., 1980). For $\underline{t^{w73}}$, the defect is believed to be in the trophectoderm, which fails to invade into and establish close contacts with the uterine decidua (Babiarz et al., 1982).

Three complementation groups, $\underline{t^{0}}$, $\underline{t^{w5}}$, and $\underline{t^{9}}$, appear to act in the embryonic tissues of the conceptus within a

few days following implantation. The t^0 complementation group, which includes t^6, acts shortly after implantation at the egg cylinder stage. Homozygous embryos show a defect in the formation of the embryonic ectoderm (Gluecksohn-Schoenheimer, 1940; Nadijcka and Hillman, 1975). On the basis of blastocyst outgrowth experiments, it has been reported that the inner cell mass cells are most affected by this mutation (Erickson and Pederson, 1975), although a similar study also demonstrated the trophectoderm to be abnormal (Wudl et al., 1977). Another study also showed defects in *in vitro* outgrowths of isolated inner cell mass cells (Hogan et al., 1980). Because these abnormalities could not be corrected in aggregates between putative homozygous and wild-type embryos, it was suggested that a generalized cell lethality exists (Wudl and Sherman, 1978). This is supported by an earlier result of the failure of putative homozygous embryos to grow in ectopic sites (Wudl et al., 1977).

The t^{w5} complementation group mutation causes death at days 7-8 of development. The embryonic endoderm becomes pyknotic and degenerates (Bennett and Dunn, 1958). Outgrowth culture experiments yielded a greater percentage of the outgrowths dying when embryos and inner cell mass cells are obtained from crosses that produced homozygoous embryos than when they are obtained from control crosses.

Since this fraction dying corresponded to the expected number of homozygous embryos, it was suggested that this mutation causes a generalized cell lethality (Wudl and Sherman, 1976; Hogan et al., 1980). However in this case it has been clearly refuted, since an embyonic stem cell line has been established from a t^{w5}/t^{w5} homozygous embryo (Magnuson et al., 1982). This cell line grows well under standard culture conditions, and differentiates to form derivatives of all three germ layers, as do wild-type embryonic stem cell lines.

The t^{9} complementation group, which includes t^{4} and t^{w18}, dies on approximately the eighth day of development. In such embryos the mesoderm is unable to migrate from the primitive streak, and such mesoderm appears abnormal (Bennett and Dunn, 1960; Moser and Gluecksohn-Waelsch, 1967; Spiegelman and Bennett, 1974). Following the injection of "abnormal" (presumptive homozygous t^{w18}/t^{w18}) embryos into testes, tumors were produced that resembled neuroblastomas, without mesodermal derivatives (a percentage did produce normal appearing tumors including mesoderm, but these were thought to be misclassified) (Artzt and Bennett, 1972). Thus it was suggested that t^{w18} blocks normal mesoderm formation. However, recently a t^{w18}/t^{w18} homozygous embryonic stem cell line has been established which, when allowed to differentiate, forms derivatives of all three

germ layers, including mesoderm (G. Martin, personal communication).

A study of the growth rate of embryos in litters segregating for $\underline{t^{w18}}$ suggested a different mode of action for the lethality (Snow and Bennett, 1978). Presumptive $\underline{t^{w18}}/\underline{t^{w18}}$ homozygous embryos had fewer cells than did their normal litter mates at 6 1/4 days of development, before any other abnormality could be noted. This was apparently due to a mitotic arrest of cells just outside the proliferative zone of the embryo, an area of high mitotic activity. Within the proliferative zone new cells are formed, however the dividing cells seem to be abnormal in their orientation, since their growth results in a thickening of the embryo instead of the normal lengthening of the embryo. Thus there may be a defect in mitosis in $\underline{t^{w18}}/\underline{t^{w18}}$ homozygotes.

Two complementation groups, $\underline{t^{w1}}$ and $\underline{t^{wLub1}}$, contain lethal loci that act late in embryonic development. Members of the $\underline{t^{w1}}$ complementation group, which includes $\underline{t^{w12}}$, die late in development, with the lethality occuring from between day 9 and term. Homozygous embryos show cell death in the ventral half of the neural tube and brain (Bennett at al, 1959a,b). Tumors produced by transplanting inner cell mass cells to the testes showed derivatives of all three germ layers for both heterozygous and homozygous embryos, although neither cartilage nor bone was present in the

homozygous tumors (Axelrod et al., 1981). A permanent fibroblast-like homozygous $\underline{t}^{w12}/\underline{t}^{w12}$ cell line was established from one of the tumors. The lethality associated with the $\underline{t}^{wLub1}$ group has not been carefully examined, but it has been reported to cause lethality at days 11-12 of development (Winking, 1979).

$\underline{t}$ haplotypes also have been identified that do not carry absolute lethalities, since $\underline{t}^{x}/\underline{t}^{x}$ offspring can be produced. Most are instead classified as semilethals since fewer $\underline{t}^{x}/\underline{t}^{x}$ animals are born than expected, and fetal wastage has been demonstrated. Homozygous embryos may show a variety of defects, including otocephaly, and can die from these defects between implantation and birth (Bennett and Dunn, 1969). Mice carring two complementing $\underline{t}$ haplotypes also show differing degrees of prenatal lethality, and have embryonic appearances similar to those of semilethal homozygous embryos (Silagi, 1962).

The third characteristic property of $\underline{t}$ haplotypes is transmission ratio distortion in the male. Male mice heterozygous for a $\underline{t}$ haplotype will transmit the $\underline{t}$ haplotype to up to 100% of his offspring. Mice derived from the wild carrying $\underline{t}$ haplotypes show extremely high distortion (Dunn, 1957), whereas in the laboratory the transmission ratio of $\underline{t}$ haplotypes can be lowered by the laboratory genetic background (Bennett et al., 1983). The genotype of the

female also appears to modulate the degree of transmission ratio distortion in the male (Demin et al., 1978), and alterations in the the time of fertilization similarly affect the degree of distortion (Braden, 1958; McGrath and Hillman, 1980b,c). Females heterozygous for t haplotypes transmit both forms of chromosome 17 to their offspring in Mendelian ratios.

The mechanism that causes transmission ratio distortion is unknown. The preferential fertilization is not due to a lack of wild-type sperm, as the segregation ratio of wild-type and t-haplotype sperm is normal during meiosis (Hammerberg and Klein, 1975b), and approximately equal numbers of wild-type and t haplotype sperm are present in the ejaculate of heterozygous mice (Silver and Olds-Clarke, 1984). This is distinct from a similar system in Drosophila, Segregator Distorter (SD), in which there is an apparent "killing" of the wild-type sperm by the SD sperm (Hartl and Haraizumi, 1976). Numerous differences have been reported between sperm from heterozygous (+/t) and wild-type (+/+) mice. No structural differences could be found between wild-type and t haplotype containing sperm (Hillman and Nadijcka, 1978a,b), but abnormal mobility of sperm from heterozygous mice has been demonstrated (Olds-Clarke, 1973; Katz et al., 1979, Tessler et al., 1981). Sperm from heterozygous males show a quicker penetration of eggs in

vitro (Olds-Clarke and Carey, 1978), and such sperm also show increased energy usage than do wild-type sperm (Ginsberg and Hillman, 1974; Nadijcka and Hillman, 1980b). Thus a difference in energy production or usage may contribute to transmission ratio distortion. Another study indicates that a difference exists in glycosyltransferase activity in heterozygous (+/*t*) and homozygous (*t*/*t*) sperm when compared to wild-type sperm (Shur and Bennett, 1979). This was shown to be due to less inhibitory activity in *t* sperm, and it was suggested that this may have a role in transmission ratio distortion (Shur, 1981). In addition, the various sperm antigens found to be associated with *t* haplotypes (Bennett et al., 1972; Yanagisawa et al., 1974, see below), as well as the *Tcp-1* gene (Silver, 1981b, see below) may have a role in this phenomenon.

A fourth effect of *t* haplotypes on mice is sterility in male mice that carry two complete *t* haplotypes. This sterility is thought to take two different forms. In male mice carrying two complementing *t* haplotypes, sperm are produced, but have been reported to have structural defects (Dooher and Bennett, 1977) although this has been disputed (Olds, 1971; Hillman and Nadijcka, 1980; Nadijcka and Hillman, 1980a). It has also been found that a reduced number of sperm are present in the ejaculate of sterile t^x/t^y males, and it is suggested that this reduction alone

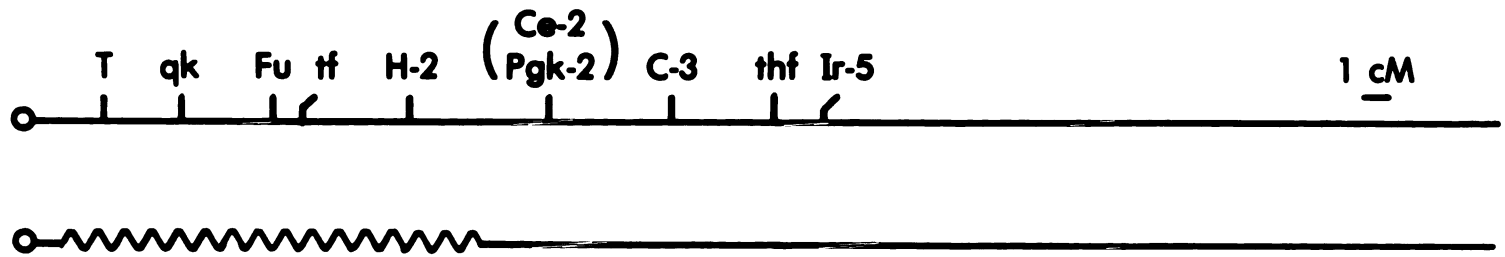
is sufficient to explain the sterility (Tucker, 1980). However, when such sperm are used in an *in vitro* fertilization assay, they are still defective compared to wild-type sperm (McGrath and Hillman, 1980a). A second form of sterility is found in male mice carrying two semilethal *t* haplotypes. In such males, spermatogenesis is greatly perturbed by microtubular defects, and normal appearing sperm are not present (Dooher and Bennett, 1974). Mice carrying a combination of a lethal haplotype and a semilethal haplotype have a phenotype like those carrying two complementing lethalities (Bennett, 1975).

A final property of *t* haplotypes is the suppression of recombination in the proximal region of chromosome 17 (see Figure 1). As genetic loci were mapped near *T* on chromosome 17, they were examined for recombination with *t* haplotypes. It was discovered that when a *t* haplotype was present in heterozygotes, the recombination with the wild-type chromosome between *T* and the fused (*Fu*) (Dunn and Caspari, 1945; Dunn and Gluecksohn-Waelsch, 1953b), tufted (*tf*) (Lyon and Phillips, 1956), and the histocompatibility-2 complex (*H-2*) (Hammerberg and Klein, 1975a) loci was greatly reduced. When a *t* haplotype is present only about 1/1000 recombinant offspring can be detected over this distance of approximately 15 centimorgans, a reduction in the normal recombination frequency of over 100-fold. The area of

Figure 1. Mouse Chromosome 17

The chromosome 17 genotype of a mouse heterozygous for a complete t haplotype (+/t) is shown. Genetic markers are indicated on the wild-type (upper) chromosome, spaced according to relative recombination frequencies (reviewed in Klein, 1979; and Roderick and Davisson, 1981). These markers are T (brachyury), qk (quaking), Fu (fused), tf (tufted), H-2 (the histocompatability-2 complex), Ce-2 (kidney catalase), Pgk-2 (phosphoglycerate kinase-2), C-3 (complement component-3), thf (thin fur), and Ir-5 (immune response-5). Parentheses indicate that Pgk-2 and Ce-2 have not been mapped relative to each other. The zigzag line indicates the region of t DNA present in a complete t haplotype-containing (lower) chromosome, thus defining the t complex.

CHROMOSOME 17



recombination suppression is limited within the chromosome, however, as free recombination can occur proximally between *t* haplotypes and the centromere (Forejt et al., 1980), and distally between *t* haplotypes and the phosphoglycerate kinase-2 (*Pgk-2*) (Nadeau and Phillips, 1984) and kidney catalase-2 (*Ce-2*) loci (Artzt, 1984). An additional result which came out of the *H-2* recombination studies was that there are a limited number of *H-2* haplotypes that are associated with *t* haplotypes, as compared to the extremely high level of polymorphism found in wild-type mice (Hammerberg and Klein, 1975c; Strum et al., 1982; Nizetic et al., 1984).

There have been two theories as to the cause of this recombination suppression in *t* haplotypes. It has been proposed that differences exist between *t* haplotypes and wild-type chromosomes in their middle repetitive DNA (Lyon et al., 1979a) or in primary gene order (Silver and Artzt, 1981). Since inversions are known to suppress recombination (Sturtevant, 1926), evidence for inversions was sought by two cytogenetic methods. First, an inversion the size of the *t* complex should show inversion loops during chromosome pairing in meiosis, and second, these chromosomes should yield anaphase bridges in the germ cells. However, although both effects were sought, neither was observed (Womack and

Roderick, 1974; Tres and Erickson, 1982). This discrepancy between the genetic and cytological data is discussed below.

It was discovered, however, that recombination between two *t* haplotypes can occur at apparently normal levels (Silver and Artzt, 1981). This finding was made possible by the discovery of a spontaneous *tf* mutation in a complete *t* haplotype. In a female mouse containing this *t* (*tf*) chromosome in combination with a distal *t* haplotype containing *T* in its proximal wild-type region, recombination occured between *T* and *tf*. When this experiment was repeated using *t* haplotypes containing distinguishable *H-2* complexes, it was found that in *t* haplotypes the gene order was *T* - *H-2* - *tf*, as opposed to the *T* - *tf* - *H-2* order found in wild type chromosomes (Artzt et al., 1982b; Pla and Condamine, 1984). Additional studies indicate that the entire *H-2* complex appears to be inverted in *t* haplotypes (Shin et al., 1983a; Shin et al., 1984).

C. Gene products of *t* haplotypes and the *t* lethal loci

Because the mouse *H-2* complex lies within the area of recombination suppression of *t* haplotypes, it has been proposed that *t* haplotypes maintain this whole region as a "supergene" complex in wild mice (Snell, 1968). It was also speculated that, like the *H-2* complex, different *t* complex genes in *t* haplotype similarly encode cell surface antigens

(Gluecksohn-Waelsch and Erickson, 1970). This suggestion that *t* haplotypes affect cell surface antigens appeared to be consistent with data from morphological studies of the *t* lethalities, which suggested that each of the lethalities is due to defects in cell-cell interactions (Artzt and Bennett, 1975). Thus a search for cell surface antigens encoded by *t* complex genes began. Since the *t* complex was known to affect both sperm and embryos, and sperm were easier to obtain in large quantities than embryos, antisera were raised first against sperm from *T* and *t* haplotype containing mice. The first antiserum reported was thought to be specific for products of the *T* locus. This antiserum reacted against sperm from mice that carried *T* (either heterozygous with wild-type, *T*/+, or heterozygous with a *t* haplotype, *T*/*t*) but not with wild-type (+/+) mice (Bennett et al., 1972). Similar antisera were prepared which were reported to recognize sperm carrying t^0, t^9, t^{w1}, t^{w2}, t^{w5}, and t^{w32} (Yanagisawa et al., 1974; Artzt and Bennett, 1977). The differences in the antigens detected by many of these antisera are thought to be due to different terminal carbohydrates (Chang and Bennett, 1980).

An antiserum raised against teratocarcinoma cells (the F9 cell line), which have characteristics similar to those of early embryonic cells, was also tested against *t* haplotype sperm (Artzt et al., 1973). It was found that

twice as many sperm from a heterozygous +/$\underline{t}^{12}$ mouse as from a wild-type +/+ mouse were required to remove the anti-F9 specificity from this antisera, thus the antigen was thought to be the wild-type product of a $\underline{t}^{12}$-specific gene (Artzt et al., 1974). Furthermore, the molecule believed to be recognized by this serum was reported to be identical in size and structure to H-2 molecules, and to be similarly associated with a $beta_2$ microglobulin-like molecule (Vitetta et al., 1975).

These results, however, have been disputed. Although putative homozygous embryos of a given t haplotype are labelled with anti-sperm antisera raised against that haplotype, each serum labelled embryos at the morula stage (Kemler et al., 1976). Thus the antigen recognized may not be in any way related to the specific embryological defect. Since t haplotypes suppress recombination over a large region, the gene encoding such an antigen may be anywhere along the t complex, and not necessesarily the lethal locus. Furthermore, attempts to repeat such experiments in another laboratory by raising antisera against sperm has failed (Gable et al., 1979; Goodfellow et al., 1979). It was argued that in these experiments, different, more sensitive techniques were used for the assay (anti-globulin immunoassay instead of sperm cytotoxicity) and that the

techniques used previously may have lead to spurious positive results.

The anti-F9 antiserum, which was reported to be the product of the wild-type t^{12} gene, indeed showed decreased staining of putative t^{12}/t^{12} embryos, however it showed a similar pattern on putative t^{w5}/t^{w5} embryos, thus negating any claim for specificity to the t^{12} haplotype (Kemler et al., 1976). Another study showed no difference in embryos in litters segregating for t^{12} or a number of other embryonic lethals, although nondefinitive differences were noted for t^{w5} and T^{Orl} (Erickson and Lewis, 1980). Furthermore, the contention that the F9 antigen is an H-2-like molecule has been withdrawn, and it is now thought to be a high molecular weight glycoprotein (Artzt et al., 1982b; Chang et al., 1983).

A different approach has also been taken to find protein products of the t complex. This involved comparing two-dimensional gels of *in vitro* labeled testis proteins from congenic mice. Congenic mice are inbred mice that are created by certain mating schemes to differ only at defined loci (see Green, 1981, for a discussion of congenic mice). Such congenic mice were created for t haplotypes by the repeated backcrossing of a t haplotype to an established inbred line. After 20 such backcrosses, the probability that a locus unlinked to the t complex is not derived from

the inbred strain is approximately 10^{-6}. However, there is a 13% chance that a locus 10cM away from the *t* complex is not derived from the inbred strain. A comparison of many independently derived congenic lines greatly increases the probability that any differences seen between congenic mice are indeed due to the presence of the *t* haplotype. Such comparisons were performed for these protein studies.

A 63 kilodalton protein was initially identified, which has an acidic form when a *t* haplotype is present, and has a basic form when a wild-type chromosome is present (Silver et al., 1979). *In vitro* translation of testis RNA showed this protein to be a direct translation product of a *t* complex gene, named *t* complex protein 1 (*Tcp-1*) (Danska and Silver, 1980). The *t* haplotype acidic form of this gene is referred to as $Tcp\text{-}1^{a}$, and the wild-type basic form as $Tcp\text{-}1^{b}$. Further studies identified seven more polymorphic proteins mapping to the *t* complex (Silver et al., 1983a), five of which are direct translation products.

The function and relatedness of the *t* complex lethal genes has long been a cause for speculation. After the discovery of apparently normal recombination between different *t* haplotypes, the different lethal genes were mapped. The genes studied (t^{w73}, t^{wLub1}, t^{w18} [t^{9}], t^{w32} [t^{12}], t^{w5}, t^{w12} [t^{w1}], and t^{0} [t^{6}]) were found to be nonallelic, and to map throughout the 15 cM span of the *t*

complex (Artzt et al., 1982a; Condamine et al., 1983; Artzt, 1984). The recombinant *t* chromosomes created in these experiments were then used in a variation of the genetic *cis-trans* test to determine whether the lethal genes showed a functional relationship. It was found that mice carrying a certain *t* haplotype containing two lethals in *cis* on one chromosome, when in combination with the appropriate "reciprocal" *t* haplotype carrying no lethals, shows 100% complementation, compared to mice carrying the parental combinations of *t* chromosomes with the lethal genes in *trans*, which show 70-80% complementation (Shin et al., 1983b). These data were considered to provide evidence supporting the hypothesis that the *t* lethal genes are interrelated and part of the same "cistron." However, alternative explanations are also plausible. Since the degree of complementation between two *t* haplotypes is known to vary with genetic background (Klyde, 1970), the difference between 70-80% and 100% complementation could be attributable to this effect. Furthermore, the differences between the recombinant *t* haplotypes used in this study are not limited to the lethal loci but also extend to undefined portions of their parental *t* haplotypes. Deleterious genes within these regions could also lead to the differences in complementation. In fact, this was propsed in this study and examples were presented that show this effect (Shin et

al., 1983b). The proper control for this experiment would entail having identical genetic backgrounds for the tests of the lethal loci in *cis* and in *trans*. This is difficult since each lethal locus is present not only in the background of the laboratory mouse in which the *t* haplotype is carried, but also within the context of the *t* haplotype with which it is associated. The finding of two particular combinations of these recombinant *t* haplotypes that give full complementation is not convincing as proof of functional relationship between the *t* haplotype lethal loci, especially since almost all other combinantions yield poorer complementation (P. Mains and L. Silver, personal communication).

At present, there is no convincing evidence for specific functions or gene products associated with any of the lethal genes. In analogy to the manner in which genes that affect enzyme expression have been classified (Paigen, 1980), it has been proposed the *t* haplotype lethal gene loci may act by similar mechanisms (Lyon, 1981). Genes causing lethality in early development presumably act through malfunction of a molecule essential for development. This molecule(s) can either be a direct protein product of the lethal gene locus, the lethal locus being a structural gene. Alternatively, this locus may in some way modify a protein or produce products that regulate expression of a protein,

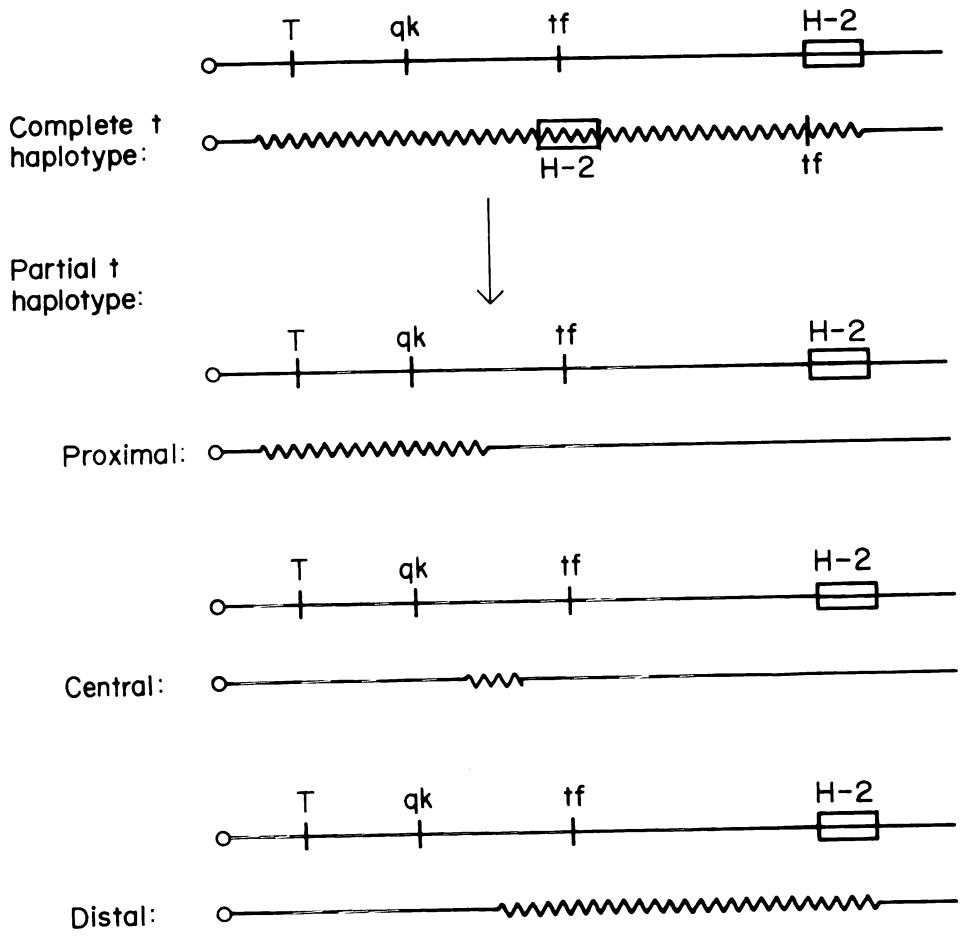
the locus thus encoding a processing, regulatory, or temporal gene.

D. Partial t haplotypes

Despite the strong suppression of recombination between wild-type and t DNA, rare crossing over does occur in the region between T and tf, and this produces what are known as partial t haplotypes (see Figure 2). These were found as "exceptions" in the balanced lethal crosses used to maintain t haplotypes. In a cross of the genotypes T/t^{x} x T/t^{x} only tailless (T/t^{x}) offspring would be expected. However, at a rate of 0.2%, exceptional offspring are produced, having the normal tailed phenotype (Dunn et al., 1962). Breeding analysis of these animals showed that in addition to containing the original t haplotype, these animals also carry a new t haplotype (t^{x}/t^{n}) (Dunn and Gluecksohn-Waelsch, 1953a). The "mutation" that creates these new haplotypes, apparently from the original t haplotype, also alters the properties of the haplotype. They no longer carry the parental lethal gene, do not have a high transmission ratio in the male, are fertile when homozygous, and do not suppress crossing over between T and tf (Dunn and Gleucksohn-Waelsch, 1953b; Lyon and Phillips, 1959; Dunn et al., 1962).

Figure 2. Schematic Representation of t Complex Region Genotypes

Representative genotypes with a complete t haplotype (top) and the three classes of partial t haplotypes (below) are given with some of the markers shown in Figure 1. The straight line represents wild-type DNA, the zigzag line represents t haplotype DNA. The inversion of tf and H-2 in t haplotype relative to wild-type DNA (Artzt et al., 1982) is indicated.


T
qk
tf
H-2
Complete t haplotype:
H-2
tf
Partial t haplotype:
T
qk
tf
H-2
Proximal:
T
qk
tf
H-2
Central:
T
qk
tf
H-2
Distal:

It was proposed that this "mutation" is actually recombination between a t haplotype and wild-type within the t complex region (Lyon and Phillips, 1959). Proof of this was obtained from crosses utilizing the tf gene as a marker to determine if crossing over occurs during the generation of these exceptions (Lyon and Meredith, 1964a,b,c). Mice of the genotype (Ttf/t^x) were mated inter se. The normal tailed exceptions (t^x/t^n), when each chromosome was bred out, were shown to be t^x/t^ntf. Thus, the change from t^x to t^n was accompained by crossing over in the T-tf interval, with the new t haplotype generally retaining the tail interaction factor and losing the parental lethality. Since T is proximal to tf, these chromosomes, which retained the "t-like" properties in this region, are known as proximal partial t haplotypes. Such chromosomes allow crossing over with wild-type in their distal extent, where the tufted gene is located. In the proximal region, crossing over is still suppressed from T to the quaking (qk) mutation (Bennett et al., 1979).

In the same series of experiments described above, Lyon and Meredith found that a cross of a t haplotype-containing normal tailed, nontufted mouse (+tf/t^x) to a short tailed, tufted mouse (Ttf/+tf) generated a t haplotype-containing short tailed, nontufted mouse (Ttf/+t^n). A similar event occured in a cross of a t haplotype-containing tailless,

nontufted mouse (*Ttf*/*t*^{x}) to a normal tailed, tufted mouse (+*tf*/+*tf*), which generated a *t* haplotype-containing short tailed, nontufted mouse (*Tt*^{n}/+*tf*). These chromosomes are apparently the consequence of the reciprocal events to the ones described above, since they do not carry the gene responsible for tail interaction, but still carry the embryonic lethal gene. These haplotypes are known as distal *t* haplotypes. These chromosomes allow crossing over with wild-type in their proximal extent, marked by the *T* gene, and continue to suppress crossing over with *tf*. A third class of partial *t* haplotypes have been derived from proximal and distal *t* haplotypes. These are the central *t* haplotypes, which do not contain the tail interaction locus or the lethal gene, and which allow crossing over both proximally and distally (Dunn and Bennett, 1968; Dunn and Bennett, 1971b; Lyon and Mason, 1977; Lyon et al., 1979a; Lyon, 1984).

Since the partial *t* haplotypes genetically separate the tail interaction locus from the lethal genes, it was clear that at least these effects are mediated by independent genes. Partial *t* haplotypes have been extensively used to analyze the sterility and transmission ratio distortion properties of *t* haplotypes. Males that are homozygous for proximal *t* haplotypes are fully fertile (Dunn and Gluecksohn-Waelsch, 1953a; Lyon and Meredith, 1964a),

however a proximal *t* haplotype in combination with a complete *t* haplotype shows reduced fertility (Braden and Gluecksohn-Waelsch, 1958; Dunn and Bennett, 1969; Bennett and Dunn, 1971; Lyon and Mason, 1977; Erickson et al., 1978; Hammerberg, 1981; Hammerberg, 1982). Thus there are thought to be at least two loci responsible for sterility in *t* haplotypes, one mapping proximally (denoted *t* complex sterility locus-1: *tcs-1*) and one distally (*tcs-2*). Two gene doses of *tcs-1* apparently have no effect, whereas two doses of *tcs-1* and one of *tcs-2* render an animal partially sterile, and two doses of both leads to complete sterility (see Silver, 1981a). An anomolous result obtained was that proximal *t* haplotypes in combination with a certain *t* haplotype, t^6, were fully fertile. Although t^6 was previously thought be like all complete *t* haplotypes, it was proposed that t^6 is lacking in the proximal sterility factor found in other complete *t* haplotypes (Lyon and Mason, 1977). Thus in the compound heterozygotes for a proximal haplotype and t^6, animals would only have one dose of *tcs-1* and one dose of *tcs-2* and thus be fertile. Proof that t^6 differs from complete *t* haplotypes has been obtained in this study.

The genetic control of segregation distortion was also shown to be regulated by multiple loci. Many different investigators have reported on the transmission ratios of partial *t* haplotypes when they were present isolated from

other t haplotypes or in combinations of t haplotypes. Isolated proximal partial t haplotypes show two different phenoytypes: either a normal transmission ratio or a low transmission ratio, ranging from 10-40% (Dunn et al., 1962; Lyon and Meredith, 1964a; Lyon and Mason, 1977; Lyon, 1984). Distal partial t haplotypes show a variable ratio, but when results are averaged a normal ratio results (Lyon and Mason, 1977; Styrna and Klein, 1982). Central haplotypes show a low ratio (Dunn and Bennett, 1968; Lyon and Mason, 1977; Lyon, 1984). When combinations of proximal and distal haplotypes are present in a mouse in the cis or trans orientations, a variety of results are obtained. When a proximal and a distal t haplotype are present together, in some experiments they are transmitted at equal ratios, in other studies the proximal haplotype is transmitted at a high level, and in yet other studies the distal haplotype is transmitted at a high level (Lyon and Mason, 1977; Hammerberg, 1982; Styrna and Klein, 1982; Lyon, 1984). Central haplotypes have the property of being transmitted equally when present in combination with a complete t haplotype, and equally when present in combination with a low ratio proximal haplotype (Dunn and Bennett, 1968; Lyon, 1984).

To explain these results, models have been proposed regarding interaction of t haplotype loci to produce

segregation distortion. One model contends that transmission ratio distortion is controlled by two loci, one mapping proximally and one distally, which may act in a distorter-responder relationship (Gluecksohn-Waelsch, 1971; Demin et al., 1978; Styrna and Klein, 1982; Bennett et al., 1983). This model fails, however, to predict which *t* haplotype, when combinations of *t* haplotypes are present in a mouse, will be transmitted at a high ratio, or why central haplotypes in combination with complete *t* haplotypes show a normal transmission ratio.

An alternate model, which answers the above critism, has also been proposed. This model contends that there is a locus, present in the central region of *t* haplotypes (thus defining central haplotypes) which acts as a responder locus (*t* complex responder, *Tcr*) to the action of at least three distorter loci (*t* complex distorters, *Tcd-1*, *Tcd-2*, and *Tcd-3*) (Lyon and Mason, 1977; Lyon, 1984). The model states that heterozygosity for *Tcr* is absolutely necessary for distortion of transmission ratio and that the degree to which a *t* haplotype (either partial or complete) is transmitted at an abnormal ratio depends on which of the distortion loci are present.

This model is thus able to predict both whether a male carrying combinations of partial *t* haplotypes will show transmission ratio distortion, and which chromosome will be

transmitted at a high ratio (Lyon, 1984). For example, a chromosome that carries Tcr will be transmitted at a high ratio if Tcd-1 and Tcd-2 are present in cis or trans, and at the highest level if all three distortion loci are present. Even if only Tcr is present on one chromosome, and the distorters present on the other chromosome, the chromosome containing Tcr will be transmitted at a high ratio. If Tcr is present on both chromosomes, both will be transmitted equally, regardless of the number of distorters present. In contrast, an isolated Tcr locus, or Tcr in combination with Tcd-1 or Tcd-3 will be transmitted at a low ratio. The Tcd-1 locus was proposed to map proximally, the Tcd-2 locus distally, and the Tcd-3 locus medially. Inherent in the this model is the hypothesis that different partial t haplotypes of a given class, proximal, central, or distal, may carry different lengths of t haplotype DNA (Lyon and Meredith, 1964a; Lyon and Mason, 1977). However, this model has been criticized as involving circular reasoning, since the partial t haplotypes were proposed to have different transmission ratio distortion factors and thus different lengths based on their transmission ratio distorting properties, without any independent verification of the amount of t haplotype DNA they contain. Proof that partial t haplotypes can indeed carry different lengths of t haplotype DNA has been obtained in this study.

Partial t haplotypes have also been used to examine the structure of the t complex. After the discovery of the Tcp-1 polymorphism between t haplotypes and wild-type, partial t haplotypes were examined for the presence of this marker. A potential problem in this analysis is that the partial t haplotypes have not been bred onto congenic strains. Background differences in the mice containing the partial t haplotypes could obscure the mapping if that background contained alleles like those in t haplotypes. However, the Tcp-1 allele found in t haplotypes has thus far been found in all complete t haplotypes and is absent from both the inbred and noninbred mice that do not contain t haplotypes. Thus this allele can be used as a marker in studies on t haplotypes.

All proximal haplotypes carry this marker whereas all distal t haplotypes do not, thus mapping the Tcp-1 gene to the proximal region of t haplotypes. Suprisingly, however, in 4 out of the 15 proximal t haplotypes examined a duplication of this protein was found (the chromosomes expressed both $Tcp\text{-}1^{a}$ and $Tcp\text{-}1^{b}$) (Silver et al., 1980). This provided evidence that the crossing over events that lead to the generation of partial t haplotypes can be, in a large number of cases, unequal, as had been proposed earlier (Lyon and Meredith, 1964b; Lyon and Bechtol, 1977).

One of the t haplotype complementation groups, t^9, consists entirely of haplotypes which have arisen from other t haplotypes (t^4 and t^9 from t^{12}, Dunn and Gluecksohn-Waelsch, 1953a; t^{w18} from t^{w11} (t^{w5}), Bennett and Dunn, 1960; t^{w30} from t^{w12}, Dunn et al., 1962; t^{w52} from t^{w5}, Bennett et al, 1976; and t^{ks1} from t^{w5}, V. Bode, personal communication). These haplotypes are unique to t lethals in that they allow crossing over with tf and H-2, and do not distort transmission ratio when heterozygous with wild-type. In the cases of t^{w30}, t^{w52}, and t^{ks1}, it was shown directly that they arose by recombinantion between a t haplotype and wild-type because they picked up the tf marker from the wild-type chromosome during their generation. In analogy to the theory and finding of unequal crossing over between t and wild-type DNA described above, it has been proposed that an unequal crossing over to create members of the t^9 group could create a lethality by either duplication or deletion of genes (Lyon and Meredith 1964b, Silver, 1983).

E. Other t complex genes affecting spermatogenesis and embryogenesis

In addition to T, there are a number of other mutations in wild-type chromosome 17 which, like some of the t haplotype genes, affect spermatogenesis or embryonic

development. Three genes that map in the *t* complex are known to affect spermatogenesis: quaking (*qk*), hybrid sterility (*Hst*) and T-associated sex reversal (*Tas*). The *qk* mutation was first observed as a neurological mutation (Sidman et al., 1964). Homozygous mice had a distinct tremor, and histological examination revealed severely deficient myelination in the central nervous system. Homozygous males were found to be sterile, with a defect in spermatid differentiation, possibly of lipid metaoblism, which leads to a virtual azoospermia (W. Bennett et al., 1971).

The *Hst* gene was discovered in an a study designed to examine the genetic basis for male sterility in F1 offspring of crosses between wild mice and laboratory mice (Forejt and Ivanyi, 1975). Two different alleles of this gene exist in laboratory strains: one which causes sterility ($Hst\text{-}1^{s}$), and one which allows fertility ($Hst\text{-}1^{f}$). This gene has been mapped to the area between *T* and *H-2*. In sterile males, testis weight was significantly less than in fertile males, and spermatogenesis appeared to break down at the spermatogonia stage.

The *Tas* gene was discovered during an attempt to backcross the T^{hp} mutation onto the C57BL/6J inbred strain (Washburn and Eicher, 1983). T^{hp} acts as an allele of *T* in that it shortens the tail of heterozygotes, interacts with *t*

haplotypes to yield tailless offspring, and is homozygous lethal (Johnson, 1974; Johnson, 1975). It differs from *T*, however, in that it seems to be a deletion of a number of other genes. T^{hp} exhibits pseudominance of *gk*, does not express *Tcp-1*, and is unable to be inherited maternally (Johnson, 1975; Bennett et al., 1975; Silver et al., 1979; see below). Because of the latter property, male mice carrying T^{hp} also carry the Y chromosome from the AKR/J strain in which T^{hp} was discovered. On the fourth to fifth backcross of T^{hp} onto C57BL/6J, the presence of hermaphrodites and a shortage of males was noted. Examination of embryos showed that this was a sex-reversal phenomenon, with XY (male) mice developing ovotestes or true ovaries. Since numerous backcrosses onto the C57BL/6J background were required to elicit this phenotype, multiple genes elsewhere in the genome in C57BL/6J must interact to cause this effect. The *Tas* gene was defined as causing this sex-reversal of the T^{hp} mutation (with its associated AKR/J-derived Y chromosome) on the C57BL/6J background.

Two additional genes mapping to the *t* complex affect embryonic development: fused (*Fu*) and *t*-associated maternal effect (*Tme*). The *Fu* gene was discovered in a laboratory stock of mice, and maps between *T* and *tf*. Like *T*, *Fu* causes a shortening of the tail in heterozygotes (Reed, 1937). *Fu* exhibits incomplete penetrance, which varies with genetic

background. Fused homozygotes are viable on some backgrounds, and lethal on others (Dunn and Gluecksohn-Waelsch, 1954) Homozygous embryos show duplications in the posterior neural tube (Theiler and Gluecksohn-Waelsch, 1956). Two alleles of fused have also been identified. Kinky (Fu^{Ki}) was found by a mouse fancier, and causes a "kinky" short tail in heterozygotes (Caspari and David, 1940). Kinky homozygotes are lethal at days 9-10 of development, and show disorganization and duplication of embryonic tissues (Dunn and Caspari, 1945; Gluecksohn-Schoenheimer, 1949). Knobbly (Fu^{Kb}) arose in a mutagenesis experiment, and causes a "knobbly" short tail in heterozygotes (Lyon, 1977). Knobbly may show incomplete penetrance, or possibly a low transmission ratio in the male. Knobbly homozygotes die at nine days of development, and show initial defects in the embryonic ectoderm (Jacobs-Cohen et al., 1984).

The t-associated maternal effect (Tme) was first identified in the T^{hp} mutant described above. Only males carrying T^{hp} could pass on the chromosome. When females carrying T^{hp} pass this chromosome on to their offspring, the embryos die at approximately 14 days of development (Johnson, 1974). Defects such as edema and polydactyly were noted. This maternal effect lethality could be rescued in aggregation chimeras between maternally-derived T^{hp} embryos

and wild-type embryos (Bennett, 1978). Such rescued chimeras, if female, still could not pass on the *Thp* mutation. Nuclear transplant experiments have indicated that the defect is nuclear, not cytoplasmic (McGrath and Solter, 1984). A partial *t* haplotype, *tᵂᴸᵘᵇ²*, has been described that exhibits an identical maternal effect (Winking and Silver, 1984). It has been proposed that the recombination event that created this partial *t* haplotype, which occured proximally in the same region as the *Thp* deletion, also created the maternal effect at or near the recombination point.

F. Evolution of *t* Haplotypes

The question of how and when *t* haplotypes originated and why they are maintained in wild mouse populations has led to much speculation. The finding of a relatively low level of DNA polymorphisms in the *H-2* region in *t* haplotypes led to the proposal that all *t* haplotypes originated from a common ancestor (Shin et al., 1982). A study of the serological relatedness of the *H-2* antigens in *t* haplotypes also led to this hypothesis (Nizetic et al., 1984). Some researchers suggest that the common ancestor is most likely to be *tᵂ⁵*, since other *t* haplotypes have been reported to arise from it (Dunn, et al., 1973; Bennett et al., 1976), whereas others believe the ancestor was a nonlethal

chromosome (Nizetic et al., 1984) . An alternate proposal was based upon the high degree of homology between different *t* haplotypes and their distinction from wild-type in DNA studies on the *H-2* region and in a 2-D gel analysis of testicular proteins (Silver, 1982; Silver et al., 1983b). It was suggested that *t* haplotypes represent introgressions into mouse subspecies by genetic material from a distinct species. The *t* complex region from this species would be normal in the context of the its species of origin, and would only exhibit the *t*-specific phenotypes in the background of *Mus musculus*. Thus it was suggested that all *t* haplotypes carrying the same lethality and *H-2* haplotype arose from the same ancestor.

The time at which *t* haplotypes originated has been thought to predate the separation of *Mus musculus* into different subspecies, since *t* haplotypes are found in at least two (Dunn et al., 1973), and possibly three (as reviewed in Sage, 1981), different subspecies of *Mus musculus*. A study that identified similar deletions in the *E*$_{alpha}$ gene in *t* haplotypes isolated from different *Mus musculus* subspecies supports the theory of *t* haplotype origin before subspeciation (Dembic et al., 1984).

The maintainence of *t* haplotypes in mouse populations is considered to be due to the transmission ratio distortion properties of *t* haplotypes (see Dunn, 1964). This has led

to speculation concerning the origin of the t haplotype lethal genes. It has been argued that the t lethal genes comprise a group of fuctionally related genes that govern stage-specific transitions during embryonic development (Bennett, 1975; Shin et al., 1983b). However, it has been pointed out that recombination suppressing chromosomes which have the advantage of transmission ratio distortion may act as a lethal sink and pick up sporadic, recessive embryonic lethalities at no harm to the survival of the chromosome (Paigen, 1980; Tres and Erickson, 1982). In fact, in a transmission ratio distorting chromosome that is also homozygous sterile, recessive lethalities are actually an advantage since they decrease the number of sterile males in the population, who cannot contribute to the spread of the chromosome (Lewontin and Dunn, 1960; Lewontin, 1962; Lewontin, 1968). Thus the t haplotype lethal genes may be secondary events in the evolution of t haplotypes.

G. Intent of this research

In order to obtain a better understanding of t haplotypes and the t complex, DNA probes recognizing t complex sequences have been sought. The characterization of the genomic DNA of t haplotype-containing and wild-type mice with these probes allows the comparison of various t haplotypes to each other and to the wild-type form of the t

complex. These comparisons have allowed a molecular characterization of the t complex region of chromosome 17. The comparison of t haplotypes with wild-type supports the view that t haplotypes are a variant of this chromosomal region, but that they do not differ greatly from the wild-type form of this region. Partial t haplotypes have also been characterized, and it has been shown that partial t haplotypes can contain different amounts of t haplotype DNA, thereby accounting for their different phenotypes. The t haplotype property of transmission ratio distortion has been analyzed in light of this characterization, and support for the multiple loci model of Lyon has been obtained. These results with the partial t haplotypes are also discussed with respect to their continued suppression of recombination, and a mechanism causing this suppression is discussed. Finally, a theory concerning the origin and evolution of t haplotypes is presented.

MATERIALS AND METHODS

A. DNA isolation

1. Plasmids

a. Miniprep

This protocol was used to extract microgram amounts of plasmid DNA from 5 ml cultures of bacteria. It is an adaptation of a protocol supplied by D. Hanahan, CSHL. The bacteria in 5 ml overnight culture were pelletted by centrifugation (2,000 r.p.m. in an International centrifuge). The supernatant was removed by aspiration, and the bacterial pellet resuspended in 350 microliters of STET (8% sucrose, 0.5% Triton X-100, 50 mM EDTA, 10 mM Tris, pH 8.0) and transferred to a microfuge tube. A dash of powdered lysozyme was added, the tube vortexed briefly, placed in a boiling water bath for 40 seconds, and this was followed immediately by centrifugation for 10 minutes at room temperature. The pellet was removed with a toothpick and discarded. 200 microliters of 5 M NH_4OAc was added to the supernatant, and the tube was filled with isopropanol and mixed. The mixture was chilled at -20°C for 10 minutes, and the DNA recovered by centrifugation at 4°C. The pellet was resuspended in 100 microliters 5mM Tris, pH 7.6,

0.1 mM EDTA. 10 microliters was sufficient to perform restriction enzyme digests and gel electrophoresis. To eliminate RNA which could mask small restriction fragments, RNase was added to the digestion or to the gel loading buffer. When such preparations were used as plasmid DNA stocks, they were usually further purified by the addition of 1-2 microliters of 10 mg/ml RNase, incubated at room temperature for 30 minutes, followed by phenol/chloroform (1:1) and chloroform extractions and ethanol precipitation.

b. Maxiprep

This protocol was used to extract milligram amounts of plasmid DNA from 500 ml cultures of bacteria. The protocol was supplied by D. Hanahan, CSHL. The bacteria from a 500 ml saturated culture were pelleted by centrifugation (2,500 r.p.m. in a Sorvall centrifuge). The pellet was resuspended in 8 mls of 50 mM Tris, pH 8.0, 50 mM EDTA, and 15% sucrose; and transferred to an SW41 ultracentrifuge tube. 0.1 mls of a fresh 10 mg/ml solution of lysozyme was added, and the mixture left for 5 minutes on ice. 0.25 mls of 20% SDS was added, mixed by inverting the tube several times, and left 10 minutes on ice. 1 ml of 5 M KAc was added, mixed by inversion, and left on ice 1 hour to overnight. This lysate was cleared by ultracentrifugation at 30,000 r.p.m. for 30 minutes at 4°C in an SW41 rotor. Supercoiled plasmid DNA was purified by equlibrium centrifugation in cesium

chloride-ethidium bromide gradients as described by Maniatis et al., 1982.

2. Phage

a. Miniprep

This protocol was used to extract microgram amounts of phage DNA from 10 ml bacterial lysates. It is an adaptation of a protocol supplied by C. Doyle, CSHL. 100 microliters of a phage stock made from a 1 ml plug suspension was mixed with 200 microliters of a stationary culture of plating bacteria (grown with the addition of 0.2% maltose to the medium) and 200 microliters of 10 mM $MgSO_4$, 10 mM $CaCl_2$, and 10 mM Tris, pH7.6 in a 50 ml sterile culture tube, and was incubated at 37°C for 15 minutes. 10 mls of medium was added, and the mixture incubated in a shaking 37°C incubator until lysis was achieved (usually 9-12 hours). A few drops of chloroform was added, and the bacterial debris was pelleted by low speed (2,000 r.p.m. in an Inernational centrifuge) centrifugation. The supernatants were decanted into Ti50 ultracentrifuge tubes. 100 microliters of 10 mM Tris, pH 7.6, 100 microlites of 1 M $MgSO_4$, and 20 microliters of 10 mg/ml DNAse was added, mixed by inversion, and incubated at 37°C for 20-30 minutes. The tubes were then filled with 0.5 M EDTA (1-2 mls), and centrifuged in a Ti50 rotor at 32,000 r.p.m. for 1 hour at 4°C. The

supernatant was decanted or aspirated away, the pellet was dried briefly, and then resuspended in 0.5 mls of SM (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris, pH 7.6, 0.01% gelatin) and transferred to a microfuge tube. 5 microliters of 10% SDS and 5 microliters of 0.5 M EDTA were added, and the mixture incubated at 68°C for 20 minutes. One phenol/chloroform (1:1) and one chloroform extraction was performed to remove proteins. One-half volume of 7.5 M NH_4OAc was added, followed by one volume of isopropanol. The DNA was allowed to precipitate for a few hours or overnight at -20°C or 30 minutes in a dry ice-ethanol bath. DNA was recovered by centrifugation for 15 minutes, and the DNA resuspended in 100 microliters of 5 mM Tris, pH 7.6, 0.1 mM EDTA. 10 microliters was sufficient to perform restriction enzyme digestion and gel electrophoresis. To eliminate RNA which could mask small restriction fragments, RNAse was added to the digests or to the gel loading buffer.

b. Maxiprep

This protocol was used to extract milligram amounts of phage DNA from 500 ml bacterial lysates. It is an adaptation of protocols described by Maniatis et al., 1982. 5 microliters of a plate lysate stock of phage were mixed with 5 OD_{600} units of bacteria, grown with the addition of 0.2% maltose to the medium, in SM. The mixture was incubated for 20 minutes at 37°C, and 500 mls of medium was

added and the mixture incubated with shaking at 37°C until lysis occured, usually 6-9 hrs. 10 mls of chloroform was added and shaking incubation continued for 15 minutes. 50 microliters of 10 mg/ml DNase and 50 microliters of 10 mg/ml RNase were added and the mixture was incubated for 30 minutes at room temperature. 29.2 grams of NaCl was added, dissolved by swirling, and the mixture was left at 4°C for 60 minutes. Debris was removed by centrifugation (4,000 r.p.m. in a Sorvall centrifuge) for 15 minutes, and 10% weight/volume ratio of PEG 6,000 was added. The phage were precipitated by stirring at 4°C for 90 minutes to overnight, and were recovered by centrifugation (4,000 r.p.m. in a Sorvall centrigure) for 20 minutes. The supernatant was decanted away, and the pellet allowed to dry briefly upsidedown. The pellet was resuspended in 6 mls TM (50 mM Tris, pH 7.8, 10 mM $MgSO_4$), and two chloroform extractions were performed. A step gradient was constructed in SW41 ultracentrifuge tubes by layering, from the bottom up, 3 mls of 40% glycerol in TM, 1 ml 5% glycerol in TM, and the phage suspension. Centrifugation in an SW41 rotor was performed at 35,000 r.p.m. for 1 hour at 4°C. The pellet was resuspended in 1 ml TM, and 0.5 ml aliquots added to two microfuge tubes. To each aliquot, 1 microliter of a mixture of 10 mg/ml DNase, 1 mg/ml RNase was added, and incubated at 37°C for 30 minutes. 25 microliters of 0.5 M EDTA was

added, followed by 15 microliters of 20% SDS and 15 microliters of 20 mg/ml predigested pronase. The mixture was incubated at 37°C for 1 hour, followed by two phenol/chloroform (1:1) extractions and one chloroform extraction. One-half volume of 7.5 M NH_4OAc was added, followed by one volume of isopropanol, and the DNA allowed to precipitate at -20°C for two hours, or in a dry ice-ethanol bath for 30 minutes. DNA was recovered by centrifugation, washed in 70% ethanol, and resuspended in 0.5 mls of 5 mM Tris, pH 7.6, 0.1 mM EDTA.

3. Mammalian genomic DNA

a. Mouse organs

This protocol is an adaptation of that described by Silver, 1982. It was used to extract high molecular wieght DNA from organs of adult mice, or from whole newborn mice. Samples were homogenized in 50 mM Tris (pH 8.0), 50 mM EDTA, and 250 mM sucrose on ice. It was found that recovery of DNA from fresh samples was facilitated by the addition of 0.2% Triton X-100 in the first homogenization. A crude nuclear fraction was recovered by centrifugation (2,000 r.p.m. in an International centrifuge). Nuclei were washed by homogenization in the same buffer, recovered by centrifugation, and resuspended in 2-10 mls of 50 mM Tris, pH 8.0, and 50 mM EDTA. SDS was added to 2%, and pronase

(self-digested at 37°C for 3 hr prior to use) was added to 1 mg/ml. Proteolytic digestion was allowed to proceed at 37°C overnight, usually 8-12 hrs. Sample volumes were increased to 5-15 mls with 50 mM Tris and 50 mM EDTA, and a single phenol extraction, followed by extractions with phenol/chloroform/isoamyl alcohol (50:48:2) were performed to extract proteins until a clean interface could be obtained. A single chloroform extraction was performed to remove residual phenol. One-fourth volume of 10 M NH_4OAc was added to the sample, followed by two volumes of -20°C absolute ethanol. The precipitated DNA was recovered by spooling, dipped in 70% ethanol to wash, allowed to dry briefly, and resuspended in 5 mM Tris (pH 7.6) and 0.1 mM EDTA by gentle rocking overnight at 4°C. The purified DNA was stored at 4°C.

b. Cell lines

This protocol was supplied by L. Silver, Princeton. Cell pellets were homogenized in 50 mM Tris, pH 8.0, 50 mM EDTA, and 100 mM NaCl. SDS and pronase addition, proteolytic digestion, and DNA purification proceeded as described for mouse organs.

c. Mouse tails

This protocol is an adaptation of one supplied by R. Brinster, Phil. A 2-3 cm length of tail was removed from a

mouse and placed in a microfuge tube containing 0.6 mls of 50 mM NaCl, 50 mM EDTA, and 50 mM Tris, pH 8.0. The tail was chopped finely with scissors, 30 microliters of 10 mg/ml collagenase added, and the mixture incubated at 37°C for 1 hour. 35 microliters of 20% SDS was added and mixed, followed by 75 microliters of 20 mg/ml predigested pronase. The mixture was incubated overnight at 37°C overnight. The sample was then extracted with equal volumes of phenol, phenol/chloroform (1:1) and chloroform. One-tenth volume of 3 M NaAc was added, mixed, and the tube was filled with cold (-20°C) isoproponal. If a precipitate did not form and condense after the isoproponal was mixed, the tube was rocked gently at 4°C for 10-20 minutes to obtain this precipitate. DNA was spooled onto a heat-sealed glass micropipette, dipped in 70% ethanol to wash, allowed to dry briefly, and resuspended in 0.5 mls of 5 mM Tris, pH 7.6, 0.1 mM EDTA by rocking overnight at 4°C. 150 microliters of this preparation usually contained 5-10 micrograms of DNA, sufficient to restriction enzyme digest for blot-hybridization analysis.

B. RNA isolation

This protocol was supplied by N. Sarvetnick, CSHL. A solution of 5 M guanidium isothiocyanate, 50 mM Tris, pH 7.6, 10 mM EDTA, 5% beta-mercaptoethanol was prepared by

first disolving the guanidium isothiocyanante in water by stirring overnight, followed by the addition of the other components. Fresh spleens were homogenized by a mechanical homogenizer in 12 mls of the guanidium solution. The homogenizer was rinsed in 3 mls of the guanidium solution, and the homogenate and the rinse were mixed in a Corex tube, the volume increase to the top of the tube with the guanidium solution, and centrifuged at 10,000 r.p.m. (in a Sorvall centrifuge) for 20 minutes. The supernatant was decanted into a clean tube, one-tenth volume (1.65 mls) of 20% Sarkosyl was added to it, and the solution was brought to 65°C for 2-3 minutes. CsCl was added to 0.1 g/ml (1.7 g), and a step gradient was constructed in an SW27 tube by layering 6 mls of 5.7 M CsCl, the extract (approximately 17.5 mls), and the tube filled with of 0.1 g/ml CsCl, 2% Sarkosyl in the guanidium solution. The solution was centrifuged in an SW27 rotor at 25,000 r.p.m. for 16 hours at 20°C.

The supernatant was aspirated away, the tube cut off 2-3 cm from the bottom, and the pellet scraped from the tube in 0.5 mls of 5 mM EDTA, 0.5% Sarkosyl, 5% beta-mercaptoethanol, prepared in DEPC-treated water. The suspension was brought to 37°C for 20 minutes, vortexed to disolve the pellet, and phenol/chloroform (1:1) extracted 3 times, and chloroform extracted once. One-tenth volume

DEPC-treated 3 M NaOAc (the original protocol calls for 3 M LiCl, which was not available) was added, followed by 2.5 volumes absolute ethanol. RNA was precipitated overnight at -20°C, centrifuged, disolved in DEPC-treated water, and reprecipitated in 0.3 M NaOAc with 2.5 volumes of ethanol.

C. Isolation of recombinant phage from genomic libraries

All techniques performed similar to those described in Maniatis et al., 1982, except for the hybridization conditions. Briefly, approximately 800,000 phage were plated (50,000 phage per plate). Nitrocellulose was used to make lifts of the phage, and such lifts were hybridized to probe in the same manner as used for Southern blots, below, except a prewashing in 50mM Tris (pH 8.0), 1 M NaCl, 1 mM EDTA, and 0.1% SDS at 41°C was performed before hybridization. Positive spots were picked by withdrawing a plug of phage plagues and agar from the plate with the wide end of a sterile pasteur pipette. These plugs were resuspended in 1 ml of SM containing a drop of chloroform, and the phage were replated at a density of 200-300 per plate. A second round of screening proceeded, and individual plaques were isolated by withdrawing small plugs of phage plaques and agar with the narrow tip of a sterile pasteur pipette, and resuspended in 1 ml of SM containing a drop of chroloform. Plate stocks of the phage were made by

the plate lysate techinque. Confluent lysis was achieved on freshly poured plates, and the phage harvested by the addition and subsequent withdrawal of SM to the plate. Chloroform was added, and debris removed by low speed centrifugation (2,000 r.p.m. in an International centrifuge). Phage were stored in SM containing a drop of chloroform at 4°C.

D. Subcloning

Fragments of recombinant clones were subcloned as described by Maniatis et al., 1982, except fragments were not purified from the vector before ligation. Transformation of the recombinant DNA into E. coli DH1 or DH5 was performed as described by Hanahan, 1983.

E. Restriction enzyme digestion

1. Digestion conditions

The DNA to be digested was diluted so that the final concentration of DNA would not exceed 100 mg/ml. The buffers and incubation temperatures used were as given by Maniatis et al., 1982; except BglII digests were performed in a buffer consisting of 20 mM glycine, pH 9.5, 20 mM $MgCl_2$, 150 mM NaCl, and 6 mM beta-mercaptoethanol; and TaqI digests were performed in a buffer consisting of

10 mM Tris, pH 8.4, 6 mM $MgCl_2$, 100 mM NaCl, and 6 mM beta-mercaptoethanol.

2. Cloned DNA

DNA from purified recombinant phage or plasmids was digested with 2-fold excess units of restriction enzyme for 2-3 hours.

3. Genomic digests

Mouse genomic DNA was usually digested as follows. 20 micrograms of DNA (enough for 3 lanes on a gel) was diluted to 300 microliters. 34 microliters of 10x buffer was added, followed by 10 units of restriction enzyme. The tube was tapped to mix, and the sample incubated for 30 minutes, tapped, and incubated 30 more minutes. 10 more units of enzyme were added, and the sample was incubated 3 hours to overnight. A final 10 units of restriction enzyme were added, 17 microliters removed and added to test digest (see below) and both samples incubated 3 hours to overnight.

To test for complete digestion, an aliqout of the above reaction was added to 200 ng plasmid (pBR322 or pUC12) or viral (SV40 or lambda phage) DNA, incubated as described above, and electrophoresed on an agarose gel, ethidium bromide-stained, and examined. When the test DNA was cut to completion, it was assumed that the genomic DNA was also cut to completion. If the test DNA was not completely digested,

another aliquot of restriction enzyme was added and the test digest repeated.

F. DNA gel electrophoresis and Southern blot-hybridization techniques

1. Agarose gel electrophoresis

Samples in small volumes were prepared for electrophoresis by the addition of one-third volume of 4x gel loading buffer (3.2% SDS, 40 mM Tris, pH8.0, 40 mM EDTA, 40% sucrose, dashes of bromophenol blue and xylene cyanol) was added. Samples in large volumes were first precipitated by the addition of one-half volume of 7.5 M NH_4OAc, 50 mM EDTA followed by 2 volumes of ethanol, chilled, the DNA recovered by centrifugation, and then disolved in 1x gel loading buffer (0.8% SDS, 10 mM Tris, pH8.0, 10 mM EDTA, 10% sucrose, dashes of bromophenol blue and xylene cyanol). For the analysis of cloned DNA, 200ng to 1 microgram of DNA was usually loaded per lane. For genomic DNA, 5 to 10 micrograms of DNA was loaded per lane.

Agarose was made up in 1x Loenings buffer (146 mM Tris base, 120 mM $NaH_2PO4 \cdot H_2O$, 4.4 mM Na_2EDTA). In general, for analysis of large (over 8kb) fragments of DNA, 0.7% gels were used, medium (2-8 kb) fragments 1.0% gels were used, and small (0.5-2 kb) fragments 1.5% gels were used. Gels

were run until marker dyes migrated the desired length at 0.5-0.8 V/cm.

To visualize the DNA, gels were stained in 1 microgram/ml ethidium bromide for 30-60 minutes, destained in water if faint fragments were being detected, and examined and photographed under ultraviolet light.

2. Transfer to nitrocellulose

The DNA in the gels was denatured by placing the gel in several volumes of 1 M NaOH, 1.5 M NaCl for 1 hour; neutralized by placing the gel in several volumes of 1 M Tris, pH 8.0, 1.5 M NaCl, for 1 hour, and equilibrated in several volumes of 10x SSC (1x SSC contains 150 mM NaCl, 15 mM Na_3Citrate) for 15 minutes. A piece of nitrocellulose was cut to the size of the gel and wet in 10x SSC. A capillary blot was set up in a pyrex dish containing 10x SSC, consisting of a wick of Whatman 3MM paper leading from the dish to a supported glass plate. The gel was inverted onto the wick-covered glass, covered with the nitrocellulose, follwed by 3 pieces of 3MM paper cut to the size of the gel, a stack of paper towels, and another glass plate weighted down with two weights of approximately 100 gram apiece. Capillary transfer was allowed to proceed overnight, after which the nitrocellulose was removed,

washed by repeated application of 2x SSC from a squirt bottle, and baked at 80°C for 2 hours under vacuum.

3. Hybridization conditions

Blots were prehybridized in hybridization solution (50% formamide, 50 mM HEPES, pH 7.0, 2x Denhart's solution, 3x SSC, 333 ng/ml denatured salmon sperm DNA) in a Seal-a-meal bag for 1 hour to overnight at 41°C on a rocking platform (100x Denhardt's solution consists of 2% Ficoll, 2% polyvinylpyrrolidone, and 2% BSA). 3 x 10^6 c.p.m. of nick-translated probe per ml of hybridization solution was boiled to denature the DNA, chilled on ice, and injected into the hybridization bag, the hole was heat-sealed, and the probe distributed throughout the blot by rubbing the mixture around the bag. The blot was hybridized to the probe for 2-3 days at 41°C on a rocking platform.

4. Nick-translation of probes

To make radioactive probes, 250 nanograms of DNA was diluted to 4.5 microliters. 1.5 microliters of 10x nick-translation buffer (0.5 M Tris, pH 7.2, 0.1 M $MgSO_4$, 5 mM dithiothreitol), 1.5 microliter of 10x cold nucleotides (200 micromolar dATP, dGTP, dTTP), and 6 microliters of labelled nucleotide (dCTP, specific activity 800 Ci/mmole) was added on ice. 0.5 microliters of

DNase (0.1 micrograms/ml) and 1 microliter of DNA polymerase 1 (2-5 units/microliter) was added, the solution mixed, and placed at 12-14°C for 60-90 minutes. The reaction was stopped by the addition of 3 volumes of gel loading buffer, and labelled probe was separated from unincorporated numcleotides using G-50 spin columns as described by Maniatis et al., 1982. Specific activities of 1-5 x 10^8 c.p.m./microgram of DNA were obtained by this procedure.

5. Washing conditions

Following hybridization, the blot was removed from the bag, rinsed in 2x SSC, and washed in 500 mls of 2x SSC, 0.5% SDS, rotating at room temperature, for 40-60 minutes. The wash was changed,at 50°C, twice with 0.1x SSC, 0.1% SDS for 40-60 minutes, and twice with 0.1x SSC for 20-30 minutes. The blot was dried on 3MM paper.

6. Exposure to film

Washed blots were exposed to X-ray film, using an intensifying screen, overnight at -70°C. Most blots were then placed back on film for periods ranging from 6-14 days.

G. RNA gel electrophoresis and Nothern blot-hybridization techniques

1. Agarose gel electrophoresis

RNA was separated on formaldehyde gels as described by Maniatis et al., 1982. The gel was not stained prior to blotting.

2. Transfer to nitrocellulose

Follwing electrophoresis, the gel was soaked in 20x SSC for 60 minutes, and blotted as described for the DNA blots above, except 20x SSC was used as the transfer buffer.

3. Hybridization conditions

Hybridization was performed in a manner similar to that for the DNA blots above, except that a different prehybridization mix (50% formamide, 1x Denhart's solution, 5x SSC, 400 ng/ml denatured salmon sperm DNA, and 0.1% SDS) and hybridization mix (the prehybridization mix plus 10% dextran sulfate) were used, and hybridization was only allowed to proceed overnight. Probes, washing, and film exposure were performed as described for the DNA blots.

H. Mouse genetics

1. Animals

The complete t haplotypes t^0, t^{w32}, t^{w12}, t^{w2}, and t^{w5} are reviewed by Bennett, 1975. The origin of the complete t haplotype t^{wLub1} is described by Winking, 1979, and Winking and Silver, 1984; t^{Lmb} by Silver et al., 1984b; t^{Tuw2}, t^{Tuw7}, t^{Tuw8}, t^{Tuw10}, t^{Tuw11}, t^{Tuw12}, t^{Tuw15}, t^{Tuw18}, t^{Tuw20}, t^{Tuw21}, t^{Tuw23}, t^{Tuw24}, t^{Tuw25}, t^{Tuw26}, t^{Tuw28}, and t^{Tuw29} by Klein et al., 1984. The origin of the t^{Tuw32} chromosome, which was found in this study not to be a complete t haplotype, is also described by Klein et al., 1984. t^{wLub4}, t^{wLub6}, t^{wLub7}, and t^{wLub9} are complete t haplotypes which were found in wild mice (H. Winking, personal communication).

The origins of the various partial t haplotypes are described in the following references: t^6 - Carter and Phillips, 1950, and Silver et al., 1984b; t^{h20} - Lyon and Bechtol, 1977; t^3 and t^4 - Dunn and Gluecksohn-Waelsch, 1953; t^{w18} - Bennett and Dunn, 1960; t^{h2} and t^{h18} - Lyon and Meredith, 1964a; t^{h17} - Lyon and Mason, 1977; t^{h45}, t^{h49}, t^{h51}, t^{lowH}, and t^{low3H} - Lyon, 1984; t^{ae5} - Vojtiskova et al., 1976; Tt^{Orl} - Moutier, 1973a,b, and Silver, et al., 1983a; t^{Tu2} and t^{Tu4} - Styrna and Klein, 1981; t^{s6} - Silver and Artzt, 1981; t^{wLub2} - Winking and Silver, 1984; t^{h44} and

t^{h53} - Fox et al., 1985. t^{ks1} and t^{ks2} were derived from recombinant chromosomes in a Ttf/t^{w5} balanced lethal stock (V. Bode, personal communication); t^{low2H} was derived from a T-tf recombinant chromosome from a mouse carrying $Tt^{h17}/t^{h2}tf$ (M. Lyon, personal communication).

The inbred strain WLA76 was derived from a pair of wild Mus domesticus mice trapped in Paris, France (J.-L. Guenet, personal communication). The origins of the other inbred strains are reviewed by Staats, 1981.

2. Phenotype and genotype determination

Tail lengths of mice were checked at birth and again at weaning. The presence of the tufted phenotype was noted by ease of loss of hair in response to physical pulling at four weeks or later, and the striped patterns of sequential hair loss in adult mice. When these methods yielded ambiguous results or could not be applied, the genotype was determined by analysis of the genomic DNA of the mouse using the probes identified in this study. Genotypes of mice used in the genetic experiments were also verified by DNA analysis.

RESULTS

A. Identification of DNA probes that map to the t complex

When this study was initiated, it was apparent that, except for the H-2 complex region genes, the number of previously cloned sequences available that might map to the t complex was limited. A number of possible approaches to obtain new probes were considered. Screening random clones (Kao et al., 1982) was impractical, since the t complex represents only approximately 1% of the genome (15cM out of a total of 1500cM). Procedures have been described using fluorescence-activated sorting of chromosomes to obtain chromosome-specific probes (Davies et al., 1981; Kunkel et al., 1982), but this is difficult with mouse chromosomes, and still only approximately one-third of the chromosome 17 clones would have originated from the t complex region. Another procedure available for obtaining chromosome-specific probes involves screening clones derived from interspecies somatic cell hybrids with species-specific repetitive sequences (Gusella et al., 1980), but again only one-third would have originated from the t complex, and to obtain useful probes this procedure requires the subcloning of unique sequences from each of the clones isolated. To overcome these difficulties, a technique used to clone DNA from dissected Drosophila salivary gland polytene

chromosomes (Scalenghe et al., 1981) was adapted for the cloning of DNA from dissected regions of mammalian chromosomes (Rohme et al., 1984). Chromosomal fragments from the proximal part of mouse chromosome 17 were dissected and cloned in microscale. A majority of these clones could be expected to be derived from the t complex region, and clones containing repetitive DNA could easily be identified and screened out of the study. The analysis of these clones is described below.

These microdissection-derived clones were provided by the laboratory of H. Lehrach, EMBL. They contain EcoRI restriction fragments of the microdissected chromosome fragments cloned into the lambda phage vector NM641, and had been screened as plaques against total mouse DNA. Any hybridizing clones were presumed to contain highly repetitive sequences and were not analyzed further. For the remaining clones the insert sizes were determined, and only clones containing inserts of 200 bases or more were received for analysis. Forty eight clones met these criteria. In many cases, the insert of the clone had been transferred to the EcoRI site of the plasmid vector pUC9 before it was received; others were received as recombinant phage in the lambda vector, and the inserts were then transferred to the EcoRI site of the plasmid vector pUC13.

Chromosome mapping experiments performed with somatic cell hybrids indicated that 95% of the clones tested were indeed derived from chromosome 17 (Rohme et al., 1984). The one clone that was found not to map to chromosome 17 was not analyzed further. In order to ascertain whether the remaining clones were derived from the t complex region, a search for restriction fragment length polymorphisms (RFLPs) that distinguish t haplotype and wild-type DNA was initiated. The mapping of probes to the t complex was achieved by comparing the hybridization patterns of the probe to restriction digest blots of genomic DNA from mice congenic for t haplotypes. These congenic mice were constructed (J.-L. Guenet, personal communication; Silver, 1982) by backcrossing the t haplotypes onto the 129/SvJ strain of inbred mice. In such mice, the only differences in the DNA between 129 and 129/t mice are the t haplotype sequences or sequences closely linked to the t complex. Thus, the identification of a RFLP between 129 and 129/t DNA maps the sequence identified by the probe to the t complex region.

The initial screening of these clones on Southern blots revealed that the clones fell into three classes. Twenty three of the clones hybridized to single-copy or low-copy number sequences in the mouse genome, eight clones recognized repetitive sequences, and sixteen clones failed

to hybridize to any sequences. Twenty of the single to low copy clones were then used in this study as probes to find sequences mapping to the t complex. Eight of these clones were found to identify polymorphisms between t and wild-type DNA. Twelve clones were classified as not identifying a polymorphism since none of ten different restriction enzymes used yielded a RFLP. These restriction enzymes were BamHI, BglII, EcoRI, HindIII, KpnI, PstI, SstI, TaqI, PvuII, and XbaI. The localization of such nonpolymorphic clones is uncertain; they may map to chromosome 17 outside of the t complex region, or they map map within the t complex but not in a region in which DNA polymorphisms could be identified with the restriction enzymes used here. Additionally, the two nonpolymorphic clones whose chromosomal localization has not been determined may map to a chromosome other than chromosome 17. A summary of these data is presented in Table 1.

A finding of a polymorphism was then verified on a panel of congenic mice to map the sequence to the t complex. These congenic mice consisting of 129/SvJ wild-type, and four different complete t haplotypes, t^0, t^{w2}, t^{w5}, and t^{w12}, on the 129/SvJ background. Four other complete t haplotypes, t^{w32}, t^{wLub1}, t^{wLub7}, and t^{Lmb}, were also examined from F1 mice with 129/SvJ. These clones were then used as probes on blots of DNA from as many seventeen other

Table 1. Search for RFLPs between *t* and Wild-type with Microdissection-derived Clones

The size of each clone is given in base pairs. Clones that have been mapped to chromosome 17 through the use of somatic cell hybrids are indicated: Y - yes, nd - not determined. Parentheses surround symbols for restriction enzymes with which the hybridizing restriction fragments could not be detected. Enzymes that are useful in finding RFLPs between *t* haplotype and wild-type DNA (positive enzymes) are divided into those that were not examined in detail (not dtl.) and those that were used to identify *t*-specific restriction fragments (*t*-spcf.). Restrition enzymes are BamHI (B), BglII (G), HindIII (H), KpnI (K), PstI (P), EcoRI (R), SacI (S), TaqI (T), PvuII (V), and XbaI (X).

TABLE 1

RFLPs between *t* and wild-type

Clone	Size	17	Negative	Positive not dtl.	Positive *t*-spcf.
Tu17	300	nd	T,G,X,S,V,P,B,(K,H,R)		
Tu18	250	nd	T,G,X		V
Tu23	600	Y	T,G,X,S,K,V,P,H,B,R		
Tu48	250	Y	G,V,R	X,S,P,T	B
Tu54	1300	nd	G,R	S,P,H,T,X	K
Tu65	550	Y	T,G,X,S,K,V,P,H,B,R		
Tu66	450	Y		G	T,B
Tu80	350	Y	T,G,X,R,S		B
Tu94	350	Y	T,V,H,K,B,S,P,G,X,(R)		
Tu101	600	Y	T,G,X,S,K,V,P,H,B,R		
Tu106	750	Y	T,G,X,S,K,V,P,H,B,R		
Tu108	400	Y	R	T,G,K	V
Tu111	2600	nd	T,G,K,V,B,R	S,H	P
Tu115	300	Y	T,G,X,S,K,V,P,H,B,(R)		
Tu116	1000	Y	T,G,X,S,K,V,P,H,B,R		
Tu122	400	nd	G,V,R		T
Tu145	1550	Y	T,G,X,S,K,V,P,H,B,R		
Tu174	250	nd	T,(G,X,S,K,V,P,H,B,R)		
Tu177	550	Y	T,G,X,S,V,P,H,B,R,(K)		
Tu180	400	Y	T,G,X,V,P,H,B,K,(S,R)		

Repetitive

Tu2, Tu5, Tu6, Tu38, Tu43, Tu44, Tu92, Tu96

No hybridization

Tu22, Tu24, Tu36, Tu37, Tu55, Tu61, Tu64, Tu67, Tu74, Tu88, Tu120, Tu127, Tu156, Tu162, Tu179, Tu182

inbred strains of mice to see if these restriction fragments were specific to t haplotype DNA. The strains tested were A/J, AKR/J, BALB/cJ, CBA/CaJ, C3H/HeJ, C57BL/6J, C57BL/10J, C58/J, DBA/1J, DBA/2J, LG/J, LT/ChReJ, NZB/B1NJ, RF/J, SM/J, SWR/J, and WLA76. Restriction fragments identified as t-specific were named by the number of the clone identifying them, followed by sequential letters if multiple t-specific fragments were observed. A summary of these data can be found in Tables 2 and 3.

Three of these eight clones recognized single-copy sequences in the mouse genome by which wild-type and t haplotype alleles could be distinguished. Tu48 is a 250 base pair genomic restriction fragment. This probe recognizes a RFLP in DNA digested with BamHI. In wild-type DNA a 6.1kb restriction fragment hybridizes with the probe, in t haplotype DNA a 5.9kb (T48) restriction fragment hybridizes (see Figure 3A). Polymorphisms between t haplotype and wild-type DNA were also found with this probe using SacI, PstI, TaqI, and XbaI, but have not been extensively characterized.

Tu111 is a 2.6kb genomic restriction fragment. This probe recognizes a RFLP in PstI digests. In wild-type DNA a 6.0kb restriction fragment hybridizes with the probe, and in t haplotype DNA two restriction fragments, 4.4kb and 1.6kb, (T111), hybridize with the probe (see Figure 3B). These

Table 2. Identification of *t*-Specific Restriction Fragments

The absence (-) of the *t*-specific fragments described in the text from inbred mouse strains and their presence (+) in complete *t* haplotypes is indicated. nd - not determined.

TABLE 2

Genotype	T48	T66A	T66B	T66C	T122	T111	T80 ABC	T18	T54	T108
Wild-type:										
129/SvJ	-	-	-	-	-	-	-	-	-	-
A/J	-	-	-	-	-	-	-	-	-	-
AKR/J	-	-	-	-	-	nd	-	-	nd	-
BALB/cJ	-	-	-	-	-	-	nd	nd	-	-
CBA/CaJ	-	-	-	-	-	nd	-	-	-	-
C3H/HeJ	-	-	-	-	-	-	-	-	-	-
C57BL/6J	-	-	-	-	-	nd	-	-	nd	-
C57BL/10J	-	-	-	-	-	nd	nd	nd	nd	-
C58/J	-	-	-	-	-	nd	nd	nd	nd	-
DBA/1J	-	-	-	-	-	nd	nd	nd	nd	-
DBA/2J	-	-	-	-	-	-	-	-	-	-
LG/J	-	-	-	-	-	nd	-	nd	nd	-
LT/ChReJ	-	-	-	-	-	nd	nd	nd	nd	-
NZB/B1NJ	-	-	-	-	-	-	-	nd	nd	-
RF/J	-	-	-	-	-	-	nd	nd	-	-
SM/J	-	-	-	-	-	-	nd	nd	nd	-
SWR/J	-	-	-	-	-	-	nd	-	-	-
WLA76	-	-	-	-	-	-	-	-	-	-
Complete t haplotypes:										
t0	+	+	+	+	+	+	nd	nd	+	+
tw2	+	+	+	+	+	+	+	nd	+	+
tw5	+	+	+	+	+	+	+	nd	+	+
tw12	+	+	+	+	+	+	nd	nd	+	+
tw32	+	+	+	+	+	+	+	+	+	+
twLub1	+	+	+	+	+	+	nd	nd	+	+
twLub7	+	+	+	+	+	+	+	+	+	+
tLmb	+	+	+	+	+	+	+	+	+	+

Table 3. Summary of t-Specific Restriction Fragments

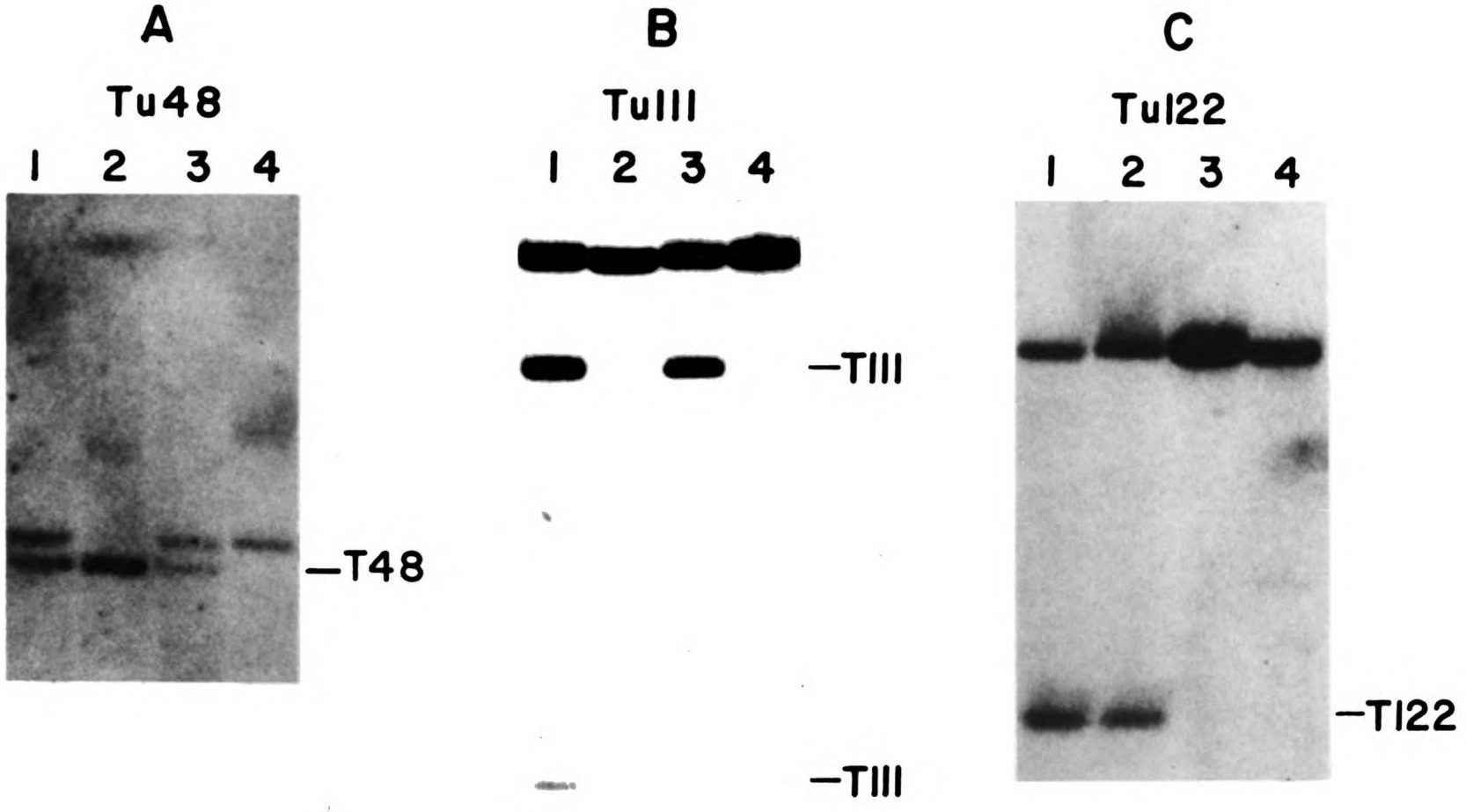
The sizes of the restriction fragments identified by each clone that recognizes a t-specific restriction fragment are listed for wild-type (129/SvJ) and t haplotype (usually t^0/t^{w5}) DNA. The t-specific restriction fragments are identified in the far right column.

TABLE 3

Clone	Enzyme	129	t	t-specific
Tu18	PvuII	11	11	
		10.1	4.4	- T18
Tu48	BamHI	6.1	5.9	- T48
Tu54	KpnI	17.5	17	
		17	16	
		16.5	13.5	
		16	13	
		13.5	10.3	- T54
		13		
Tu66	TaqI	9.7	6.2	- T66A
		2.9	4.4	
		1.9	2.9	
		1.7	1.7	
			0.8	- T66C
Tu66	BamHI	20	21	
		12.5	20	
		12	17.5	- T66B
		6.3	14	
		5.8	12.8	
			8.2	
			7.0	
			5.8	
Tu80	BamHI	17.5	11.2	- T80A
		10.5	10.5	
		9.9	9.9	
		8.0	7.2	- T80B
		6.2	6.4	- T80C
Tu108	PvuII	15	15	
		12	13.5	
		7.8	12	
		6.4	9.4	- T108
		5.5	6.3	
			6.1	
Tu111	PstI	6.0	4.4	- T111
			1.6	- T111
Tu122	TaqI	5.1	1.9	- T122

Figure 3. *t*-Specific Restriction Fragments (I)

Hybridization patterns of restriction fragments of genomic DNA using the indicated clones as probes. The *t*-specific restriction fragments indicated in the text are indicated to the right of the panel. Sizes of these fragments are given in the text and Table 3.
(A) Hybrdization of Tu48 to BamHI digested DNA. Lane 1 - +/t^{wLub7}; lane 2 - t^{0}/t^{w5}; lane 3 - +/t^{3}; lane 4 - +/t^{h17}.
(B) Hybridization of Tu111 to PstI digested DNA. Lane 1 - +/t^{w18}; lane 2 - +/t^{Tu2}; lane 3 - +/t^{w12}; lane 4 - +/+.
(C) Hybridization of Tu122 to TaqI digested DNA. Lane 1 - +/t^{4}; lane 2 - +/t^{h17}; lane 3 - +/t^{h53}; lane 4 - +/+.


A
Tu48
1 2 3 4
—T48
B
TuIII
1 2 3 4
—TIII
—TIII
C
Tu122
1 2 3 4
—T122

restriction fragments might be accounted for by the presence of a PstI site in t haplotype DNA within the hybridizing sequence accounting for the polymorphism. Polymorphims between t haplotype and wild-type DNA have also been found with this probe in SacI and HindIII digests.

Tu122 is a 400 base pair genomic restriction fragment. This probe recognizes a RFLP in TaqI digests. In wild-type DNA, a 5.1kb restriction fragment hybridizes with the probe, and in t haplotype DNA a 1.9kb (T122) restriction fragment hybridizes to the probe (see Figure 3C).

Five of the eight polymorphic clones recognized multiple restriction fragments in the mouse genome. Tu18 is a 250 base pair genomic restriction fragment. This probe recognizes a RFLP between 129 and t haplotype DNA digested with the enzyme PvuII. When wild-type DNA is present, 11kb and 10.1kb restriction fragments are recognized, whereas when t haplotype genomic DNA is present 11kb and 4.4kb (T18) restriction fragments are recognized (see Figure 4A). Although this probe recognizes two restriction fragments in PvuII blots, only a single restriction fragment is detected in digests with other enzymes. Whether this clone recognizes a single copy sequence containing a PvuII restriction site, or whether there are two copies of this sequence in the genome, one of which could not be detected

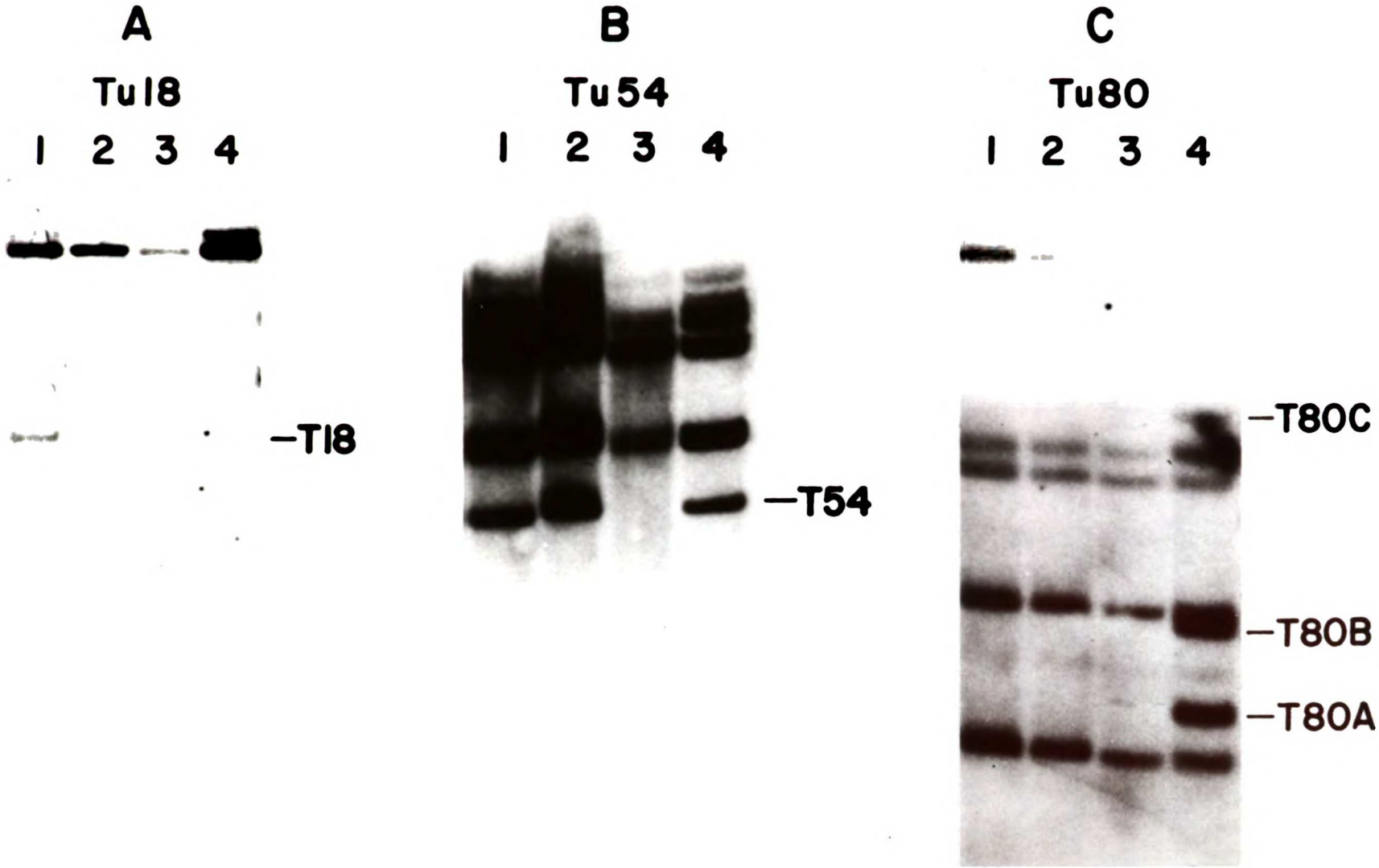
Figure 4. t-Specific Restriction Fragments (II)

Hybridization patterns of restriction fragments of genomic DNA using the indicated clones as probes. The t-specific restriction fragments indicated in the text are indicated to the right of the panel. Sizes of these fragments are given in the text and Table 3.

(A) Hybridization of Tu18 to PvuII digested DNA. Lane 1 - +/t^{ks1}; lane 2 - +/+; lane 3 - +/t^{Lmb}; lane 4 - +/+.

(B) Hybridization of Tu54 to KpnI digested DNA. Lane 1 - +/t^{6}; lane 2 - +/t^{h17}; lane 3 - +/t^{4}; lane 4 - +/t^{h20}.

(C) Hybridization of Tu80 to BamHI digested DNA. Lane 1 - +/t^{h53}; lane 2 - +/Tt^{Orl}; lane 3 - +/t^{Tu2}; lane 4 - +/t^{w18}.


A
Tu18
1 2 3 4
—T18
B
Tu54
1 2 3 4
—T54
C
Tu80
1 2 3 4
—T80C
—T80B
—T80A

on the screening blots with other enzymes, cannot be determined at this time.

Tu54 is a 1.3kb genomic restriction fragment. This probe recognizes RFLPs in digests with a number of enzymes, and the easily recognizable polymorphism in KpnI digests was chosen for characterization. This probe recognizes multiple restriction fragments in the genome, but a 10.3kb (T54) KpnI restriction fragment hybridizes to this probe only when t haplotype DNA is present (see Figure 4B). In studies with this and the other clones describe below, the wild-type homologues of the t-specific restriction fragments could not be identified due to the presence of multiple hybridizing sequences. This probe also identifies polymorphisms between the wild-type strains 129/SvJ and DBA/2J with XbaI, and 129/SvJ and CBA/J with TaqI, but these were not extensively characterized.

Tu66 is a 450 base pair genomic restriction fragment. This probe recognizes RFLPs in digests with number of different enzymes. In TaqI digests, 6.2kb (T66A) and 0.8kb (T66C) restriction fragments are found only when t haplotype DNA is present (see Figure 5A). BamHI digests show a 17.5kb (T66B) restriction fragment only associated with the presence of t DNA (Figure 5B). BglII and TaqI digests were also found to yield polymorphisms between t haplotype and wild-type DNA, but these were not extensively characterized.

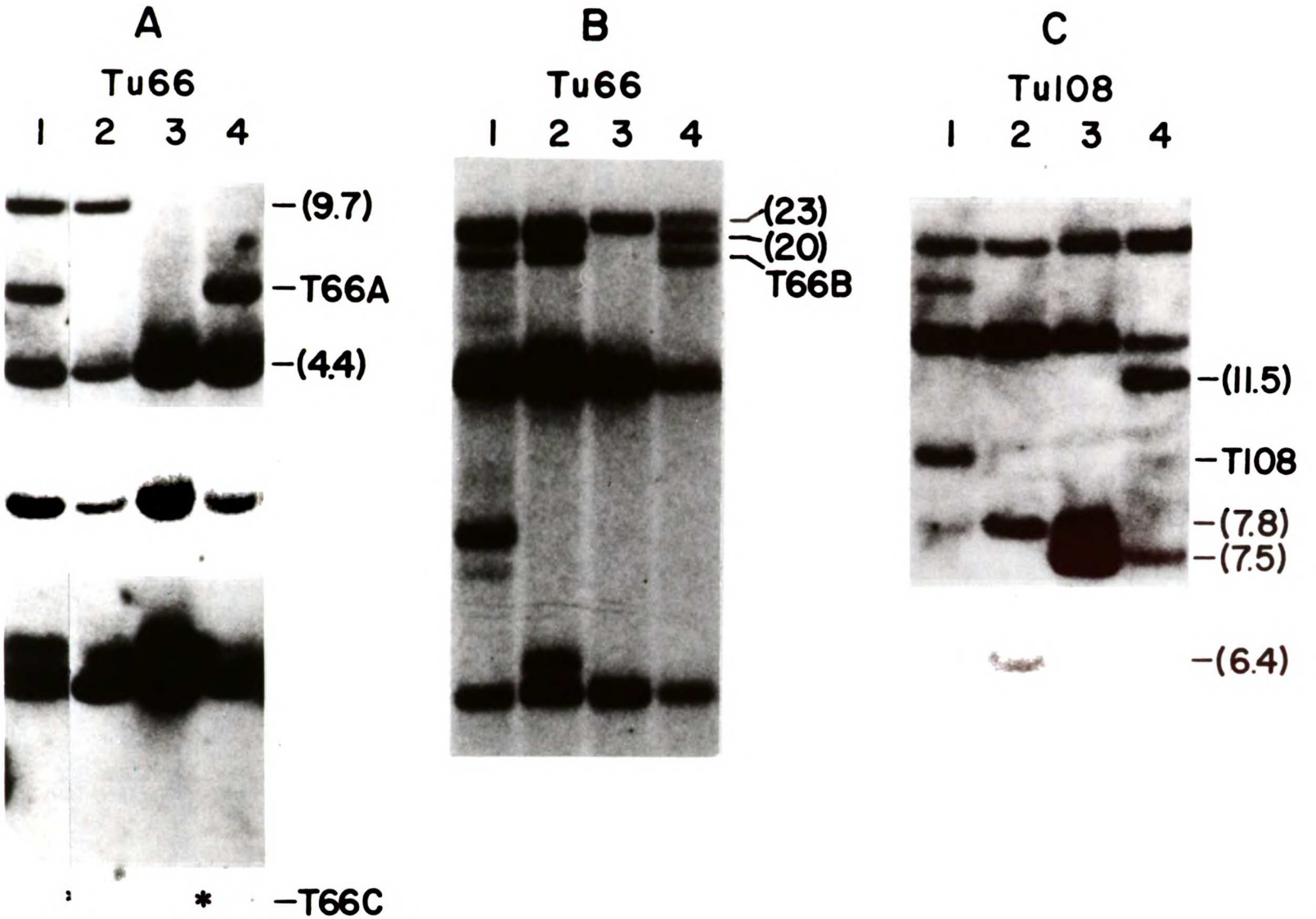
Figure 5. t-Specific Restriction Fragments (III)

Hybridization patterns of restriction fragments of genomic DNA using the indicated clones as probes. The t-specific restriction fragments indicated in the text are indicated to the right of the panel. Sizes of these fragments are given in the text and Table 3. Positions of restriction fragments that are polymorphic between the 129/SvJ and DBA/2J strains are indicated to the right of the panel, identified by their sizes in parentheses.

(A) Hybridization of Tu66 to TaqI digested DNA. An asterisk (*) highlights the presence of a faint restriction fragment in lanes 1 and 4. Lane 1 - +/t^{3}; lane 2 - +/t^{Tu2}; lane 3 - +/+; lane 4 - +/t^{w32}.

(B) Hybridization of Tu66 to BamHI digested DNA. Lane 1 - +/t^{4}; lane 2 - +/t^{lowH}, lane 3 - t^{h44}/t^{h44}; lane 4 - +/t^{h49}.

(C) Hybridization of Tu108 to PvuII digested DNA. Lane 1 - +/t^{h20}; lane 2 - +/t^{w18}; lane 3 - t^{h53}/t^{h53}; lane 4 - +/+.


A
Tu66
1 2 3 4
—(9.7)
—T66A
—(4.4)
—T66C
B
Tu66
1 2 3 4
—(23)
—(20)
T66B
C
Tu108
1 2 3 4
—(11.5)
—T108
—(7.8)
—(7.5)
—(6.4)

Polymorphisms were also observed between wild-type strains using this clone. 129/SvJ and C57BL/6J mice carry a 9.7kb hybridizing TaqI restriction fragment, whereas C3H/HeJ and DBA/2J mice (and t haplotypes) carry a 4.4kb hybridizing TaqI restriction fragment (see Figure 5A). Additionally, 129/SvJ and C57BL/6J mice carry a 20kb BamHI fragment, whereas DBA/2J and BALB/cJ mice carry a 23kb BamHI fragment (see Figure 5B).

Tu80 is a 350 base pair restriction fragment. This probe recognizes RFLPs between t haplotypes and wild-type DNA in BamHI digests. When t haplotype DNA is present, this probe recognizes 11.2kb (T80A), 7.2kb (T80B), and 6.4kb (T80C) restriction fragments not found in wild-type DNA (see Figure 4C).

Tu108 is a 400 base genomic restriction fragment. This probe recognizes RFLPs in digests with a number of restriction enzymes. In PvuII digests, numerous polymorphic restriction fragments are identified, including a prominent 9.4kb (T108) restriction fragment which is associated only with the presence of t haplotype DNA (see Figure 5C). Digests performed with KpnI yield only a single hybridizing band in both wild-type and t haplotype DNA which is polymorphic between 129 and t haplotypes. Wild-type DNA exhibits a 10kb hybridizing restriction fragment, and t haplotypes an 11kb hybridizing restriction fragment (see

Silver and Olds-Clarke, 1984). BglII and TaqI digests also yield polymorphisms between t haplotype and wild-type DNA, but these were not characterized. The sequences identified by this probe were highly polymorphic between wild-type strains, with at least five different restriction patterns found in the wild-type strains in PvuII digests, including 7.8kb and 6.4kb restriction fragments present in 129/SvJ, and 11.5kb and 7.5kb restriction fragments in DBA/2J (see Figure 5C).

In many of the hybridization experiments performed, wild-type DNA from two different inbred strains, 129/SvJ and DBA/2J, was examined. The sizes of of the restriction fragments identified by these clones in these wild-type strains are listed in Table 4(A,B,C,D). A summary of the enzymes which yield RFLPs between these two strains with a given clone is also given in this table.

Two other probes were used to identify t complex sequences. The first probe identified in this study that mapped to the t complex was a genomic clone of an alpha globin pseudogene, referred to as alpha-pseudogene4. This gene, one of the two pseudogenes of the five characterized alpha globin related sequences in the mouse genome (Leder et al., 1980), had been previously mapped to chromosome 17 in studies utilizing somatic cell hybrids (Leder et al., 1981; Popp et al., 1981). Initial screening of the DNA from the

Table 4(A,B,C,D). Wild-type Restriction Fragment Sizes

The sizes of the restriction fragments identified by the microdissection-derived clones on the blots used for screening for RFLPs is indicated for 129/SvJ and DBA/2J with the restriction enzymes listed. The summary indicates enzymes which identify RFLPs between these two inbred strains.

TABLE 4A

Clone	BamHI 129	BamHI DBA	BglII 129	BglII DBA	HindIII 129	HindIII DBA	KpnI 129	KpnI DBA	PstI 129	PstI DBA
17	14.5	14.5	nd	nd	2.7	2.7	nd	nd	15.5	15.5
18	nd	nd	5.9	5.9	nd	nd	nd	nd	nd	nd
23	17	17	7.4	7.4	25	25	23	23	13	13
	10	10	6.5	6.5	19	19	15	15	5.9	5.9
	9.8	9.8	5.5	5.5	18	18	13	13	5.4	5.4
	7.9	7.8	5.2	5.2	13	13	11	11	4.3	4.3
	6.2	6.2	4.8	4.8	8.7	12	5.6		3.6	3.6
		4.7	4.4	4.4	4.4	7.6	2.7		3.4	3.4
			2.7	2.7		5.1			2.7	2.7
						2.7			1.3	1.3
48	6.1	6.1	4.1	4.1	nd	nd	nd	nd	8.6	8.6
			1.3	1.3						
54	nd	nd	16	16	18	18	17.5	17.5	20	20
			14	14	17	17	17	17	11.5	11.5
			12.5	12.5	13	13	16.5	16.5	10	10
			9.6	9.6	11.5	11.5	16	16	9.0	9.0
			9.4	9.4	11	11	13.5	13.5	8.6	8.6
			6.0	6.0	9.0	9.0	13.0	13.0	5.7	5.7
			3.5	3.5	8.7	8.7			5.1	5.1
					4.4	4.4			4.3	4.3
					3.9	3.9				
65	13.5	13.5	6.4	6.4	11	11	5.3	5.3	2.8	2.8
					6.5	6.5				
66	20	23	16	16	nd	nd	nd	nd	nd	nd
	12.5	12.5	12.5	12.5						
	12	12	9.3	9.3						
	6.3	6.3	8.4	8.4						
	5.8	5.8	6.1	6.1						
80	17.5	17.5	7.3	7.3	nd	nd	nd	nd	2.7	2.7
	10.5	10.5	6.2	6.2					1.8	1.8
	9.9	9.9	5.7	5.7						
	8.0	8.0	5.1	5.1						
	6.2	6.2								

TABLE 4B

Clone	PvuII 129	PvuII DBA	SacI 129	SacI DBA	TaqI 129	TaqI DBA	XbaI 129	XbaI DBA	Summary of RFLPs in wild-type
17	7.0	7.0	18	18	4.9	4.9	6.6	6.6	-
18	11 10.1	11 10.1	nd	nd	2.7	2.7	14	14	-
23	14 8.5 6.3 2.3	14 8.5 6.3 2.3	10.5 7.0 6.3 5.0 4.8 4.5	10.5 7.0 6.3 5.0 4.8 4.5	nd	nd	15.3 14.7 2.8 2.5 2.4 1.9	15.3 14.7 2.8 2.5 2.4 1.9	BamHI HindIII KpnI
48	5.4	5.4	2.2	2.2	1.2	1.2	7.2	7.2	-
54	nd	nd	21 16 15 12 11 10.5 8.2 7.5 6.9 5.5 3.9	21 16 15 12 11 10.5 8.2 7.5 6.9 5.5 3.9	15 11 9.0 8.5 7.5 4.3	15 11 9.0 8.5 7.5 4.3	14 13.5 11 10 9.3 7.6 6.9 6.7 6.0 5.5 3.4 1.5	14 11 10 9.3 8.4 7.6 6.9 6.7 6.0 5.5 3.4 1.5	XbaI
65	1.4 1.2	1.4 1.2	3.7 2.6	3.7 2.6	nd	0.7 0.4	1.8 1.4	1.8 1.4	-
66	nd	nd	nd	nd	9.7 2.9 1.9 1.7	4.4 2.9 1.9 1.7	nd	nd	BamHI TaqI
80	8.6 8.3 6.2 2.4	8.6 8.3 6.2 2.4	17 11 10 8.0 6.0	17 11 10 8.0 6.0	4.4 4.1 1.9	4.4 4.1 1.9	2.4 2.3 1.9	2.4 2.3 1.9	-

TABLE 4C

	BamHI		BglII		HindIII		KpnI		PstI	
Clone	129	DBA	129	DBA	129	DBA	129	DBA	129	DBA
94	14	14	4.4 4.0	4.4 4.0	11.5 5.1	11.5 5.1	20	20	13.5	13.5
101	8.1	8.1	7.3	7.3	20 13.5	20 13.5	21	21	8.0	8.0
106	9.8	9.8	11	11	8.3 4.5	8.3 4.5	25	25	12	12
108	nd	nd	16 13 8.2 7.6 6.8 5.3	16 13 12.5 8.2 6.8 5.3	nd	nd	10	10	nd	nd
111	24	24	2.5	2.5	18 4.7	18 4.7	30	30	6.0	6.0
115	12	12	nd	nd	1.3	1.3	25	25	2.2	2.2
116	12	12	4.0	4.0	2.6 2.3	2.6 2.3	25 3.3	25 3.3	3.9	3.9
122	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
145	14 2.5	14 2.5	6.9 1.1	6.9 1.1	8.8 7.8 4.1 3.8 1.0	8.8 7.8 4.1 3.8 1.0	30	30	14 3.6 1.8	14 3.6 1.8
174	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
177	15	15	nd	nd	16 13	16 13	nd	nd	6.7 2.2	6.7 2.2
180	3.4	3.4	3.9	3.9	2.3	2.3	18	18	6.2	6.2

TABLE 4D

Clone	PvuII 129	PvuII DBA	SacI 129	SacI DBA	TaqI 129	TaqI DBA	XbaI 129	XbaI DBA	Summary of RFLPs in wild-type
94	6.9 2.0	6.9 2.0	17.5	17.5	5.0	5.0	6.5	6.5	-
101	nd	nd	6.8	6.8	10.1	4.9	6.6	6.6	TaqI
106	nd	nd	9.7	9.7	nd	nd	13.5	13.5	-
108	15 12 7.8 6.4 5.5	15 12 11.5 7.5	nd	nd	12 6.5 4.0 2.5 2.2	12 6.5 4.0 2.5 2.2	nd	nd	PvuII BglII
111	9.6	9.6	6.0 2.3	6.0 2.3	3.4	3.4	nd	nd	-
115	nd	nd	11.5	11.5	6.1	7.8	1.8	1.8	TaqI
116	18 2.4	17 2.4	12.5	12.5	nd	nd	7.0	7.0	PvuII
122	nd	nd	nd	nd	5.1	5.1	nd	nd	-
145	12 6.6 4.0	12 6.6 4.0	4.3 2.7	4.3 2.7	nd	nd	9.0 1.5 1.3	9.0 1.5 1.3	-
174	nd	nd	nd	nd	nd	nd	2.9	2.9	-
177	1.9	1.9	14	14	4.4	4.4	6.4 2.4	6.4 2.4	-
180	nd	nd	nd	nd	nd	nd	3.5	3.5	-

congenic mice was performed with the enzymes PvuII, TaqI, and EcoRI. Figure 6 shows the hybridization pattern with the probe. Lanes 1, 4, and 7 contain 129 (+/+) DNA, lanes 2, 5, and 8 contain 129/t (+/t) DNA, and lanes 3, 6, and 9 contain t/t DNA. In the lanes in which the DNA was digested with PvuII (lanes 1-3) or EcoRI (lanes 7-9), no differences in the hybridization pattern were observed between wild-type animals and those that carry a t haplotype. But a distinct restriction fragment length polymorphism was observed in TaqI digests (lanes 4-6). As indicated in Table 5, all complete t haplotypes examined carry the same 5.2kb restriction fragment, which is not found in the genomes of any of the inbred strains studied (but see below). Furthermore, mice carrying two complete t haplotypes exhibit only this t-specific restriction fragment and no wild-type fragments (Figure 6, lane 6). Thus this TaqI restriction fragment represents the alpha-pseudogene4 homologous sequence that must be located within t haplotype DNA. A TaqI restriction fragment length polymorphism was also observed between different inbred strains. 129/SvJ, BALBc/J, C3H/HeJ, and LT/ChRe mice carry two alpha-pseudogene4 homologous TaqI restriction fragments, 3.8 and 1.4kb, whereas C57BL6/J, C58/J, DBA/1J, DBA/2J, LG/J, SWR/J, and NZB/B1NJ mice carry a single 3.4-kb fragment (see Table 5 and below).

Figure 6. Identification of Hba-ps4 RFLP

Hybridization of alpha-pseudogene4 probe to restriction fragments of genomic DNA, digested with the indicated restriction enzymes. Lanes 1, 4, and 7 - +/+; lanes 2, 5, and 8 - +/t; lanes 3, 6, and 9 - t/t.

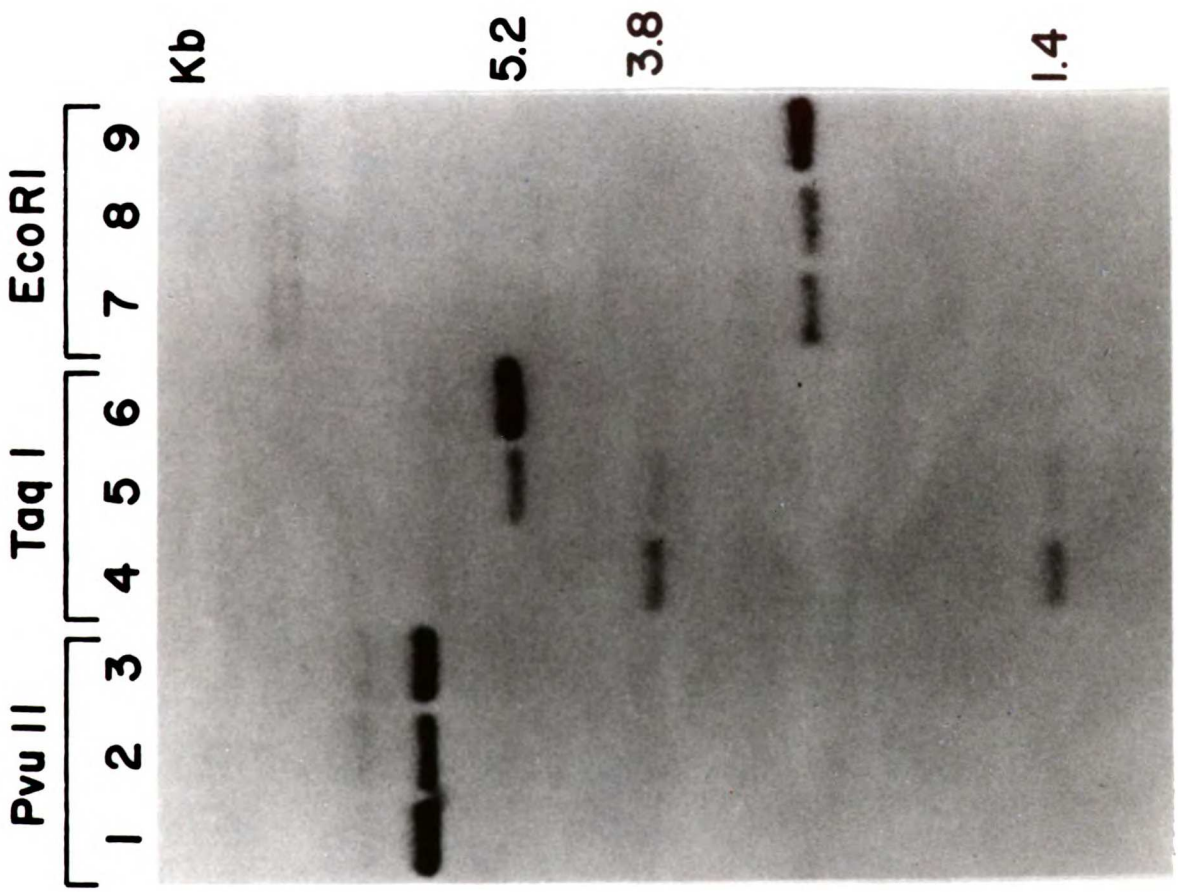

Pvu II
Taq I
EcoRI
1 2 3 4 5 6 7 8 9
Kb
5.2
3.8
1.4

Table 5. Hba-ps4 Alleles in Inbred Mice and t Haplotypes

The presence of the wild-type Hba-ps4^{b} or ^{c} alleles in inbred mice, and the Hba-ps4^{t} allele in complete t haplotypes is indicated on the left. The presence (+) or absence (-) of the Hbs-ps4^{t} allele in partial t haplotypes is indicated on the right. Partial t haplotypes not carrying Hba-ps4^{t} may contain Hba-ps4^{b} or ^{c} except in the case of t^{h20}, which is deleted for this locus (0).

TABLE 5

Genotype	Hba-ps4	Genotype	Hba-ps4
Wild-type:		Proximal	
129/SvJ	c	*t*ae5	-
BALB/cJ	c	*t*h45	-
C3H/HeJ	c	*Tt*Or1	-
C3HeJ/FeJ	c	*t*h51	-
C57BL/6J	b	*t*ks2	-
C58/J	b	*t*h44	-
DBA/1J	b	*t*h53	-
DBA/2J	b	*t*h49	-
LG/J	b	*t*h2	-
LT/ChReJ	c	*t*3	-
NZB/B1NJ	b	*t*Tu4	-
SWR/J	b	*t*4	-
		*t*ks1	-
t haplotypes:		*t*w18	-
Complete		Central	
*t*O	t	*t*lowH	-
*t*w2	t	*t*low2H	-
*t*w5	t	*t*low3H	-
*t*w12	t		
*t*w32	t	Distal	
*t*Lmb	t	*t*h18	t
*t*wLub1	t	*t*Tu2	t
*t*wLub7	t	*t*h17	t
*t*Tuw11	t	*t*s6	t
*t*Tuw12	t	*t*wLub2	t
*t*Tuw28	t	*t*6	t
		*t*h20	0

The genetic locus identified by the alpha-pseudogene4 probe has been named hemoglobin alpha, pseudogene, alpha-4, or Hba-ps4. The allele defined by the C3H pattern (3.8 and 1.4kb restriction fragments) will be called Hba-ps4^{c}, the allele defined by the C57BL/6J pattern (a 3.4kb restriction fragment) will be called Hba-ps4^{b}, and the allele associated with *t* haplotypes (a 5.2kb restriction fragment) will be called Hba-ps4^{t}. Using these polymorphisms in the inbred strains described here, the mapping of this sequence to the *t* complex region in wild-type strains of mice has been confirmed using recombinant inbred strains and H-2 congenic mice (D'Eustachio et al., 1984). In this study it was also found that the SM/J strain of inbred mice was unique among inbred strains in that it carried the Hba-ps4^{t} allele, previously thought to be specific to *t* haplotypes.

A cDNA for an alpha-crystallin gene, pMalphaA2Cr, had been previously cloned from mouse lens RNA (King et al., 1982). Using polymorphisms identified in inbred mouse strains, this sequence was mapped to the *t* complex region using recombinant inbred and H-2 congenic mice (Skow, 1984). There are three alleles of this gene in wild-type, two found in the majority of strains (identified as 11kb or 10kb restriction fragments in digestions performed with HincII), and one found in only a single inbred strain (identified as a 9kb HincII restriction fragment) (L. Skow, personal

communication). Examination of t haplotypes revealed that t haplotypes exhibited two of these alleles, one of the common alleles (the 11kb HincII fragment), and the rare allele (the 9kb restriction fragment) (see Figure 7). In contrast to the allele distribution found in inbred mice, almost half of the t haplotypes carried the rare allele of this gene (Table 6). A BamHI RFLP was also found between t haplotypes and between wild-type strains, and the alleles identified followed the same distribution as the HincII alleles.

B. Mapping DNA markers to subregions of t haplotypes

With the characterization of the markers described above, it was then to possible use partial t haplotypes to map the restriction fragments identified by these clones. In turn, the partial t haplotypes could also be characterized with these clones (see Table 7, Figure 8).

T48 is the most proximal of the t haplotype markers described here. This conclusion was based on a number of observations. T48 is absent from t^6 and all proximal haplotypes derived from t^6, but is present in all other proximal haplotypes. It is absent from all of the central and distal haplotypes. Since t^6 is known to differ from complete t haplotypes at the proximal end (Silver et al., 1983b), and the T48 restriction fragment is present in the proximal haplotypes derived from complete t haplotypes but

Figure 7. alpha-Crystallin Alleles in *t* Haplotypes

Hybridization of the alphaACr2 probe to HincII digested DNA. A 2 kb hybridizing restriction fragment which is present together with the 9kb allele is not shown. Lane 1 - *t*^{Tuw7}/*t*^{Tuw7}; lane 2 - *t*^{Tuw8}/*t*^{Tuw8}; lane 3 - *t*^{Tuw10}/*t*^{Tuw10}; lane 4 - *t*^{Tuw15}/*t*^{Tuw15}; lane 5 - +/*t*^{Tuw21}; lane 6 - +/*t*^{Tuw29}; lane 7 - +/*t*^{wLub7}; lane 8 - +/*t*^{w32}; lane 9 - *t*^{w2}/*t*^{w2}; lane 10 - *t*^{Tu4}/*t*^{Tu4}; lane 11 - +/*t*^{0}.

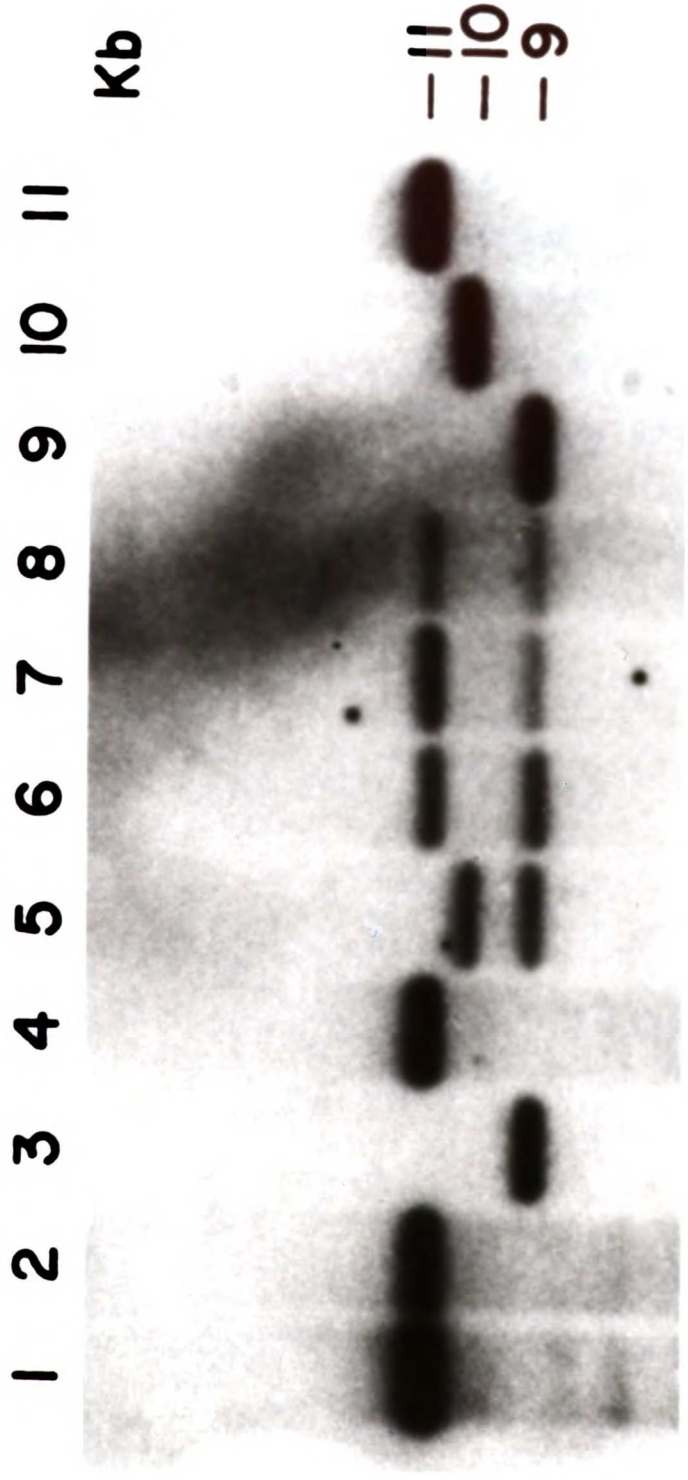

Kb
11
10
9
1 2 3 4 5 6 7 8 9 10 11

Table 6. alpha-Crystallin Alleles in *t* Haplotypes

The presence of the 11kb or the 9kb HincII restriction fragment identified by the alphaACr2 cDNA clone in complete *t* haplotypes is indicated. The t^{Tuwn} mice were not examined on an inbred background, however an examination of the aACr2 alleles present in the genetic background of these mice allowed a determination of the allele they carry.

TABLE 6

Haplotype	AlphaCr	Haplotype	AlphaCr
t0	11	tTuw8	11
tLmb	11	tTuw10	9
tw2	9	tTuw11	11
tw5	11	tTuw12	11
tw12	11	tTuw15	11
tw32	9	tTuw18	11
twLub1	11	tTuw20	11
twLub4	9	tTuw21	9
twLub6	9	tTuw23	11
twLub7	9	tTuw24	9
twLub9	11	tTuw25	9
tTuw2	9	tTuw26	11
tTuw7	11	tTuw29	11

Table 7. Presence of *t*-Specific Restriction Fragments in Partial *t* Haplotypes

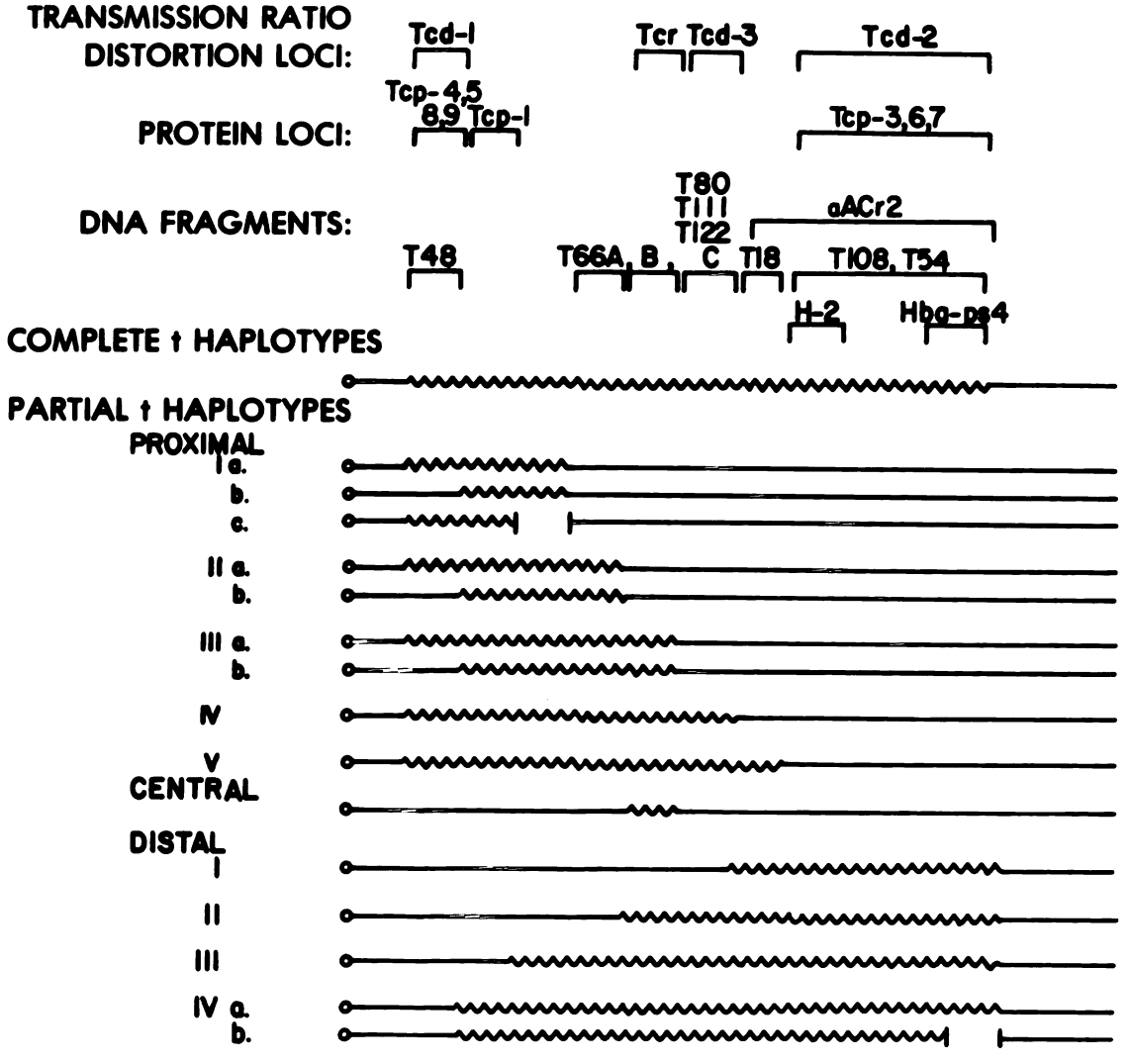
The presence (+) or absence (-) of the *t*-specific restriction fragments described in the text in partial *t* haplotypes is indicated. nd - not determined. The classes and subclasses of the partial *t* haplotypes indicated in Figure 8 are given preceeding the names of the haplotypes.

TABLE 7

Genotype	T48	T66A	T66B	T66C	T122	T111	T80 ABC	T18	T54	T108
Partial t haplotypes:										
Proximal										
I										
a *tae5*	+	-	-	-	-	-	nd	nd	nd	-
b *th45*	-	-	-	-	-	-	nd	nd	nd	-
c *Tt0r1*	+	-	-	-	-	-	-	nd	nd	-
II										
a *th51*	+	+	-	-	-	-	-	nd	nd	-
tks2	nd	+	nd	-	-	nd	nd	nd	nd	-
b *th44*	-	+	-	-	-	-	nd	nd	nd	-
th53	-	+	-	-	-	-	-	-	nd	-
III										
a *th49*	+	+	+	-	-	nd	-	nd	nd	-
b *th2*	-	+	+	-	-	-	nd	nd	nd	-
IV										
t3	+	+	+	+	+	+	+	-	-	-
tTu4	nd	+	nd	nd	+	nd	+	nd	nd	nd
V										
t4	+	+	+	+	+	+	+	+	-	-
tks1	+	+	+	+	+	nd	nd	+	nd	-
tw18	+	+	+	+	+	+	+	+	-	-
Central										
tlowH	-	-	+	-	-	-	-	-	nd	-
tlow2H	-	-	+	-	-	-	-	nd	nd	-
tlow3H	-	-	+	-	-	-	-	-	nd	-
Distal										
I										
th18	-	-	-	-	-	-	-	+	nd	+
tTu2	-	-	-	-	-	-	-	+	+	+
II										
th17	-	-	+	+	+	+	+	+	+	+
ts6	-	-	+	+	+	nd	nd	nd	nd	+
III										
tWLub2	-	+	+	+	+	+	+	nd	nd	+
IV										
a *t6*	-	+	+	+	+	+	+	+	+	+
b *th20*	-	+	+	+	+	nd	+	nd	+	+

Figure 8. Genetic Map of *t* Haplotypes

The relative extent of *t* haplotype DNA in the various *t* haplotypes was determined by the presence in each haplotype of *t*-specific markers. The zigzag line indicates *t* haplotype DNA, a gap marked by vertical lines indicates a deletion. The partial *t* haplotypes are grouped into the general classes (proximal, central, and distal) and subdivided on the basis of their relative lengths. The partial *t* haplotypes belonging to these subclasses are given in Table 7. The positions of the distortion loci (*Tcd-1,2,3* and *Tcr*) are described by Lyon, 1984; the positions of the protein loci (*Tcp-1* and *Tcp-3-9*) by Silver et al., 1983b; and the position of the class I-like *H-2* genes by Silver, 1982. Several of the classifications were made on the basis of data not presented here. t^{h45} does not carry any of the DNA markers studied, but is placed in the first proximal subclass because it carries $Tcp\text{-}1^{a}$; t^{6} and t^{h20} (distal subclass IV) and t^{wLub2} (distal subclass III) carry the same proximal DNA markers but are placed in different subclasses because t^{6} and t^{h20} carry $Tcp\text{-}1^{a}$ whereas t^{wLub2} does not (Silver et al., 1983b and L. Silver, personal communication).


TRANSMISSION RATIO DISTORTION LOCI:
Tcd-1
Tcr Tcd-3
Tcd-2
PROTEIN LOCI:
Tcp-4,5
8,9 Tcp-1
Tcp-3,6,7
DNA FRAGMENTS:
T80
T111
T122
αACr2
T48
T66A, B, C
T18
T108, T54
H-2
Hbα-ps4
COMPLETE t HAPLOTYPES
PARTIAL t HAPLOTYPES
PROXIMAL
I a.
b.
c.
II a.
b.
III a.
b.
IV
V
CENTRAL
DISTAL
I
II
III
IV a.
b.

not those derived from $\underline{t^6}$, T48 maps to this region of difference between $\underline{t^6}$ and complete $\underline{t}$ haplotypes. Although $\underline{t^6}$ fits the original definition of a complete $\underline{t}$ haplotype, the data clearly indicate that $\underline{t^6}$ is wild-type in the most proximal region associated with complete $\underline{t}$ haplotypes and thus must be considered a long distal $\underline{t}$ haplotype.

T66A appears to map distal to T48 in $\underline{t}$ haplotypes. This marker is present in a subset of all proximal $\underline{t}$ haplotypes, including some derived from $\underline{t^6}$. It is present in $\underline{t^6}$ and two other distal haplotypes, but not in any of the central haplotypes.

T66B appears to map distal to T66A. This marker is present in a subset of the proximal haplotypes that carry T66A. It is present in some distal haplotypes that do not carry T66A, as well as all that do, and is present in all central haplotypes.

T66C, T80ABC, T111, and T122 are present in identical sets of the partial $\underline{t}$ haplotypes, and map distal to T66B. All six markers are present in only a few proximal haplotypes, which represent a subset of those that carry T66B. They are present in the distal haplotypes in which T66B is present, but they are not present in the central haplotypes.

T18 appears to map distal to T66C and the other markers described above. This marker is present in the smallest

subset of proximal haplotypes, a subset of those that carry the T66C group, which are also similar in that they all belong to the $\underline{t^9}$ lethal complementation group. T18 is present in the distal haplotypes that do not carry the T66C group of markers, as well as all that do, but is not present in the central haplotypes.

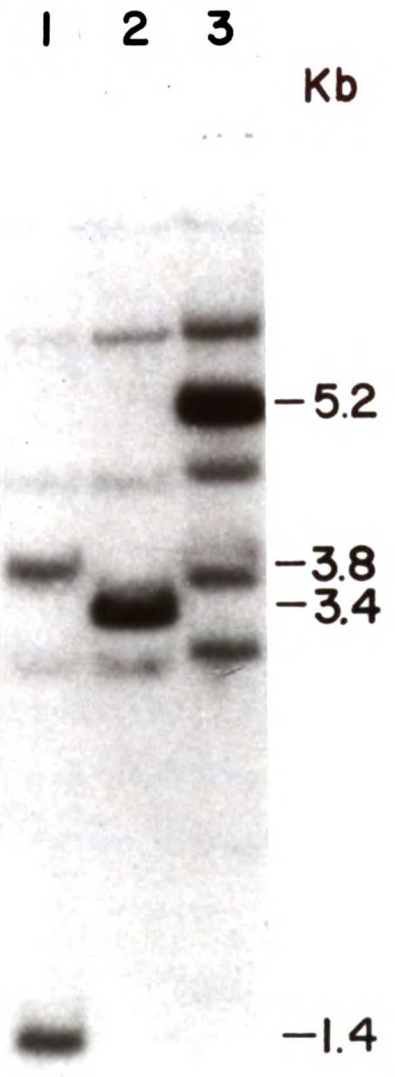
T108 and T54 are the most distal markers described. They appear to be present in all of the distal haplotypes, but in none of the central or proximal haplotypes.

The Hba-ps4 locus maps distally with T108 and T54. It differs from these two markers, however, in that the $\underline{t^{h20}}$ distal t haplotype does not carry $\underline{\text{Hba-ps4}^t}$ (Table 5). Further investigation revealed that $\underline{t^{h20}}$ was deleted for the Hba-ps4 sequence: heterozygous mice containing $\underline{t^{h20}}/\underline{\text{Hba-ps4}^c,^b}$, or t carry only the Hba-ps4 allele present on the non-$\underline{t^{h20}}$ chromosome (Figure 9). Although the t haplotype allele identified with this clone was later found to be present in a wild-type strain, the results here are nevertheless consistent with it mapping in the distal region.

The sequence recognized by the alpha-crystallin probe was mapped in a slightly different manner. Because there are two different alleles in t haplotypes, and since neither of the alleles is t-specific, the analysis described above could not be used. However, it was found that whereas $\underline{t^{w2}}$

Figure 9. Deletion of *Hba-ps4* from t^{h20}

Hybridization pattern of the alpha-pseudogene4 probe to TaqI digested genomic DNA from mice of the following genotypes. Lane 1 - (*Hba-ps4*^{c})+/t^{h20}; lane 2 - (*Hba-ps4*^{b})+/t^{h20}; lane 3 - (*Hba-ps4*^{t})t^{s6}/t^{h20}. The *Hba-ps4* allele of chromosome heterozygous with t^{h20} is indicated in parentheses.


1
2
3
Kb
5.2
3.8
3.4
1.4

complete t haplotype carries the 9kb HincII allele (Figure 7, lane 9), the t^{Tu4} proximal partial t haplotype, derived from t^{w2}, does not carry the 9kb HincII allele (Figure 7, lane 10). Therefore the alpha-crystallin sequence maps in the region distal to the t haplotype DNA in t^{Tu4}, in the region where T18, T108, T54, and Hba-ps4 map.

C. Analysis of t complex region genomic clones from the t^{w5} haplotype

In the wild-type form of chromosome 17, the H-2 complex has been well characterized. Since the H-2 complex is an integral part of the t complex, a study of the DNA from this region may yield information about the structure and organization of the t complex. A genomic library was supplied by laboratory of H. Lehrach, EMBL, constructed with DNA from the embryonic stem cell line homozygous for the t^{w5} haplotype (Magnuson et al., 1982) in the lambda vector EMBL3 (Frischauf et al., 1984). This library was screened for H-2 class I sequences with the pH2IIa cDNA probe, which recognizes approximately 30 genes in the mouse genome (Steinmetz et al., 1981, Steinmetz et al., 1982a; Winoto et al., 1983, Weiss et al., 1984). Twenty four independent clones (B1, D5, D6, F1,G1, G2, G3, H2, K1, K2, L6, P3, R2, R3, S4, S6, T1, T3, T4, T5, V3, V4, V5, W7, Y2, and Y3), as determined by comparisons of restriction digests, were

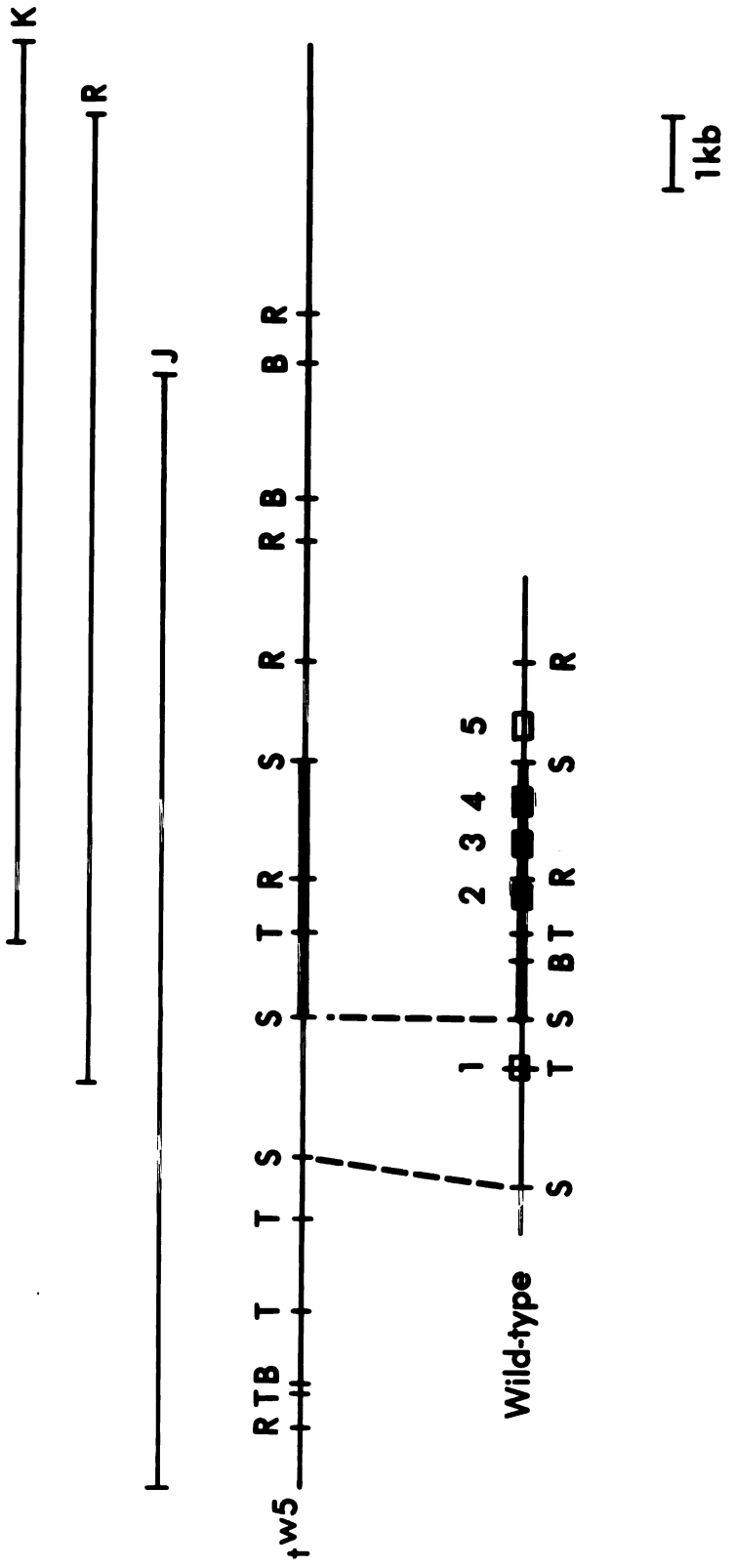
obtained, with an average insert size of 15kb. Since class I genes comprise 3.3kb (including introns) of DNA, each clone carries on the average of 12kb of flanking DNA.

It has been suggested (Lyon et al., 1979a) that the recombination suppression between t haplotype and wild-type DNA may be due to differences in middle repetitive DNA. Additionally, the suggestion that t haplotypes may be derived from a different species than Mus also leads to the hypothesis that a difference in the structure or organization of middle repetitive DNA may exist (Silver, 1982). To examine this possibility, Southern blot of the DNA from the t^{w5}-derived class I H-2 clones, digested with the enzymes SalI, BamHI, and EcoRI, were probed with both wild-type genomic DNA, and genomic DNA containing a t haplotype. If a difference in middle repetitive DNA exists between t haplotypes and wild-type, restriction fragemnts might be noted which hybridize to a probe made from t haplotype containing genomic DNA but not to wild-type genomic DNA. No reproducible differences could be noted in the hybridization to the restriction fragment of these clones (data not shown).

The t^{w5} genomic library was also screened with a genomic clone for the H-2 class II E_{alpha} gene (Steinmetz et al., 1982b). Three overlapping genomic clones were obtained, which span 20kb of DNA. Figure 10 indicates that

Figure 10. Restriction Map of the t^{w5} E_{alpha} Gene Region

Three recombinant lambda phage clones, J, K, and R, were isolated from a t^{w5}/t^{w5} genomic library using the E_{alpha} probe. These clones were restriction mapped by single and double digestion with BamHI (B), EcoRI (R), SalI (S), and SstI (T), followed by blot-hybridization with the probe. The comparison to the E_{alpha} gene restriction map of wild-type expressing strains (derived from results of Steinmetz et al., 1982b and Mathis et al., 1983a) is shown. The positions of the five exons in the wild-type gene are indicated by boxes. The region of the wild-type gene that the probe was derived from is shown by a bold line. A bold line also indicated the region that the probe hybridizes to in the t^{w5} gene. The exon 1 region in wild-type, including the SstI site, appears deleted from t^{w5} (indicated by broken lines), in a manner similar to that observed in wild-type strains which do not express this gene (Mathis et al., 1983b, Hyldig-Nelson et al., 1983).


K
R
J
1kb
RTB T T S S T R S R B R B R
t^w5
Wild-type
S T S B T R S R
1 2 3 4 5

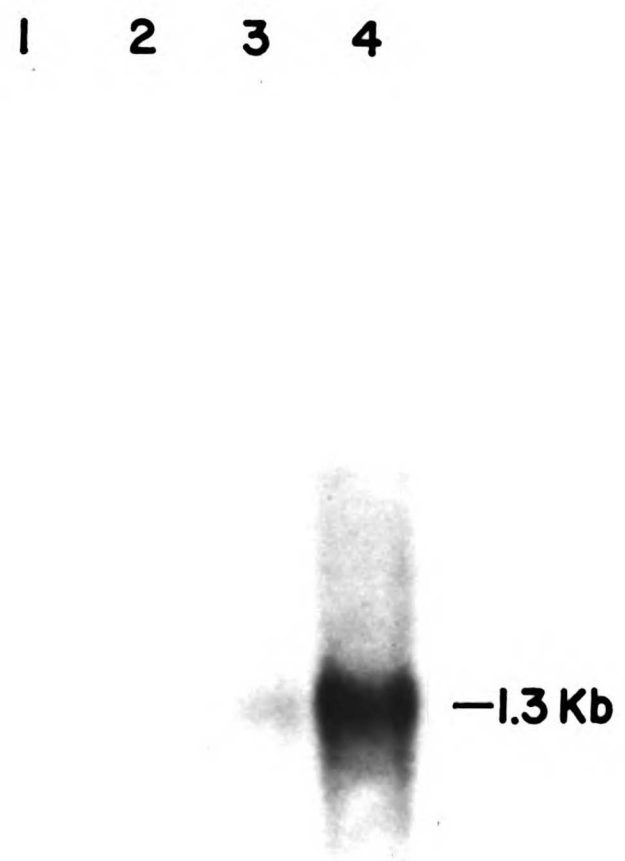
a restriction map of the clones reveals close similarities with the wild-type restriction maps of the gene (Steinmetz, et al., 1982b; Mathis et al., 1983a; Mathis et al., 1983b; Hyldig-Nielsen et al., 1983). It has been found that a few inbred strains have deleted the DNA region encompassing the 1st exon of the E_{alpha} gene and thus cannot produce the E_{alpha} messenger RNA (Mathis et al., 1983b, Hyldig-Nielsen et al., 1983). The t^{w5} E_{alpha} gene also appears deleted in this region. Southern blot analysis indicated that half of the t haplotypes share this deletion, whereas the others appear normal. Nothern blot analysis of spleen RNA from two t haplotypes, one containing this deletion (t^{w5}) and the other not (t^{w12}), supports the contention that the deletion in the t haplotypes causes a loss of transcription of this gene as it does in wild-type (Figure 11). Studies in another laboratory confirm and extend these results (Dembic et al., 1984). 50% of a large sample of t haplotypes contain this deletion, whereas only 4% of wild mice do not express this gene (Klein and Figueroa, 1981).

D. Analysis of recombination between a t haplotype containing a deletion and a distal t haplotype

A genetic experiment in the laboratory generated mice of the genotype Tt^{s6}/t^{h20}. These mice thus had a t haplotype genotype of a distal t haplotype (t^{s6}) in

Figure 11. Analysis of E_{alpha} Transcripts

Hybridization of the E_{alpha} probe to spleen RNA. Longer exposures showed no detectable transcripts in lanes 1 and 2. The mice from which the RNA in lanes 2 and 3 was isolated were congenic on the 129/SvJ genetic background, which does not express the E_{alpha} gene (lane 1), whereas C3H/HeJ mice express this gene (lane 4). The transcript detected was the size expected for the E_{alpha} transcript (Mathis et al., 1983). 10 micrograms of RNA was loaded in each lane, except in lane 4 which was accidentally overloaded. Rehybridization to a ribosomal RNA probe indicated approximately equal amounts of RNA were present in lanes 1-3, and excess amounts in lane 4.
Lane 1 - 129; lane 2 - (129)+/t^{w5}; lane 3 - (129)+/t^{w12}; lane 4 - C3H.

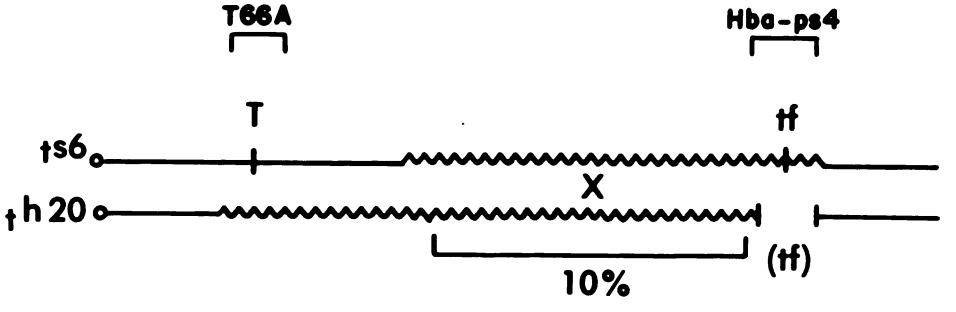

1
2
3
4
—1.3 Kb

combination with a longer distal t haplotype containing a deletion of Hba-ps4 (t^{h20}). To examine the gene order in these two haplotypes, females of this genotype were mated to +tf/+tf male mice and the offspring examined by DNA analysis (see Figure 12). In this experiment, it was believed that the recombination frequency between T66A (marking the proximal region of the chromosome), an H-2 complex region probe, and Hba-ps4 (which maps near tufted) could be measured in order to substantiate by molecular means the inversion in the distal region of t haplotypes, because preliminary evidence suggested that these haplotypes contained distinguishable H-2 complexes (L. Silver, personal communication). However, a more thorough examination of the genomic DNA from t^{s6} and t^{h20} digested with numerous restriction enzymes using the general class I H-2 probe pH2IIa (Steinmetz et al., 1981) and the class II probe for the E_{alpha} gene (Steinmetz et al., 1982b) failed to yield any polymorphisms between these two haplotypes. In fact, since both haplotypes were derived from the t^6 haplotype (t^{h20} as a mutation, t^{s6} as a t/t recombinant with t^{w12}), the H-2 regions may actually be identical. A recent study (Shin et al., 1984) has demonstrated that the Tla region of the H-2 complex in t^{s6} is indeed derived from t^6 and not t^{w12}.

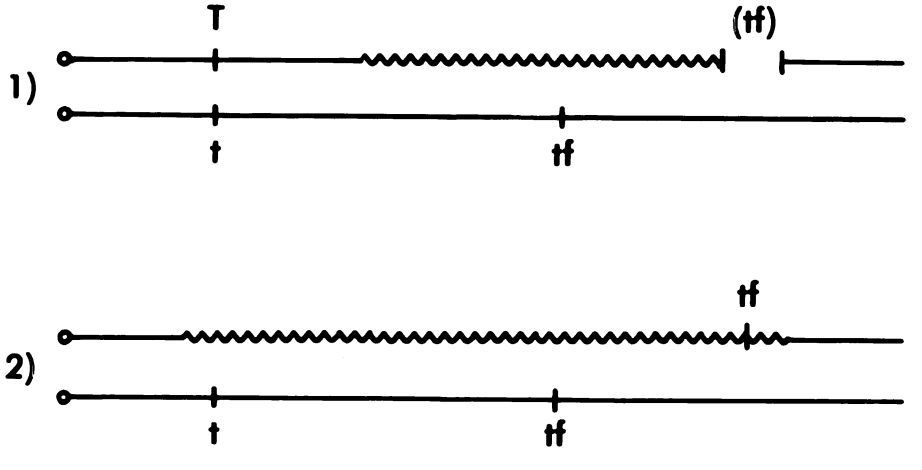
Figure 12. Recombination Between $\underline{t^{s6}}$ and $\underline{t^{h20}}$

Female $\underline{Tt^{s6}}/\underline{t^{h20}}$ mice (A - parental genotype) were mated to $\underline{+tf}/\underline{+tf}$ male mice, and the offspring were examined for recombination between T66A and Hba-ps4 (Table 8). 6 recombinant offspring were identified out of 59 examined, yielded a recombination frequency of 10%. The two types of recombinant genotypes are indicated in (B).

A. Parental genotype:



B. Recombinant genotypes:



The offspring of these mice could therefore only be typed for the presence of the T66A restriction fragment, which could have been contributed only by the t^{h20} chromosome, and for the Hba-ps4^{t} allele, which could only have been contributed by the t^{s6} chromosome. Three parental females were used in the experiment. 59 offspring were examined (see Table 8). 53 offspring were of the parental type: 27 carried the same markers as t^{h20}, and 26 the same markers as t^{s6}. 6 recombinants were collected, 3 of which had the proximal region of t^{s6} (lacking the T66A marker) and the distal region of t^{h20} (deleted for Hba-ps4) (Figure 12, recombinant genotype 1), and 3 having the reciprocal combination of markers (Figure 12, recombinant genotype 2). This yields a recombination frequency of 10%. One of the recombinants has been maintained. This chromosome, t^{sf1}, is of the second class of recombinant genotypes (offspring number 3 from female 0195).

E. Analysis of a unique viable *t* haplotype

In a study of wild mice aimed at identifying new *t* haplotypes, two examples of unique viable haplotypes were found in Haifa, Israel (Klein et al., 1984). The *t* haplotype identified in one of these mice was maintained in the laboratory and named t^{Tuw32}. In contrast to all other wild-derived *t* haplotypes examined, this *t* haplotype

Table 8. Recombination between $\underline{t^{s6}}$ and $\underline{t^{h20}}$

Female mice of the genotype $\underline{Tt^{s6}}/\underline{t^{h20}}$ were mated to +tf/+tf male mice, and t/t recombination in the offspring was detemined by examining the offspring for T66A (present in $\underline{t^{h20}}$) and $\underline{Hba\text{-}ps4^{t}}$ (present in $\underline{t^{s6}}$) (see Figure 12). The table list the female parent of each mouse, and the presence of the $\underline{t^{20}}$ markers (denoted a), the $\underline{t^{s6}}$ markers (denoted b), or a set of markers generated by recombination, indicated by an asterisk (*).

TABLE 8

Female	Mouse	66A	Hba-ps4
0164	01	a	a
	02	b	b
	07	a	a
	08	b	b
	09	a	a
	10	a	a
	11*	b	a
	12	a	a
	14	b	b
0166	01*	a	b
	02	a	a
	03	b	b
	04	b	b
	05	a	a
	06	a	a
	07	b	b
	08	b	b
	09	b	b
	10	a	a
	11	a	a
	12	b	b
	13	a	a
	14	a	a
	15	a	a
	16	b	b
	17	b	b
	18	b	b
	19	a	a
	20	a	a
	21	b	b

Female	Mouse	66A	Hba-ps4
0166	22	b	b
	23*	b	a
	24	a	a
	25	a	a
	27*	a	b
	28	b	b
	29	b	b
	30	b	b
	31	b	b
	32	a	a
0195	01	a	a
	02	b	b
	03*	b	a
	04	b	b
	05	a	a
	06*	b	a
	07	b	b
	08	b	b
	09	b	b
	10	b	b
	11	a	a
	12	a	a
	13	a	a
	14	b	b
	15	b	b
	16	a	a
	17	a	a
	18	a	a
	19	a	a

contained only a subset of the t-specific restriction fragments (see Figure 13A). In addition, it contained the allele of the Hba-ps4 found in all t haplotypes but only a single wild-type strain, the 9kb HincII allele of alpha-crystallin which is found in 50% of the t haplotypes but only a single inbred strain, an E_{alpha} gene deletion which is found in 50% of t haplotypes, and an $H\text{-}2^{k}$ locus antigenic specificity unique to t haplotypes which has been found in 50% of t haplotypes (Nizetic et al., 1984).

To determine if these t haplotype-like alleles are linked together by recombination suppression as they are in complete t haplotypes, female mice of the genotype t^{Tuw32}/Ttf were mated to +tf/+tf males, and offspring were examined for recombination between T, tf, and the DNA markers T48, Hba-ps4, alpha-crystallin, and E_{alpha} (see Figure 13B). The data in Table 9 show that apparently free recombination occurs between T48/T, Hba-ps4/tf, and alpha-crystallin/E_{alpha}.

This mouse was originally identified on the basis of the presence of tct (wild males were mated to +/T females, and the production of tailless offspring indicated the presence of a t haplotype). To discover whether this haplotype carried transmission ratio distortion loci, male mice of the genotype t^{Tuw32}/Tt^{h17} (transmission ratio distortion factor genotype ?,?,?,?/-,Tcr,Tcd-3,Tcd-2) and

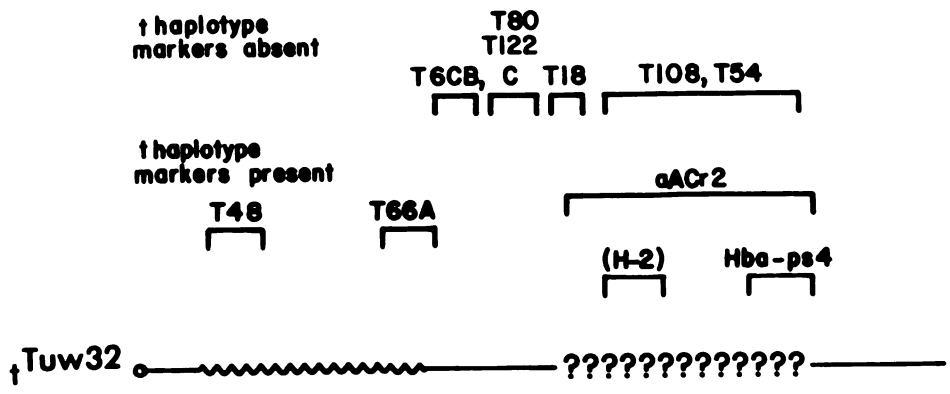
Figure 13. Characterization of t^{Tuw32}

(A). Homozygous t^{Tuw32} mice were examined for the presence of the markers used in this study. The markers absent from this chromosome are indicated above, and the markers present below. The presence or absence of the T111 marker was not determined. aACr2 indicates the 9kb HincII allele, Hba-ps4 indicates the $Hba\text{-}ps4^{t}$ allele, and (H-2) indicates the deleted form of the E_{alpha} gene. The zigzag line indicates the probable presence of t DNA in the proximal region, the straight line the absence of t DNA in the central region, and the question marks (?) indicate the unknown compostition of the distal region.

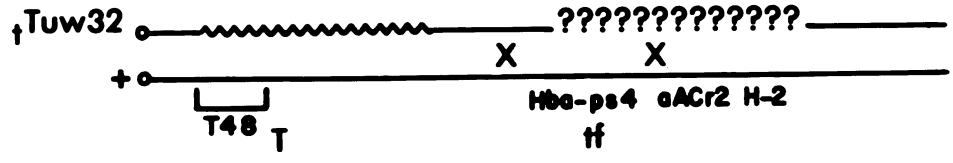
(B). Recombination between the T48, T, Hba-ps4, tf, alpha-crystallin (aAcr2), and E_{alpha} (indicated by H-2) was determined in offspring from t^{Tuw32}/Ttf females (Table 9). Recombination was found between T48,T; Hba-ps4,tf; and aACr2,H-2; yielding the same gene order found in wild-type.

(C). The transmission ratio of each chromosome from t^{Tuw32}/Tt^{h17} and t^{Tuw32}/Tt^{h18} male mice was determined by examining the tail length of offspring generated by mating to wild-type females (transmission of t^{Tuw32} yields normal tails, transmission of Tt^{h17} or Tt^{h18} yields short tails). t^{Tuw32}/Tt^{h17} male mice gave 4 normal tailed and 25 short tailed offspring, whereas t^{Tuw32}/Tt^{h17} male mice gave 31 normal tailed and 30 short tailed offspring.

A.


t haplotype markers absent
T80
T122
T6CB, C
T18
T108, T54
t haplotype markers present
T48
T66A
αACr2
(H-2)
Hba-ps4
tTuw32
??????????????

B.


tTuw32
??????????????
X
X
+
T48
T
Hba-ps4 αACr2 H-2
tf

C.

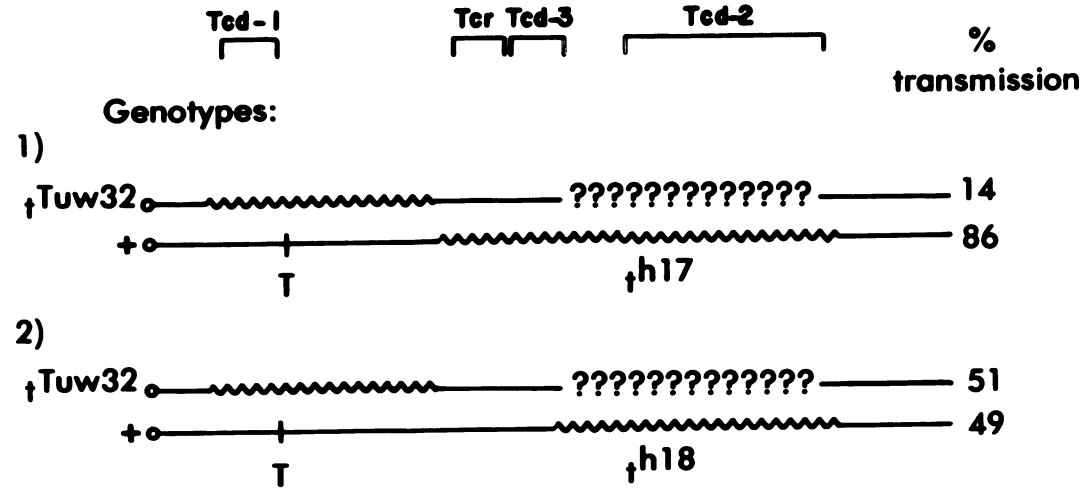

Tcd-1
Tcr Tcd-3
Tcd-2
%
transmission
Genotypes:
1)
tTuw32
??????????????
14
+
86
T
th17
2)
tTuw32
??????????????
51
+
49
T
th18

Table 9. Recombination between t^{Tuw32} and Wild-type

Two female mice of the genotype t^{Tuw32}/Ttf were mated to +tf/+tf male mice, and recombination between T48, T (tail), Hba-ps4, tf, alpha-crystallin, and E_{alpha} was determined by examination of the offspring (see Figure 13B). The alleles carried by t^{Tuw32} were denoted t, the alleles carried by the wild-type Ttf chromosome were denoted -. Offspring are listed by litter, and mice carrying a recombinant set of markers are indicated by an askerisk (*).

TABLE 9

Litter #	Mouse #	48	Tail	Hba-ps4	tufted	alphaCr	Ealpha
2137	1*	nd	+	+	+	-	-
	2	+	+	+	+	+	+
	3	-	-	-	-	-	-
	4	-	-	-	-	-	-
2012	5	+	+	+	+	+	+
	6*	+	+	-	+	+	+
	8	+	+	+	+	+	+
	9	+	+	+	+	+	+
	10	+	+	+	+	+	+
	11	-	-	-	-	-	-
2191	13	-	-	-	-	-	-
	14	-	-	-	-	-	-
	15	-	-	-	-	-	-
	16	-	-	-	-	-	-
	17	+	+	+	+	+	+
	18	+	+	+	+	+	+
	19	+	+	+	+	+	+
	20	+	+	+	+	+	+
2308	21	+	+	+	+	+	+
	22	-	-	-	-	-	-
	23*	-	-	-	-	+	+
2533	24	-	-	-	-	-	-
	25	-	-	-	-	-	-
	26	+	+	+	+	+	+
	27	+	+	+	+	+	+
2780	28	-	-	-	-	-	-
	29	-	-	-	-	-	-
	30	-	-	-	-	-	-
	31	+	+	+	+	+	+
	32	+	+	+	+	+	+
	33*	+	+	-	-	-	-
3346	34	-	-	-	-	-	-
	35	-	-	-	-	-	-
	36	-	-	-	-	-	-
	37	-	-	-	-	-	-
	38	+	+	nd	+	+	+
	39	+	+	+	+	+	+
	40	+	+	+	+	+	+
3656	41	+	+	+	+	+	+
	42	+	+	+	+	+	+
	43	+	+	+	+	+	+
	44	+	+	+	+	+	+
	45*	+	+	-	-	-	-
	46	-	-	-	-	-	-

t^{Tuw32}/Tt^{h18} (?,?,?,?/-,-,-,Tcd-2) were mated to wild-type females (see Figure 13C). The male transmission ratio was measured as the proportion of short tailed offspring (from the Tt^{h17} or Tt^{h18} chromosome) to normal tailed offspring (from the t^{Tuw32} chromosome). Figure 13C shows that the presence of t^{Tuw32} allowed the t^{h17} chromosome to distort but not the t^{h18} chromosome. t^{Tuw32} thus carries the Tcd-1 locus, allowing it to raise the ratio of a chromosome (t^{h17}) which carries Tcr and Tcd-2. t^{Tuw32} does not carry the Tcr locus, since t^{Tuw32} allows a chromosome (t^{h17}) that carries Tcr to distort, and did not allow a chromosome (t^{h18}) that lacked both Tcd-1 and Tcr to distort. This experiment did not allow the determination of the presence of either the Tcd-2 or Tcd-3 loci in t^{Tuw32}.

DISCUSSION

A. Molecular examination of the t complex

When this project was begun, the only probes available that recognized sequences mapping to the t complex were those for the genes of the H-2 complex. As a result of this work, eight new probes (Tu18, Tu48, Tu54, Tu66, Tu80, Tu108, Tu111, and Tu122) and one previously described probe (alpha-pseudogene4), which recognize independent sequences in t haplotypes have been made available. Furthermore, most of these probes recognize sequences outside of the H-2 complex region, providing the first probes for the proximal and central regions of the t complex. In addition, while this work was being completed, four other probes that recognize t complex sequences have been found. Two of these, Tu119 and Tu89, were derived from microdissected chromosome fragments and were analyzed in a manner similar to that described here (B. Herrmann et al., personal communication). In t haplotypes, T119 maps between T48 and T66A, and T89 maps with T18. The two other probes are cDNA clones, pMalphaACr2 and pMK174, which recognize sequences that were mapped to the t complex by studies of the segregation of polymorphismic restriction fragments in recombinant inbred mouse strains (Skow, 1984; Mann et al., 1984). The analysis of pMalphaACr2 in t haplotypes was

performed as part of this study, and the analysis of pMK174 was performed by others in a manner similar to that described here (L. Silver and R. Elliott, personal communication). In t haplotypes, pMK174 maps between T48 and T66A, with T119.

The work with the microdissection-derived DNA probes has allowed a determination of the organization of DNA in this region. However, there may be some bias in this analysis, since the the derivation of these clones was not completely random, as they consisted of repeat-free genomic EcoRI restriction fragments. The cloning procedure was also selective for clones with small inserts, the average insert size was approximately 660 base pairs. Using these clones as probes on genomic blots, fifteen recognized one or two restriction fragments, whereas five hybridized to four to ten restriction fragments. Thus when the highly repetitive clones are screened out, 3/4 of the clones generated in this manner recognize one to two copy sequences, and 1/4 recognize low copy number sequences. Through the analysis with partial t haplotypes, the sequences recognized by these clones mapped to different regions of the t complex.

All of the sequences hybridizing to four of the multicopy clones have been shown to map to chromosome 17 (the localization of all of the sequences hybridizing to the

remaining clone, Tu54, has not been determined). One of these clones, Tu66, is unique in that it was shown to hybridize to sequences separable by the recombination events that created the partial t haplotypes. It may be a general phenomenon in the genome that related sequences are present in low copy number on a given chromosome. It is thought that a major force in genome evolution is the duplication of sequences and their eventual divergence. Such duplications may be generated by unequal crossing over, thus leading to identical but separable sequences. Further unequal crossing over between similar sequences can then create gene families. The cross-over intermediate during this unequal recombination can be resolved with recombination of flanking markers to yield further duplications or deletions, or without recombination of flanking markers to yield gene conversion events. Known examples studied at the molecular level include the globin genes and the class I H-2 genes (Bagloni, 1962; Goossens et al., 1980; Higgs et al., 1980; Leder et al., 1980; Pease et al., 1983; Mellor et al, 1983; Hess et al., 1984; Weiss et al., 1984). The significance of the multicopy sequences recognized by the clones in this study is not known. These sequences may be markers for uncharacterized gene families, they could be remnants of nonfunctional duplications of sequences during evolution, or

they may be involved in a regulatory role in genome function, such as chromosomal pairing.

This work has also allowed a determination of the average restriction fragment length in a given area of the mouse genome. Table 10 shows this value for the enzymes used in this study (except EcoRI, since the probes were all EcoRI genomic fragments) calculated from the restriction fragment sizes present in the wild-type 129/SvJ and DBA/2J hybridization experiments. Also shown are the average lengths calculated in a study of human X chromosome probes, and the expected fragment size based on relative single base and nearest-neighbor base frequency (Aldridge et al., 1984). Although these expected fragment size calculations were based on data from the human genome, it is valid to use them for comparison to mouse data since the single base and nearest-neighbor frequencies are similar for man and mouse (Fasman, 1976). It is clear that all of the average fragment lengths observed in this study are approximately equal to the expected values (within 20%) except for those obtained with the enzymes HindIII and TaqI.

The HindIII fragment lengths observed in this study were about twice those expected. In the study of the human X chromosome it was found that the observed sizes were similar to the expected values. There are two possible reasons for the variation found in this study. The first is

Table 10. Average Restriction Fragment Size in Wild-type

The sizes of the restriction fragments recognized by each microdissection-derived probe was calculated for each enzyme. These average lengths of restriction fragments were themselves than averaged for each enzyme to yield an average restriction fragment length for each enzyme. These are shown in comparison to the average restriction fragment lengths observed in a study of the human X chromosome, and an expected fragment size calculated from relative single base and nearest-neighbor base frequency (Aldridge et al., 1984). These expected lenghts are twice those calculated from the base frequency data since the restriction site distribution examined on a blot is the sum of the average fragment size from both ends of the probe, thus doubling the length, minus the probe length. This approximation is valid when the probes usesd are much shorter than the average fragment length, as is the case here (Bishop et al., 1983).

TABLE 10

Mean	Bam	Bgl	Hnd	Kpn	Pst	Pvu	Sac	Taq	Xba
Expected	11.2	5.3	3.7	17.8	6.3	6.3	10.0	2.5	6.4
Observed									
Mouse 17	11.5	6.2	8.0	19.0	7.1	6.9	9.3	4.7	6.0
Human X	12.8	6.4	4.0	12.6	7.1	6.8	9.7	4.7	8.0

that in this region of the genome, the HindIII recognition sequence occurs about two-fold less frequently than in the genome as a whole. A second possibility concerns the distribution of HindIII sites relative to EcoRI sites. Since these probes are short EcoRI genomic restriction fragments, the EcoRI recognition sequence occurs twice in a small region. Perhaps in regions in which two closely spaced EcoRI are not present, HindIII sites are distributed normally. An examination of the t complex region with probes other than those derived in this manner in addition to an examination of other regions of the genome will help distinguish between these possibilities.

The observed fragment lengths for TaqI (recognition sequence TCGA) was 4.7kb both in the present study and in the study of the human X chromosome. These values are almost twice as great as the expected value of 2.5kb. A similar phenomenon was found for the enzyme MspI (recognition sequence CCGG) in the human X chromosome study, and it was proposed that either differences in CpG distributions existed between the X chromosome and the autosomes, or base modifications prevented cleavage of some of the sites (Aldridge et al., 1984). Since the same result has been obtained for TaqI sites on mouse chromosome 17, the former explanation is unlikely to be correct, and there is no mechanism to account for the latter (TaqI is resistent to

cytosine methylation, the only known base modification in mammalian genomic DNA). However, an alternate explanation for this phenomenon is possible. The distribution of the CpG doublet may vary in the genome not from chromosome to chromosome but between repetitive sequences and low copy number sequences. A higher frequency of CpGs in repetitive sequences relative to low copy sequences would result in CpGs being further apart in the low copy sequences than predicted by these genomic averages. In fact, by nearest neighbor frequency calculations there are 0.9-1.1% CpGs in the mouse or human genome (Fasman, 1976), but mouse satellite DNA contains 3.4% CpG dimers, over three times the amount in the genomic average (Horz and Altenberger, 1981). Additionally, there seems to be a clustering of CpG dimers in the genome, possibly near the 5' end of genes (Tykocinski and Max, 1984; Bird et al., 1985). Thus the observed TaqI restriction fragment lengths may be typical of those identified with low copy number probes; the presence of a higher proportion of CpGs in satellite and possibly other repetitive DNA as well as the clustering of CpGs leads to the low expected value when the whole genome is averaged.

In addition to the studies described in this work, more microdissection-derived clones are being isolated and analyzed. Mapping of these sequences in wild-type is also proceeding, which will allow the comparison of the map order

in t haplotypes and wild-type. Thus at the present time the t complex region of chromosome 17 is the largest region of the mouse genome to undergo a detailed molecular characterization.

B. Strategies for detecting restriction fragment length polymorphisms

All of the restriction enzymes used in this study except EcoRI were found to identify at least one RFLP between wild-type strains or between t haplotypes and wild-type. The negative result with EcoRI is presumably not meaningful, since the probes were mostly small EcoRI genomic restriction fragments, and the EcoRI restriction fragments to which they are homologous often could not be detected on the Southern blots. The utility of these restriction enzymes in identifying polymorphisms can be analyzed in comparison to the data available in searches for RFLPs in the human genome. It has been suggested (Skolnick and White, 1982) and reported (Barker et al., 1984) that a greater number of polmorphisms can be identified in digests by restriction enzymes that contain the sequence CpG. This may be the result of a single base mutation, since when the cytosine residue is methylated, a deamination will result in a transition mutation to thymidine: CpG to TpG (Razin and Riggs, 1980). An obvious corrollary of this is that

restriction enzymes that contain the sequence TpG should also show an increase in identifying RFLPs. One of the restriction enzymes used here, TaqI, contains the CpG doublet. Two of the restriction enzymes used contained the TpG doublet, PstI and PvuII. It has been empirically found that restriction enzymes with six-base recognition sequences consisting of an alternating 3 purine/3 pyrimidine string have been useful in identifying RFLPs (Skolnick et al., 1984). Five of these enzymes examined, BamHI, BglII, EcoRI, HindIII, SacI, contained this pattern of bases. Finally, two of the enzymes can be categorized as miscellaneous choices, KpnI and XbaI.

Based on the number of RFLPs identified here, the relative ease of obtaining a complete digestion of DNA, and the ability to identify a polymorphic pattern on a blot due to the distribution of fragment sizes and their subsequent ease of separation on the agarose gels (0.8%) used in the screening, BglII, PvuII, and TaqI were the most useful restriction enzymes in this study. BamHI was also found to be particularly useful, but it was more difficult to obtain a complete digestion and lower percentage gels were required to achieve optimal separation of restriction fragments. PstI was valuable in that it identified a polymorphism with one probe (Tu111) that was not found with any other enzyme. Although MspI is potentially useful because it contains the

CpG doublet, it was found to yield patterns difficult to interpret since it often gave incomplete digests, possibly because of the sensitivity of MspI to methylation (Sneider, 1980; Busslinger et al., 1983; Keshet and Cedar, 1983).

C. Comparisons of *t* haplotypes and wild-type chromosomes

The data in this study allow a comparison of the sequences homologous to these probes in *t* haplotypes and wild-type DNA. No quantitative differences in DNA sequences were found in this study. All probes examined identified similar number of sequences in both complete *t* haplotypes and wild-type, and no deletions or amplifications were noted. Additionally, the search for *t*-specific repetitive elements in the t^{w5} *H-2* genomic clones was negative. This interpertation of this result is limited by the sensitivity of this experiment; such *t*-specific repetitive elements might exist but their detection may have been obscured by the presence of nearby repetitive elements common to *t* haplotypes and wild-type. Furthermore, there may be repetitive elements found in *t* haplotypes not found in the wild-type form of the *t* complex, but which also exist elsewhere in the genome. This phenomenon has been documented in *Drosophila*, in which families of middle repetitive elements occupy different chromosomal positions in different strains (Young, 1979). In this case the total

genomic probes used would not have allowed the identification of these sequences.

It was found that except for the polymorphism identified with the alpha-crystallin probe, all complete *t* haplotypes examined were identical. This infrequency of polymorphisms observed between *t* haplotypes, parallels the results obtained with the *H-2* class I cDNA probes (Shin et al., 1982; Silver, 1982) and with testis protein patterns (Silver et al., 1983b). All complete *t* haplotypes are very closely related at the present level of analysis.

Seven of the probes used in this study recognized RFLPs between the 129/SvJ and DBA/2J inbred strains, whereas eight of the probes recognized RFLPs between *t* haplotypes and wild-type. A higher percentage of the probes that recognize multiple sequences identify a polymorphism than did the probes that recognize one to two copy sequences. This is most likely due fact to the that probes that recognize multiple sequences have a larger number of chances for identifying a polymorphic restriction fragment.

The number of probes that recognize differences between *t* haplotypes and wild-type was not greatly different from the number of differences identified between two inbred strains. It may be significant, however, that only two of the probes known to identify sequences mapping to the *t* complex identified RFLPs between these wild-type strains.

The other probes that identify polymorphisms between wild-type strains may actually not map to the t complex, and a finding of no RFLP between t haplotypes and wild-type would not be meaningful. Still, at the present time there is no convincing evidence that wild-derived t haplotypes are more different in their DNA sequence organization when compared to inbred strains of mice than the closely related inbred strains are to each other.

D. Characterization of partial t haplotypes

This set of clones has been used to identify markers specific to t haplotype DNA. These markers were then used to classify a large set of independent partial t haplotypes. The partial t haplotypes made possible the mapping of these markers, and thus has allowed the separation of these markers into five different regions of t haplotype DNA.

These markers have also allowed a determination of the length of partial t haplotypes. Although it had been suggested that different partial t haplotypes may differ in their lengths of t haplotype DNA, there had been no verification of that theory. The present study has shown that there are at least five different distal extents of t DNA in the proximal t haplotypes, and four different proximal extents of t DNA in the distal t haplotypes. In

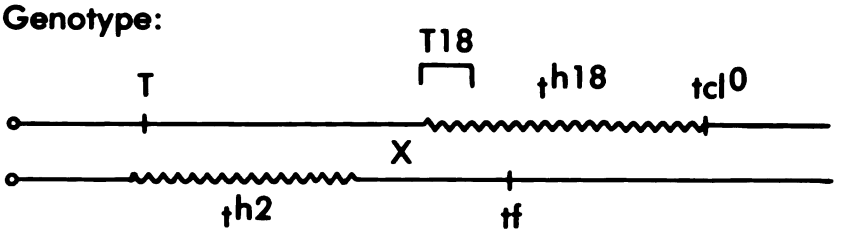
this analysis the central haplotypes appear homogeneous with respect to their proximal and distal extents of t DNA.

Confirmation of the differences in lengths between the partial t haplotypes has been obtained in genetic studies. The data in this study predict that the proximal haplotype t^{w18} contains a longer distal extent of t chromatin than does the proximal haplotype t^{h2} since t^{w18} contains the T66C, T122, T80ABC, T111, and T18 markers whereas t^{h2} does not. Furthermore, since the distal haplotype t^{h18} contains T18, the t DNA in t^{w18} should overlap that in t^{h18}, whereas the t DNA in t^{h2} should not overlap that in t^{h18}. Indeed, when recombinant offspring were produced from a mouse carrying t^{h2} and t^{h18}, the class of offspring produced through recombination between the two t haplotypes appears to contain both t haplotypes in the *cis* position (Figure 14A,B). This recombinant chromosome allows recombination between the two t haplotypes when heterozygous with a wild-type chromosome, indicating that there must be wild-type DNA between t^{h2} and t^{h18} (Lyon and Meredith, 1964b). In contrast, recombination between t^{w18} and t^{h18} produced a class of offspring that contains what appears to be a complete t haplotype carrying the t^{h18} (tcl^{0}) lethal locus (Figure 14C,D). This recombinant chromosome suppresses recombination with wild-type over its whole length, indicating that there must be overlapping t DNA in

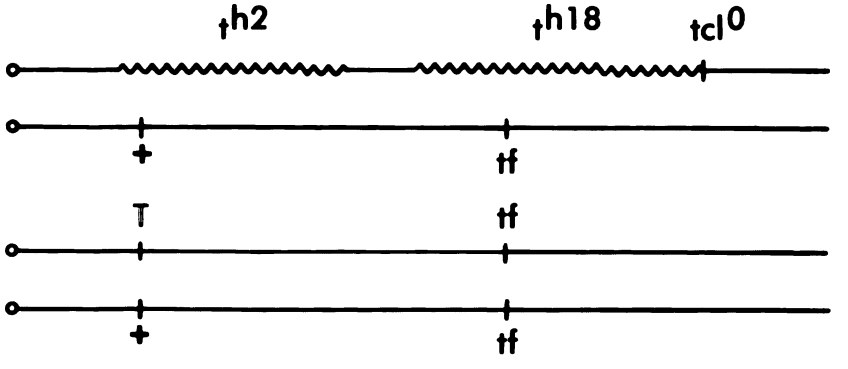
Figure 14. Differences in Lengths of Partial *t* Haplotypes

When offspring of *Tt*^{h18}/*t*^{h2}*tf* (A - parental genotype) mice were examined for recombination between *T* and *tf*, study of the two classes of recombinant offspring (B - recombinant genotypes) indicated that overlapping *t* DNA is not present in these haplotypes (Lyon and Meredith, 1964b). In contrast, when offspring of *Tt*^{h18}/*t*^{w18}*tf* (C - parental genotype) were examined for recombination between *T* and *tf*, study of the two classes of recombinant offspring (D - recombinant genotypes) indicated that overlapping *t* DNA is present in these haplotypes (Fox et al., 1985). T18 indicates the T118 marker that is absent from *t*^{h2} but present in *t*^{h18} and *t*^{w18}. *tcl*^{0} indicates the lethal locus present in *t*^{h18}, and *tcl*^{9} the lethal locus present in *t*^{w18}.

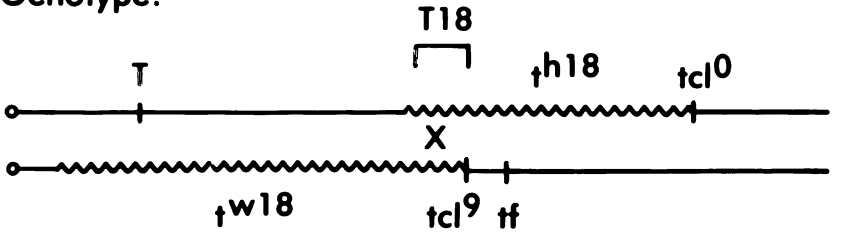
A. Parental Genotype:



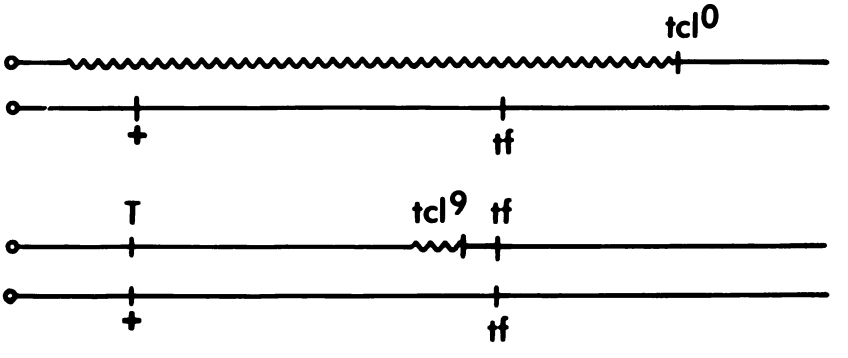
B. Recombinant Genotypes:



C. Parental Genotype:



D. Recombinant genotypes:



these haplotypes. The recripocal class of recombinants contains a small piece of t haplotype DNA that includes the t^{w18} (tcl^{9}) lethal locus, supporting the conclusion that overlapping t haplotype DNA exists in the t^{w18} and t^{h18} haplotypes, since if they were not overlapping, recombinants would contain either both parental lethal loci or neither locus (Fox et al., 1985).

Two of the breakpoints identified in the partial t haplotypes appear to have phenotypes associated with them. The t^{9} lethality is present in all of the longest class of proximal t haplotypes, extending to the T18 marker (proximal class V in Figure 8): t^{w18}, t^{4}, and t^{ks1}. These all presumably arose by recombination between wild-type DNA and a complete t haplotype. The T89 marker also maps in this same region. It has been found that the t^{w18} chromosome actually carries both the t haplotype and wild-type forms of T89 (V. Bautch, personal communication). Upon examination, it was found that t^{w18} also carries both the t and wild-type forms of T18. t^{4} and t^{ks1} appear by intensity estimates to be similarly duplicated for these markers. Additionally, the availability of a t^{w18}/t^{w18} homozygous cell line has allowed the identification of deletions of sequences homologous to the Tu94 and Tu180 clones (G. Martin, personal communication). Thus unequal crossing over leading to both duplication and deletion of DNA is associated with this

lethality, as suggested earlier (Lyon and Meredith, 1964b; Silver, 1983)

A second breakpoint also seems to give rise to distinct phenotypes. Recombination between wild-type and the complete *t* haplotype *t*^{wLub1} gave rise to the *t*^{wLub2} chromosome. This new partial *t* haplotype is associated with a maternal effect (Winking and Silver, 1984) and a recessive lethality (N. Sarvetnick, personal communication) not present in its parental chromosomes. As with *t*^{w18}, there are markers duplicated (T, T119, pMK174) and deleted (*Tcp-1*) from this chromosome (N. Sarvetnick et al., personal communication). In this case two phenotypes, a recessive lethality and a dominant maternal effect, appear associated with this unequal crossing over.

E. Genetic basis of transmission ratio distortion

In earlier studies, partial *t* haplotypes were used in attempts to determine the number, locations, and types of interactions of *t* haplotype genes responsible for the *t*-specific property of transmission ratio distortion. Several laboratories have described the results of breeding experiments set up to determine the transmission ratios in mice carrying different combinations of proximal, central, and distal *t* haplotypes. Additionally, the effect of certain proximal and central haplotypes in combination with

complete t haplotypes has also been examined. However, without a knowledge of the differences between the various partial t haplotypes, the data from the different studies appeared contradicatory.

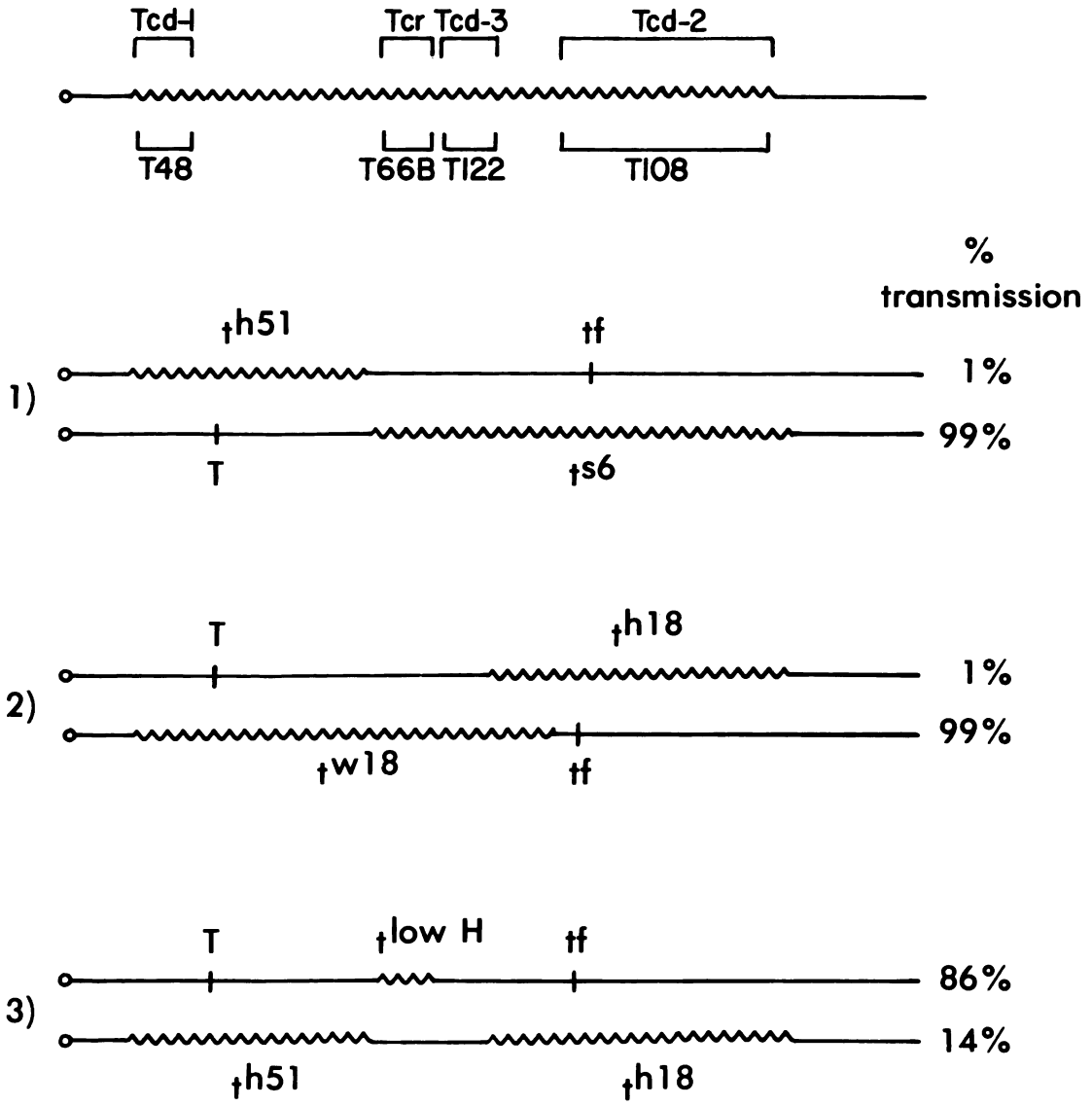
The data from the molecular studies described here allow a correlation of the physical regions of t haplotype DNA identified by these markers with the phenotypically defined loci described by Lyon (Lyon and Mason, 1977; Lyon, 1984). It is now clear that the available data support this model (see below and Figure 15). The Tcd-1 locus maps proximally with the T48 restriction fragment. Tcr is distal to Tcd-1 and maps with T66B. Tcd-3 and the markers T66C, T80ABC, T111, and T122 together map distal to Tcr. Finally, the Tcd-2 locus maps most distally with T54 and T108.

The results obtained for three of the genotypes tested by Lyon (1984) show the preeminince of Tcr in determining which chromosome will be transmitted at a high level when combinations of partial t haplotypes are present in a male mouse. As shown in Figure 15, a male mouse containing t^{h51}/t^{s6} (transmission ratio distortion loci genotype Tcd-1,-,-,-/-,Tcr,Tcd-3,Tcd-2) transmits the distal haplotype t^{s6}, the Tcr containing chromosome, to 99% of his offspring. A male mouse containing t^{w18}/t^{h18} (Tcd-1,Tcr,Tcd-3,-/-,-,-,Tcd-2) transmits the proximal haplotype t^{w18}, the Tcr containing chromosome, to 99% of his

Figure 15. Transmission Ratio Distortion in t Haplotypes

A. The correlation between the transmission ratio distortion loci described by Lyon (1984) and regions of t haplotype DNA defined by the markers characterized in this study is indicated.

B. Genotypes and transmission ratios of male mice described by Lyon (1984) to support the hypothesis that the Tcr locus determines which chromosome is transmitted at a high ratio. The lengths of the partial t haplotypes used are indicated as determined in the present study.


Tcd-1
Tcr Tcd-3
Tcd-2
T48
T66B T122
T108
%
transmission
1)
th51
tf
1%
T
ts6
99%
2)
T
th18
1%
tw18
tf
99%
3)
T
tlow H
tf
86%
th51
th18
14%

offspring. Finally, a mouse containing $t^{lowH}/t^{h51}t^{h18}$ (-,Tcr,-,-/Tcd-1,-,-,Tcd-2) transmits the central haplotype t^{lowH}, the Tcr containing chromosome, to 86% of his offspring.

F. Crossover suppression in t haplotypes

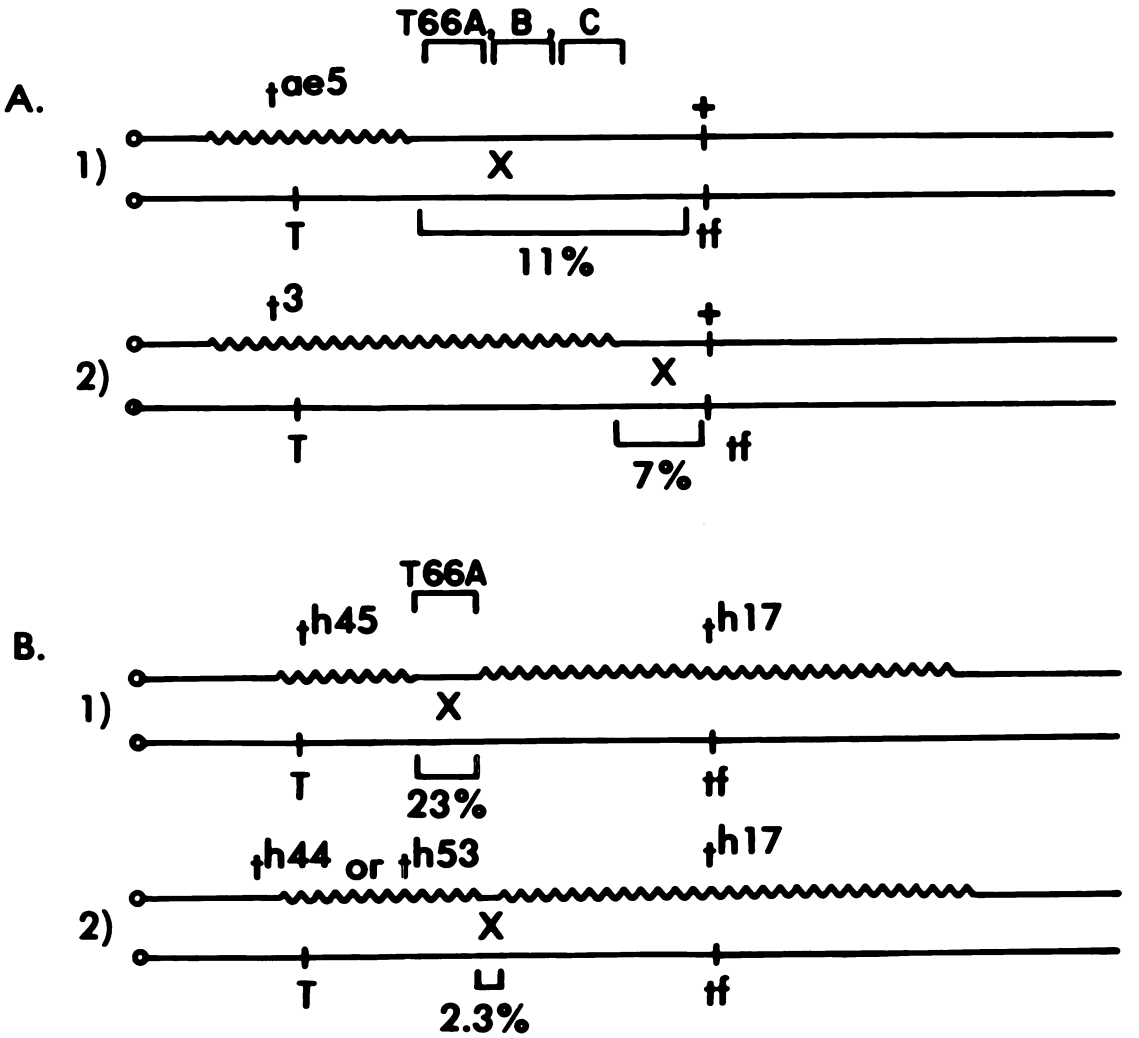
Previous studies have suggested that partial t haplotypes suppressed recombination over their own length (Bechtol and Lyon, 1978; Bennett et al., 1979; Lyon et al., 1979a). With the knowledge gained from this study concerning the different lengths of the partial t haplotypes, it is now possible to determine if the recombination suppressing property of these t haplotypes is dependent on the amount of t haplotype DNA they contain.

A significant difference in the recombinatory properties of two proximal t haplotypes, t^{ae5} and t^{3}, has been reported (Gluecksohn-Waelsch and Erickson, 1970). When in trans with a chromosome carrying the T and tf markers (Ttf/t^{x}), t^{ae5} allowed 11% recombination between T and tf whereas t^{3} allowed 7%, suggesting that they differ in their lengths of t haplotype DNA (see Figure 16A). This was confirmed here in the DNA analysis, since t^{3} was shown to carry the DNA markers T66A, T66B, and the markers mapping with T66C whereas t^{ae5} does not. Thus t^{3}, which contains a longer length of t haplotype DNA than does t^{ae5}, allows

Figure 16. Recombination Suppression by Partial *t* Haplotypes

A. Mice carrying t^{ae5}/*Ttf* allow more recombination between *T* and *tf* than do mice carrying t^{3}/*Ttf* (Gluecksohn-Waelsch and Erickson, 1970). The lengths of these partial *t* haplotype are indicated as determined in the present study, showing that t^{ae5} has less *t* DNA (it does not contain T66A,B,C) than t^{3} does.

B. t^{h45}, which does not contain the T66A marker, allows more recombination between *T* and *tf* when in *cis* to t^{h17} than do t^{h44} or t^{h53}, which do contain T66A (Fox et al., 1985).


T66A, B, C
A.
tae5
+
1)
X
T
tf
11%
t3
+
2)
X
T
tf
7%
T66A
B.
th45
th17
1)
X
T
tf
23%
th44 or th53
th17
2)
X
T
tf
2.3%

significantly less recombination between T and tf than does t^{ae5}.

The proximal haplotypes t^{h44} and t^{h53} were derived in the same cross that generated t^{h45}. They differ from t^{h45} in the DNA analysis, however, in that both carry the T66A marker whereas t^{h45} does not. To test their recombinatory properties, these proximal haplotypes were each placed in cis with the distal haplotype t^{h17}, and the recombination frequency between T and tf was determined in $t^{hn}t^{h17}$/Ttf heterozygotes (see Figure 16B, Fox et al., 1985). Among the offspring of $t^{h45}t^{h17}$/Ttf heterozygotes, 23% were recombinant between T and tf, whereas with $t^{h44}t^{h17}$/Ttf the recombination value was 2.5%, and with $t^{h53}t^{h17}$/Ttf it was 2.2%. Thus both t^{h44} and t^{h53} permit significantly less recombination between T and tf than does t^{h45}. The unusually high rate of recombination observed in these crosses is probably accounted for by the combined effect of the translocation T(1;17)190Ca associated with t^{h17} (Bechtol and Lyon, 1978) as well as a compensatory enhancement of recombination that is observed in regions adjacent to areas of recombination suppression in the t complex (Bennett et al., 1979; Lyon et al., 1979a) and other systems (Roberts, 1976).

It is clear from these studies that partial t haplotypes suppress recombination in the segment of

chromosome they occupy in proportion to the amount of t haplotype DNA they contain. The results of Silver and Artzt (1981) suggest that crossover suppression in the distal part of t haplotypes is due to a nonhomology between wild-type and t haplotype DNA, since free recombination occurs in regions homozygous for t DNA. Artzt et al. (1982) and Shin et al. (1984) showed that this mismatching is at least in part due to a chromosomal inversion in the distal region of the t complex. However, it is unlikely that this inversion is the sole cause of crossover suppression in t haplotypes, since the proximal partial t haplotypes, which do not contain this inversion, still suppress recombination.

Using the clones described here and other t complex markers, evidence has been obtained for an second inversion in t haplotypes, within this proximal region of the t complex (N. Sarvetnick et al., personal communication). The proximal haplotype Tt^{Orl} and the distal haplotype t^{wLub2} each duplicate and delete markers, and appear to be complementary to each other, i.e. markers deleted from one are duplicated in the other. The only way to generate chromosomes with both duplications and deletions is through crossing over within an inversion between t haplotypes and wild-type, and in this case apparent "reciprocal" crossover products have been identified. The limits of this inversion is defined by the markers neither duplicated not deleted,

T48 and T122. Confirmation of this inversion is being obtained through the mapping of these DNA markers in wild-type chromosomes (B. Herrmann and H. Lehrach, personal communication).

The presence of this second inversion in t haplotypes helps to explain the dilemma of the inability to obtain cytogenetic evidence for an inversion. For inversion loops to form during pairing and crossovers to occur leading to anaphase bridges, a large segment of DNA must be inverted as a unit. The presence a second inversion can disrupt these phenomena. Other inversions or other genetic rearrangements may also be present in t haplotypes, as there is no evidence concerning the genetic structure in the extreme proximal region, in the area between the proximal and distal inversions, or whether the distal inversion actually extends to the distal limit of the t complex.

To date, no partial t haplotypes have been reported in which an exchange with wild-type DNA has occured between the loci of T and qk, or between qk and H-2. These are the regions encompassed by the two inversions in t haplotypes relative to wild-type. Inversions can in principal suppress recombination in two ways: either by reduced chiasma formation or by the occurence of normal chiasmata with inviability of the crossover products. However, the data presented in this study show that there are several points

in between qk and tf where chiasmata can occur between t haplotypes and wild-type and give rise to viable products.

G. Other t haplotype properties

The loci responsible for the other characteristic t haplotype properties, tail interaction, male sterility, and embryonic lethalities, can also be examined in light of the present data. The tail interaction locus, tct, had previously been shown to map in the proximal region of t haplotypes (Lyon and Meredith, 1964a,b). In this study, this locus could be localized between T48 and T66A. The proximal limit is T48 since the t^6 haplotype and its derivitives carry tct but not the most proximal marker T48, and the distal limit is T66A since the proximal haplotypes (proximal class I in Figure 8) that do not contain T66A do carry tct.

The property of male sterility is less well understood at the genetic level than the other t haplotype properties. The available data suggest that there are two loci, one proximal and one distal, which interact to cause sterility (Silver, 1981). Since the proximal locus (tcs-1) is not found in t^6 and its derivatives, it maps to the region defined by T48, along with Tcd-1. The distal locus (tcs-2) maps in the region defined by T54 and T108, along with Tcd-2. In fact, it has been suggested that the loci causing

sterility may be the same as some of the distortion loci (Lyon, 1984).

The t haplotype lethal loci appear to be different discrete genes, which have been mapped throughout the t complex, mostly in the distal region (Artzt, 1984). The markers obtained in this study are also distributed throughout the t complex. It was originally hoped that RFLPs could be identified between t haplotypes with these probes to allow their localization relative to the lethal loci, with a goal of eventual isolation of these genes through molecular cloning of the DNA between specific markers. However the difference between t haplotypes found with the alpha-crystallin probe is the only RFLP between t haplotypes outside of H-2.

At the present time, the lethal loci made most accessible to study by the t haplotype markers are the two created by recombination between t haplotypes and wild-type, that have duplications and deletions associated with them (t^9 and t^{wLub2}). The t^{w5} lethal locus has been reported to map near $H\text{-}2^k$ (Shin et al., 1984). Since the H-2 region has been extensively analyzed in wild-type, this gene may also accessible to characterization.

Additionally, there is evidence that the alpha-pseudogene4 probe recognizes sequences linked to the Fu locus and the tf gene. The demonstation that t^{h20} is

deleted for Hba-ps4 supports an earlier contention that t^{h20} carries a deletion. This hypothesis was based on the observation that t^{h20}, which arose from t^{6}, picked up a tufted mutation, failed to complement Fu^{Kb}, but was still associated with the tail interaction factor and the parental lethal locus and H-2 haplotype (Lyon and Bechtol, 1977; Bechtol and Lyon, 1978). The simplest interpertation of the data is that Hba-ps4 is closely linked to tf and Fu in t haplotypes and all three loci were deleted during the generation of t^{h20}. Because the t^{h20} haplotype demonstrates poorer complementation than t^{6} does with the complete t haplotypes t^{w5}, t^{w32}, and t^{w1}, it was suggested that the postulated deletion of tf would also cover these lethal genes (Lyon et al., 1979b). However, it is now known that the t^{w5} and t^{w32} lethal genes do not map near tf (Artzt et al., 1982a). Furthermore, poorer complementation is not the same as complete lethality, which is what would be expected if the lethal genes were indeed deleted. A likely possibility is that the lower survival rate of t^{h20}/t^{x} embryos is a consequence of the deletion of uncharacterized genes near Fu, tf, and Hba-ps4, and is not due to a deletion of specific lethal genes.

H. Origin and maintainence of t haplotypes

From this and other studies of t haplotypes performed recently, several points about t haplotypes are now clear. t haplotypes are very closely related to each other, at least as closely related on the DNA level as are inbred strains, which probably have a recent, common origin (Ferris et al., 1982). t haplotypes also do not appear to differ greatly from wild-type mice, thus no evidence has been obtained to support the hypothesis that they originated from a different species. The theory that t^{w5} is the ancestor haplotype for the other t haplotypes cannot be valid, since t^{w5} contains a deletion in the E_{alpha} gene that many other t haplotypes do not contain.

The conclusion that restriction fragments have been identified as t-specific is qualified by fact that a limited number of wild-type mice have been examined. A thorough investigation of wild-type mice, especially including wild-derived mice, may show that these restriction fragments, like those identified by the alpha-pseudogene4 and alpha-crystallin probes, are found in non-t haplotype-containing mice. The same may also be true for the t haplotype testis proteins (Silver et al., 1983b). t haplotypes may thus have originated recently within wild mice.

The persistence and spread of t haplotypes is easily accounted for by the transmission ratio distorting property of males carrying t haplotypes. The origin of t haplotypes can be explained by the acquisition on a chromosome of the alleles of loci which interact to cause transmission ratio distortion. Since these loci have been shown to be separable, they can be maintained as a unit by a series of inversions in the t complex region, thus creating t haplotypes. This may have happened once, and the different H-2 haplotypes in t haplotypes created through evolution, or it may have happened several times, in mice with related H-2 haplotypes.

The results obtained with the t^{Tuw32} haplotype are interesting in the light of the above discussion. This wild-derived haplotype contains a subset of t haplotype associated markers and transmission ratio distortion loci. In the distal region, these markers are not present in the inverted order present in complete t haplotypes. This association may represent a stage in the evolutionary origin of t haplotypes. Molecular and genetic characterization of wild-derived mice from the region where this haplotype was derived (Haifa) and other regions should help clarify the meaning of these results.

The identification of many new t haplotype lethal complementation groups (Klein et al., 1984) makes the

contention that the t lethal loci represent the mutation of a group of genes controlling developmental stage-specific changes less likely, since the number of t haplotype lethals increases with the number examined. The lethalities associated with t haplotypes thus may be thought of as random "parasitic" mutations, since recessive lethalities have little effect on a chromosome with the advantage of transmission ratio distortion. In fact, as discussed previously, these lethalities may actually be advantageous to the survival and spread of that chromosome. Support of the contention that the t haplotype lethalities do not represent "special" developmental genes is that two lethalities, t^{w18} and t^{wLub2}, appear to have arisen through the duplication and deletion of sequences during recombination. A mutation has also been identified in a non-t haplotype containing chromosome 17 which does not complement the t^{0} lethality (Sanchez et al., 1984). It is unlikely that such events are selective for genes that control development. Inactivation by mutation of structural genes can easily lead to developmental lethality, as has been exemplified by the viral-insertion induced Mov-13 lethality, which is caused by the mutation of the alpha1(I) collagen gene (Jaenisch et al., 1983; Schnieke et al., 1983). The embryonic lethalities associated with t haplotypes may be no more illuminating for developmental

biololgy than any other accumulation of recessive lethalities within a 15cM region.

t haplotypes represent a fascinating genetic system within the mouse genome. Multiple, separable loci cause the non-Mendelian transmission of *t*-containing chromosomes. Different combinations of these loci lead to a high, normal, or low transmission ratios of the chromosome containing them. The mapping of the transmission ratio distortion loci within defined regions of *t* haplotype DNA provides a framework for further molecular and biological studies of this phenomenon. *t* haplotypes are maintained as a unit by inversions relative to wild-type. These inversions are also be useful in an experimental sense. Chromosomes containing inversions can serve as balancer chromosomes in mutagenesis studies, in a manner similar to the Muller-5 stocks of *Drosophila*. Such studies will allow a more thorough study of mammalian gene mutations and a lethal saturation analysis of this region.

REFERENCES

Aldridge, J., Kunkel, L., Bruns, G., Tantravahl, U., Lalande, M., Brewster, T., Moreau, E., Wilson, M., Bromley, W., Roderick, T., and Latt, S.A. (1984). A strategy to reveal high-frequency RFLPs along the human X chromosome. Am. J. Hum. Genet. 36: 546-564.

Artzt, K. (1984). Gene mapping within the T/t complex of the mouse. III. t-lethal genes are arranged in three clusters on chromosome 17. Cell 39: 565-572.

Artzt, K. and Bennett, D. (1972). A genetically caused ectodermal tumor in the mouse. J. Nat. Cancer Inst. 48: 141-158.

Artzt, K. and Bennett, D. (1975). Analogies between embryonic (T/t) antigens and adult major histocompatibility (H-2) antigens. Nature 256: 545-547.

Artzt, K. and Bennett, D.(1977). Serological analysis of sperm of antigenically cross-reacting T/t haplotypes and their interactions. Immunogenetics 5: 97-107.

Artzt, K., Dubois, P., Bennett, D., Condamine, H., Babinet, C., and Jacob, F. (1973). Surface antigens common to mouse cleavage embryos and primitive teratocarcinoma cells in culture. Proc. Natl. Acad. Sci. USA 70: 2988-2992.

Artzt, K., Bennett, D., and Jacob, F. (1974). Primitive teratocarcinoma cells express a differentiation anitgen specified by a gene at the T locus in the mouse. Proc. Natl. Acad. Sci. USA 71: 811-814.

Artzt, K., McCormick, P., and Bennett, D. (1982a). Gene mapping within the T/t complex of the mouse. I. t-lethal genes are nonallelic. Cell 28: 463-470.

Artzt, K., Shin, H-S., and Bennett, D. (1982b). Gene mapping within the T/t complex of the mouse. II. Anomalous position of the H-2 complex in t haplotypes. Cell 28: 471-476.

Axelrod, H.R., Artzt, K., and Bennett, D. (1981). Rescue of embryonic cells homozygous for a lethal haplotype of the T/t complex: t^{w12}. Dev. Biol. 73: 332.

Babiarz, B., Garrisi, G.J. and Bennett, D. (1982). Genetic analysis of the t^{w73} haplotype of the mouse using deletion mutations: evidence for a parasitic lethal mutation. Genet. Res. 39: 111-120.

Baglioni, C. (1962). The fusion of two peptide chains in hemoglobin Lepore and its interpertation as a genetic deletion. Proc. Natl. Acad. Sci. USA 48: 1880-1886.

Barker, D., Schafer, M., White, R., (1984). Restriction sites containing CpG show a higher frequency of polymorphism in human DNA. Cell 36: 131-138.

Bechtol, K.B. and Lyon, M.F. (1978). H-2 typing of mutants of the t^6 haplotype in the mouse. Immunogenetics 6: 571-583.

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. and Dunn, L.C. (1958). Effects on embryonic development of a group of genetically similar alleles derived from different populations of wild house mice. J. Morphol. 103: 135-157.

Bennett, D. and Dunn, L.C. (1960). A lethal mutant (t^{w18}) in the house mouse showing partial duplications. J. Exp. Zool. 143: 203-219.

Bennett, D. and Dunn, L.C. (1969). Genetic and embryological comparisons of semilethal t-alleles from wild mouse populations. Genetics 61: 411-422.

Bennett, D. and Dunn, L.C. (1971). Transmission ratio distortion genes on chromosome IX and their interactions. In Proc. Symp. Immunogenetics of the H-2 System, ed. Karger, S. (Liblice-Prague, Basel) pp. 90-103.

Bennett, D., Dunn, L.C., and Badenhausen, S. (1959a). A second group of similar lethals in populations of wild house mice. Genetics 44: 796-802.

Bennett, D., Dunn, L.C., and Badenhausen, S. (1959b). The embryological effects of four late-lethal t-alleles in the mouse which affect the neural tube and skeleton. J. Morphol. 105: 105-144.

Bennett, D., Goldberg, E., Dunn, L.C., and Boyse, E.A. (1972). Serological detection of a cell-surface antigen specified by the T (Brachyury) mutant gene in the house mouse. Proc. Natl. Acad. Sci. USA 69: 2076-2080).

Bennett, D., Dunn, L.C., Spiegelman, M., Artzt, K., Cookingham, J., and Shermerhorn, E. (1975). Observations on a set of radiation-induced dominant T-like mutations in the mouse. Genet. Res. 26: 95-108.

Bennett, D., Dunn, L.C., and Artzt, K. (1976). Genetic change in mutations at the T/t locus in the mouse. Genetics 83: 361-372.

Bennett, D., Artzt, K., Cookingham, J., and Calo, C. (1979). Recombinational analysis of the viable t-haplotype t^{38}. Genet. Res. 33: 269-277.

Bennett, D., Alton, A.K., and Artzt, K. (1983). Genetic analysis of transmission ratio distortion by t-haplotypes in the mouse. Genet. Res. 41: 29-45.

Bennett, W.I., Gall, A.M., Southard, J.L., and Sidman, R.L. (1971). Abnormal spermiogenesis in quaking, a myelin-deficient mutant mouse. Biol. Reprod. 5: 30-58.

Bird, A., Taggart, M., Frommer, M., Miller, O.J., and Macloed, D. (1985). A fraction of the mouse gemone that is derived from islands of nonmethylated, CpG-rich DNA. Cell 40: 91-99.

Bishop, D.T., Williamson, J.A., Skolnick, M.H. (1983). A model for restriction fragment length distributions. Am. J. Hum. Genet. 35: 795-815.

Bode, V.C. (1984). Ethylnitrosourea mutagenesis and the isolation of mutant alleles for specific genes located in the T region of mouse chromosome 17. Genetics 108: 457-470.

Braden, A.W.H. (1958). Influence of time of mating on the segregation ratio of alleles at the T locus in the house mouse. Nature 181: 786-787.

Braden, A.W.H. and Gluecksohn-Waelsch, S. (1958). Further studies on the effect of the T locus in the house mouse on male fertility. J. Exp. Zool. 138: 431-452.

Busslinger, M., deBoer, E., Wright, S., Grosveld, F., and Flavell, R. (1983). The sequence GGCmCGG is resistant to MspI cleavage. Nucleic Acids Res. 11: 3559-3569.

Carter, T.C. and Phillips, R.J.S. (1950). Three recurrences of mutants in the house mouse. J. Heredity 41: 252.

Caspari, E. and David, P.R. (1940). The inheritance of a tail abnormality in the house mouse. J. Heredity 31: 427-431.

Chang, C.C. and Bennett, D., (1980). Nature of antigenic determinants of T locus antigens. Cell 19: 537-543.

Chang, C.C., Sege, K., Alton, A.K., Bennett, D., and Artzt, K. (1983). Characterization of an antigen present on testicular cells and preimplantation embryos whose expression is modified by the t^{12} haplotype. J. Immunogenetics 10: 465-485.

Chesley, P. (1935). Development of the short-tailed mutant in the house mouse. J. Exptl. Zool. 70: 429-459.

Chesley, P. and Dunn, L.C. (1936). The inheritance of taillessness (anury) in the house mouse. Genetics 21: 525-536.

Condamine, H., Guenet, J-L., and Jacob, F. (1983). Recombination between two mouse t haplotypes (t^{w12}tf and t^{lub-1}): Segregation of lethal facors relative to centromere and tufted (tf) locus. Genet. Res. 42: 335-340.

Danska, J. and Silver, L.M. (1980). Cell-free translation of a T/t complex cell surface associated gene product. Cell 22: 901-904.

Davies, K.E., Young, B.D., Elles, R.G., Hill, M.E., and Williamson, R. (1981). Cloning of representative genomic library of the human X chromosome after sorting by flow cytometry. Nature 293: 374-376.

Dembic, Z., Singer, P.A., and Klein, J. (1984). E^0: a history of a mutation. EMBO Journal 3: 1647-1654.

Demin, J.S., Safronova, L.D., and Lapkina, J.A. (1978). Modifying effect of female genotype on the frequency of t-haplotypes transmission from T/t^6 males to offspring of mice. Cytology and Genetics 12: 36-39.

D'Eustachio, P., Fein, B., Michaelson, J., and Taylor, B.A. (1984). The alpha-globin pseudogene on mouse chromosome 17 is linked to H-2. J. Exp. Med. 159: 958-963.

Dobrovolskaia-Zawadskaia, N. (1927). Sur la mortification spontanee de la queue chez la souris nouveau-nee et sur l'existence d'un caractere (facteur hereditaire) non-viable. C.R. Soc. Biol. 97: 114-119.

Dobrovolskaia-Zavadskaia, N., and Kobokieff, N. (1932). Terminaison suspelvienne de la colonne vertevrale au niveau de tronc chez les souris adultes sans queue. C.R. Soc. Biol. 104: 969-971.

Dooher, G.B. and Bennett, D. (1974). Abnormal microtubular systems in mouse spermatids associated with a mutant gene at the T-locus. J. Embryol. Exp. Morphol. 32: 749-761.

Dooher, G.B. and Bennett, D. (1977) Spermiogenesis and spermatozoa in sterile mice carrying different lethal T/t locus haplotypes: A transmission and scanning electron microscopic study. Biol. Reprod. 17: 268-288.

Dunn, L.C. (1937). A third lethal in the T (Brachy) series in the house mouse. Proc. Natl. Acad. Sci. USA 23: 474-477.

Dunn, L.C. (1956). Analysis of a complex gene in the house mouse. Cold Spring Harbor Symp. 21: 187-195.

Dunn, L.C. (1957). Studies of the genetic variability in populations of wild house mice. II. Analysis of eight additional alleles at locus T. Genetics 42: 299-311.

Dunn, L.C. (1964). Abnormalities associated with a chromosome region in a mouse. I. Transmission and population genetics of the t region. Science 144: 260-262.

Dunn, L.C. and Bennett, D. (1968). A new case of transmission ratio distortion in the house mouse. Proc. Natl. Acad. Sci. USA 61: 570-573.

Dunn, L.C. and Bennett, D. (1969). Studies of effects of t-alleles in the house mouse on spermatozoa. II. Quasi-sterility caused by different combinations of alleles. J. Reprod. Fertil. 20: 239-246.

Dunn, L.C. and Bennett, D. (1971a). Lethal alleles near locus T in house mouse populations on the Jutland Penusula, Denmark. Evolution 25: 451-453.

Dunn, L.C. and Bennett, D. (1971b). Further studies of a mutation (low) which distorts transmission ratios in the house mouse. Genetics 67: 543-558.

Dunn, L.C. and Caspari, E. (1945). A case of neighboring loci with similar effects. Genetics 30: 543-568.

Dunn, L.C. and Gluecksohn-Schoenheimer, S. (1943). Tests for recombination amongst three lethal mutations in the house mouse. Genetics 28: 29-40.

Dunn, L.C. and Gluecksohn-Waelsch, S. (1953a). Genetic analysis of seven newly discovered mutant alleles at locus T in the house mouse. Genetics 38: 261-271.

Dunn, L.C. and Gluecksohn-Waelsch, S. (1953b). The failure of a t-allele (t^3) to suppress crossing-over in the mouse. Genetics 38: 512-517.

Dunn, L.C. and Gluecksohn-Waelsch, S. (1954). A genetical study of the mutation 'Fused' in the house mouse, with evidence concerning its allelism with a similar mutation 'Kink'. J. of Genetics 52: 383-391.

Dunn, L.C. and Suckling, J. (1956). Studies of the genetic variability in wild populations of house mice. I. Analysis of seven alleles at locus T. Genetics 41: 344-352.

Dunn, L.C., Bennett, D., and Beasley, A.B. (1962). Mutation and recombination in the vicinity of a complex gene. Genetics 47: 285-303.

Dunn, L.C., Bennett, D., and Cookingham, J.(1973). Polymorphisms for lethal alleles in European populations of Mus musculus. J. Mammalogy 54: 822-830.

Erickson, R.P. and Lewis, S.E. (1980). Cell surfaces and embryos: Expression of the F9 teratocarcinoma antigen in T region lethal, other lethal and normal preimplantation mouse embryos. J. Reprod. Immunol. 2: 293-300.

Erickson, R.P. and Pedersen, R.A. (1975). In vitro development of t^6/t^6 embryos. J. Exp. Zool. 193: 377-383.

Erickson, R.P., Betlach, C.J., and Epstein, C.J. (1974). Ribonucleic acid and protein metabolism of t^{12}/t^{12} embryos and T/t^{12} spermatozoa. Differentiation 2: 203-209.

Erickson, R.P., Lewis, S.E., and Slusser, K.S. (1978). Deletion mapping of the t complex of chromosome 17 of the mouse. Nature 274: 163-164.

Fasman, G., ed. (1976). Handbook of Biochemistry and Molecular Biology, 3rd ed. (CRC Press, Cleveland, Ohio) pp. 270, 273, and 317.

Ferris, S.D., Sage, R.D., and Wilson, A.C. (1982). Evidence from mtDNA sequences that common laboratory strains of inbred mice are descended from a single female. Nature 295: 163-165.

Forejt, J. and Ivanyi, P. (1975). Genetic studies on male sterility of hybrids between laboratory and wild mice. Genet. Res. 24: 189-206.

Forejt, J., Capkova, J., and Gregorova, S. (1980). T(16:17)43H as a tool in analysis of the proximal part of chromosome 17 (including T-t gene complex) of the mouse. Genet. Res. 35: 165-177.

Fox, H.S., Silver, L.M., and Martin, G.R. (1984). An alpha globin pseudogene is located within the mouse t complex. Immunogenetics 19: 129-135.

Fox, H.S., Martin, G.R., Lyon, M.F., Herrmann, B., Frishauf, A-M., Lehrach, H., and Silver, L.M. (1985). Molecular probes define different regions of the mouse t complex. Cell 40: 63-69.

Frishauf, A.M., Lehrach, H., Poutska, A., and Murray, N. (1983). Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. 170: 827-842.

Gable, , R.J., Levinson, J.R., McDevitt, H.O., and Goodfellow, P.N. (1979). Assay for antibody mediated cytotoxicity of mouse spermatozoa by ^{86}rubidium release. Tiss. Ant. 13: 177-185.

Ginsberg, L. and Hillman, N. (1974). Meiotic drive in t^n-bearing mouse spermatozoa: a relationship between aerobic respiration and transmission frequency. J. Reprod. Fertil. 38: 157-163.

Ginsberg, L. and Hillman, N. (1975). ATP metabloism in t^n/t^n mouse embryos. J. Embryol. Exp. Morphol. 33: 715-723.

Gluecksohn-Schoenheimer, S. (1940). The effect of an early lethal (t^0) in the house mouse. Genetics 26: 391-400.

Gluecksohn-Schoenheimer, S. (1949). The effects of a lethal mutation responsible for duplications and twinning in mouse embryos. J. Exp. Zool. 110: 47-76.

Gluecksohn-Waelsch, S. (1971). Models for mechanisms of segregation distortion. In Proc. Intl. Symp. The Genetics of the Spermatozoan, R.A. Beatty and S. Gluecksohn-Waelsch, eds. (University of Edinburgh), pp. 306-309.

Gluechsohn-Waelsch, S. and Erickson, R.P. (1970). The T-locus of the mouse: implications for mechanisms of development. Curr. Topics Dev. Biol. 5: 281-316.

Goodfellow, P.N., Levinson, J.R., Gable, R.J., and McDevitt, H.O. (1979). Analysis of anti-sperm sera for T/t locus-specific antibody. J. Reprod. Immunol. 1: 11-21.

Goossens, M., Dozy, A.M., Embury, S.H., Zachariades, Z., Hadjimmas, M.G., Stamatoyannapoulos, G., and Kan, Y.W. (1980). Triplicated alpha-globin loci in humans. Proc. Natl. Acad. Sci. USA 77: 518-521.

Green, E.L. (1981). Genetics and Probability in Animal Breeding Experiments. (Oxford University Press, New York).

Guenet, J-L., Condamine, H., Gaillard, J., and Jacob, F. (1981). t^{wPA-1}, t^{wPA-2}, t^{wPA-3}: Three new t haplotypes in the mouse. Genet. Res. 36: 211-217.

Hammerberg, C. (1981). The influence of T^{Orl} upon male fertility in t-bearing mice. Genet. Res. 37: 71-77.

Hammerberg, C. (1982). The effects of the t-complex upon male reproduction are due to multiple distorter genes acting upon a responder locus. Genet. Res. 39: 219-226.

Hammerberg, C., and Klein, J. (1975a). Linkage relationships of markers on chromosome 17 of the house mouse. Genet. Res. 26: 203-211.

Hammerberg, C., and Klein, J. (1975b). Evidence for postmeiotic effect of t factors causing segregation distortion in mouse. Nature 253: 137-138.

Hammerberg, C., and Klein, J. (1975c). Linkage disequilibrium between H-2 and t complexes in chromosome 17 of the mouse. Nature 258: 296-299.

Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmids. *J. Mol. Biol.* *166*: 557-580.

Hartl, D.L. and Hiraizumi, Y. (1976). Segregation distortion after fifteen years. In *The Genetics of Drosophila Melanogaster*, Vol. 1b, E. Novitski and M. Ashburner, eds. (New York: Academic Press), pp. 615-666.

Hess, J.F., Schmid, C.W., and Shen, C.-K.J. (1984). A gradient of sequence divergence in human adult alpha-globin duplication units. *Science* *226*: 67-70.

Higgs, D.R., Old, J.M., Pressley, L., Clegg, J.B., and Weatherall, D.J. (1980). A novel alpha-globin gene arrangement in man. *Nature* *284*: 652-655.

Hillman, N. and Nadijcka, M. (1978a). A comparitive study of spermiogenesis in wild-type and *T*:*t* bearing mice. *J. Embryol. Exp. Morphol.* *44*: 243-261.

Hillman, N. and Nadijcka, M. (1978b). A study of spermatozoan defects in wild-type and *T*:*t*-bearing mice. *J. Embryol. Exp. Morphol.* *44*: 263-280.

Hillman, N. and Nadijcka, M. (1980). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. I. A morphological study of spermiogenesis. *J. Embryol. Exp. Morphol.* *59*: 27-37.

Hillman, N. and Tasca, R.J. (1973). Synthesis of RNA in t^{12}/t^{12} mouse embryos. *J. Reprod. Fertil.* *33*: 501-506.

Hillman, N., Hillman, R., and Wileman, G. (1970). Ultrastructural studies of cleavage t^{12}/t^{12} mouse embryos. Am. J. Anat. 128: 311-340.

Hogan, B., Spiegelman, M., and Bennett, D. (1980). In vitro development of inner cell masses isolated from t^{0}/t^{0} and t^{w5}/t^{w5} mouse embryos. Dev. Biol. 80: 289-300.

Horz, W. and Altenberger, W. (1981). Nucleotide sequence of mouse satellite DNA. Nucleic Acids Res. 9: 683-696.

Hyldig-Nielsen, J.J., Schenning, L., Hammerling, U., Widmark, E., Lind, P., Servenius, B., Lund, T., Flavell, R., Lee, J.S., Trowsdale, J., Schreier, P.H., Zablitzky, F., Larhammar, D., Peterson, P.A., and Rask, L. (1983). The complete nucleotide sequence of the $I\text{-}E_{alpha}^{(d)}$ immune response gene. Nucl. Acids Res. 11: 5055-5071.

Jacobs-Cohen, R.J., Spiegelman, M., Cookingham, J.C., and Bennett, D. (1984). Knobbly, a new dominant mutation in the mouse that affects embryonic ectoderm organization. Genet. Res. 43: 43-50.

Jaenisch, R., Harbers, K., Schnieke, Lohler, J., Chumakov, I., Jahner, D., Grotkopp, D., and Hoffman, E. (1983). Germlime integration of moloney murine leukemia virus at the Mov13 locus leads to recessive lethal mutation and early embryonic death. Cell 32: 209-216.

Johnson, D.R. (1974). Hairpin-Tail: A case of post-reductional gene action in the mouse egg? Genetics 76: 795-805.

Johnson, D.R. (1975). Further observations of the hairpin-tail (T^{hp}) mutation in the mouse. Genet. Res. 24: 207-213.

Katz, D.F., Erickson, R.P. and Nathanson, M. (1979). Beat frequency is bimodally distributed in spermatozoa from T/t^{12} mice. J. Exp. Zool. 210: 529-535.

Kao, F.T., Hartz, J.A., Law, M.L., and Davidson, J.N. (1982). Isolation and chromosomal localization of unique DNA segments from a human genomic library. Proc. Natl. Acad. Sci. USA 79: 865-869.

Kemler, R., Babinet, C., Condamine, H., Gachelin, G., Guenet, J-L., and Jacob, F. (1976). Embryonal carcinoma antigen and the T/t locus of the mouse. Proc. Natl. Acad. Sci. USA 73: 4080-4084.

Keshet, E. and Cedar, H. (1983). Effect of CpG methylation on MspI. Nucleic Acids Res. 11: 3571-3580.

King, C.R., Shinohara, T., and Piatigorsky, J. (1982). alphaA-Crystallin messenger RNA of the mouse lens: more noncoding than coding sequences. Science 215: 985-987.

Klein, J. (1971). Cytological identification of the chromosome carrying the IXth linkage group (including H-2) in the house mouse. Proc. Natl. Acad. Sci. USA 68: 1594-1597.

Klein, J. (1975). Biology of the Mouse Histocomaptibility-2 Complex (New York: Springer Verlag).

Klein, J. (1979). Why associative recognition? In Cell Biology and Immunology of Leukocyte Function, ed. Quaster, M. (New York: Academic Press) pp. 309-314.

Klein, J., and Figueroa, F., (1981). Polymorphism of the mouse H-2 complex. Immunol. Rev. 60: 23-57.

Klein, J. and Radska, K. (1968). Deficiency of "ribosomal" DNA in t^{12} mutant mice. Proc. XII Inter. Cong. Genetics 1: 149.

Klein, J., Sipos, P., and Figueroa, F. (1984). Polymorphism of t complex genes in European wild mice. Genet. Res. 44: 39-46.

Klyde, B.J. (1970). Selection in mice for change in viability of a semilethal haplotype at the T locus. J. Heredity 61: 39-43.

Kunkel, L.M., Tantravahi, U., Eisenhard, M., and Latt, S.A. (1982). Regional localization on the human X of DNA segments cloned from flow sorted chromosomes. Nucl. Acids Res. 10: 1557-1578.

Leder, P., Hansen, J.N., Konkel, D., Leder, A., Nishioka, Y., and Talkington, C. (1980). Mouse globin system: A functional and evolutionary analysis. Science 209: 1336-1342.

Leder, A., Swan, D., Ruddle, F., D'Eustachio, P., and Leder, P. (1981). Dispersion of alpha-like globin genes of the mouse to three different chromosomes. Nature 293: 196-200.

Lewontin, R.C. (1962). Interdeme selection controlling a polymorphism in the house mouse. Amer. Nat. 96: 65-78.

Lewontin, R.C. (1968). The effects of differential viability on the popluation dynamics of t alleles in the house mouse. Evolution 22: 262-273.

Lewontin, R.C. and Dunn, L.C. (1960). The evolutionary dynamics of a polymorphism in the house mouse. Genetics 45: 705-722.

Lyon, M.F. (1977). Mouse News Letter 56: 37.

Lyon, M.F. (1981). The t-complex and the genetic control of development. Symp. Zool. Soc., London 47: 455-477.

Lyon, M.F. (1984). Transmission ratio distortion in mouse t-haplotypes is due to multiple distorter genes acting on a responder locus. Cell 37: 621-628.

Lyon, M.F. and Bechtol, K.B. (1977). Derivation of mutant t-haplotypes of the mouse by presumed duplication or deletion. Genet. Res. 30: 63-76.

Lyon, M.F. and Mason, I. (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 Meredith, R. (1964a). The nature of t alleles in the mouse. I. Genetic analysis of a series of mutants derived from a lethal allele. Heredity 19: 301-312.

Lyon, M.F. and Meredith, R. (1964b). The nature of t alleles in the mouse. II. Genetic analysis of an unusual mutant allele and its derivatives. Heredity 19: 313-325.

Lyon, M.F. and Meredith, R. (1964c). Investigations of the nature of t alleles in the mouse. III. Short tests of some further mutant alleles. Heredity 19: 327-330.

Lyon, M.F. and Phillips, R.J.S. (1959). Crossing-over in mice heterozygous for t-alleles. Heredity 13: 23-32.

Lyon, M.F., Evans, E.P., Jarvis, S.E., and Sayers, I. (1979a). t-Haplotypes of the mouse may involve a change in intercalary DNA. Nature 279: 38-42.

Lyon, M.F., Jarvis, S.E., Sayers, I., and Johnson, D.R. (1979b). Complementation reactions of a lethal mouse t-haplotype believed to include a deletion. Genet. Res. 33: 153-161.

Magnuson, T., Epstein, C.J., Silver, L.M., and Martin, G.R. (1982). Pluripotent embryonic stem cell lines can be derived from t^{w5}/t^{w5} blastocysts. Nature 298: 750-753.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1982). Molecular Cloning: A Laboratory Manual (New York: Cold Spring Harbor Laboratory).

Mann, E., Elliott, R.W., and Huhan, C. (1984). Mouse News Letter 71: 48.

Mathis, D.J., Benoist, C.O., Williams, V.E., Kanter, M.R., and McDevitt, H.O. (1983a). The murine E_{alpha} immune response gene. Cell 32: 745-754.

Mathis, D.J., Benoist, C.O., Williams, V.E., Kanter, M.R., and McDevitt, H.O. (1983b). Several mechanisms can account for defective E_{alpha} gene expression in different mouse haplotypes. Proc. Natl. Acad. Sci. USA 80: 273-277.

McGrath, J. and Hillman, N. (1980a). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. III. In vitro fertilization. J. Embryol. Exp. Morphol. 59: 49-58.

McGrath, J. and Hillman, N. (1980b). The in vitro transmission frequency of the t^{12} mutation in the mouse. J. Embryol. Exp. Morphol. 60: 141-151.

McGrath, J. and Hillman, N. (1980c). The in vitro transmission frequency of the t^{6} allele. Nature 283: 479-481.

McGrath, J. and Solter, D. (1984). Maternal T^{hp} lethality in the mouse is a nuclear, not cytoplasmic, defect. Nature 308: 550-552.

Mellor, A.L., Weiss, E., Ramachandran, K., and Flavell, R.A. (1983). A potential donor for the bm1 gene conversion event in the C57BL mouse. Nature 306: 792-795.

Mintz, B. (1964). Formation of genetically mosaic mouse embryos, and early development of "Lethal (t^{12}/t^{12})-Normal" mosaics. J. Exp. Zool. 157: 273-292.

Moser, G.C. and Gluecksohn-Waelsch, S. (1967). Developmental genetics of a recessive allele at the complex T-locus in the mouse. Dev. Biol. 16: 564-576.

Moutier, R. (1973a). Mouse News Letter 48: 38.

Moutier, R. (1973b). Mouse News Letter 49: 42.

Nadeau, J.H. and Phillips, S.J. (1984). Recombination suppression and the t^6-complex in house mice. Genetics (abstract) s76-77.

Nadijcka, M. and Hillman, N. (1975). Studies of t^6/t^6 mouse embryos. J. Embryol. Exp. Morphol. 33: 697-713.

Nadijcka, M. and Hillman, N. (1980a). Sterility in mutant (t^{Lx}/t^{Ly}) male mice. II. A morphological study of spermatozoa. J. Embryol. Exp. Morphol. 59: 39-47.

Nadijcka, M. and Hillman, N. (1980b). Differences in cAMP levels in t^n- and non-t^n-bearing mouse spermatozoa. Biol. Reprod. 22: 1102-1105.

Nizetic, D., Figueroa, F., and Klein, J. (1984). Evolutionary relationships between the t and H-2 haplotypes in the house mouse. Immunogenetics 19: 311-320.

Olds, P. (1971). Effect of the T locus on sperm ultrastructure in the house mouse. J. Anat. 109: 31-37.

Olds-Clarke, P. (1973). Nonprogressive sperm motility is characteristic of most complete t haplotypes in the mouse. Genet. Res. 42: 151-157.

Olds-Clarke, P. and Carey, J.E. (1978). Rate of egg penetration in vitro accelerated by T/t locus in the mouse. J. Exp. Zool. 206: 323-332.

Paigen, K. (1980). In The Molecular Genetics of Development. W. Loomis and R. Leighton, eds. (New York: Academic Press) pp. 419-470.

Pease, L.R., Schulze, D.H., Pfaffenbach, G.M., and Nathenson, S.G. (1983). Spontaneous H-2 mutants provide evidence that a copy mechanism analogous to gene conversion generates polymorphism in the major histocompatibility complex. Proc. Natl. Acad. Sci. USA 80: 242-246.

Pla, M. and Condamine, H. (1984). Recombination between two mouse t haplotypes (t^{w12}tf and t^{Lub-1}): Mapping of the H-2 complex relative to centromere and tufted (tf) locus. Immunogenetics 20: 177-185.

Popp, R.A., Lalley, P.A., Whitney, J.B., and Anderson, W.F. (1981). Mouse alpha-globin genes and alpha-globin-like pseudogenes are not syntenic. Proc. Natl. Acad. Sci. USA 78: 6362-6366.

Razin, A. and Riggs, A. (1980). DNA methylation and gene function. Science 210: 604-610.

Reed, S.C. (1937). The inheritance and expression of Fused, a new mutation in the house mouse. Genetics 22: 1-13.

Roberts, P.A. (1976). The genetics of chromosome aberration. In The Genetics and Biology of Drosophila, Vol. 1a, M. Ashburner and E. Novitski, eds. (New York: Academic Press), pp. 68-184.

Roderick, T.H.and Davisson, M.T. (1981). Linkage Map. In Genetic Variants and Strains of the Laboratory Mouse, M.C. Green, ed. (Stuttgart: Gustav Fisher Verlag) pp. 279-282.

Rohme, D., Fox, H., Herrman, B., Frishauf, A.M., Edstrom, J.E., Mains, P., Silver, L.M., and Lehrach, H. (1984). Molecular clones of the mouse t complex derived from microdissected metaphase chromosomes. Cell 36: 969-976.

Sage, R.D. (1981). Wild mice. In The Mouse in Biomedical Research, Vol I, H.L. Foster, J.D. Small, and J.G. Fox, eds. (New York: Academic Press) pp. 40-90.

Sanchez, E.R. and Hammerberg, C. (1984). A new t-complex embryonic lethal (tcl^0) has arisen in the T^{hp} chromosome of the mouse and is allelic to t^0. Genet. Res. 43: 27-33.

Scalenge, F., Turco, E., Edstron, J.E., Pirotta, V., and Melli, M., (1981). Microdissection and cloning of DNA from a specific region of Drosophila melanogaster polytene chromosomes. Chromosoma 82: 205-216.

Schnieke, A., Harbers, K., and Jaenisch, R. (1983). Embryonic lethal mutation in mice induced by retrovirus insertion into the alpha1(I) collagen gene. Nature 304: 315-32.

Shin, H-S., Stavenezer, J., Artzt, K., and Bennett, D. (1982). Genetic structure and origin of t haplotypes of mice, analyzed with H-2 cDNA probes. Cell 29: 969-976.

Shin, H-S., Flaherty, L., Artzt, K., Bennett, D., and Ravetch, J. (1983a). Inversion in the H-2 complex of t-haplotypes in mice. Nature 306: 380-383.

Shin, H-S., McCormick, P., Artzt, K., and Bennett, D. (1983b). Cis-trans test shows a functional relationship between non-allelic lethal mutations in the T/t complex. Cell 33: 925-929.

Shin, H-S., Bennett, D., and Artzt, K. (1984). Gene mapping within the T/t complex of the mouse. IV: The inverted MHC is intermingled with several t-lethal genes. Cell 39: 573-578.

Shur, B.D. (1981). Galactosyltransferase activities on mouse sperm bearing multiple t^{lethal} and t^{viable} haplotypes of the T/t complex. Genet. Res. 38: 225-236.

Shur, B.D. and Bennett, D. (1979). A specific defect in galactosyltransferase regulation on sperm bearing mutant alleles of the T/t locus. Dev. Biol. 71: 243-259.

Sidman, R.L., Dickie, M.M., and Appel, S.H. (1964). Mutant mice (quaking and jimpy) with deficient myelination in the central nervous system. Science 144: 309-311.

Silagi, S. (1963). Some aspects of the relationship of RNA metabolism to development in normal and mutant embryos cultivated in vitro. Exp. Cell Res. 321: 149-152.

Silver, L.M. (1981a). Genetic organization of the mouse t complex. Cell 27: 239-240.

Silver, L.M. (1981b). A structural gene (Tcp-1) within the mouse t complex is separable from effects on tail length and lethality but may be associated with effects on spermatogenesis. Genet. Res. 38: 115-123.

Silver, L.M. (1982). Genomic analysis of the H-2 complex region associated with mouse t haplotypes. Cell 29: 961-968.

Silver, L.M. (1983). Reevaluation of the evidence for the generation of new lethal t haplotypes by mutation. Immunogenetics 18: 91-96.

Silver, L.M. and Artzt, K. (1981). Recombination supression of mouse t haplotypes due to chromatin mismatching. Nature 290: 68-70.

Silver, L.M. and Olds-Clarke, P. (1984). Transmission ratio distortion of mouse t haplotypes is not a consequence of wild-type sperm degeneration. Dev. Biol. 105: 250-252.

Silver, L.M., Artzt, K., and Bennett, D. (1979). A major testicular protein specified by a mouse T/t complex gene. Cell 17: 275-284.

Silver, L.M. White, M., and Artzt, K. (1980). Evidence for unequal crossing over within the mouse T/t complex. Proc. Natl. Acad. Sci. USA 77: 6077-6080.

Silver, L.M., Lukralle, D., and Garrels, J.I. (1983a). T^{Orl} is a novel, variant form of mouse chromosome 17 with a deletion in a partial t haplotype. Nature 301: 422-424.

Silver, L.M., Uman, J., Danska, J., and Garrels, J.I. (1983b). A diversified set of testicular cell proteins specified by genes within the mouse t complex. Cell 35: 35-45.

Silver, L.M., Lukralle, D., Garrels, J.I. and Willison, K. (1984). Persistance of a lethal t haplotype in a laboratory stock of outbred mice. Genet. Res. 43: 21-25.

Skolnick, M.H. and White, R. (1982). Strategies for detecting and characterizing restriction fragment length polymorphisms (RFLP's). Cytogenet. Cell Genet. 32: 58-67.

Skolnick, M., Willard, H., Menlove, L. (1984). Report of the committee on human gene mapping by recombinant DNA techniques. Human gene mapping 7. Cytogenet. Cell Genet. 32: 58-67.

Skow, L.C. (1984). Sequences which hybridize to mouse alpha-crystallin cDNA are located near $H\text{-}2^{k}$. Genetics (abstract) s101.

Smith, L.J. (1956). A morphological and histochemical investigation of a preimplantation lethal (t^{12}) in the house mouse. J. Exp. Zool. 132: 51-83.

Sneider, T. (1980). The 5'-cytosine in CCGG is methylated in two eucaryotic DNAs and MspI is sensitive to methylation at this site. Nucleic Acids Res. 8: 3829-3840.

Snell, G. (1968). The H-2 complex of the mouse. Observations and speculations concerning its comparitive genetics and polymorphism. Folia Biol. 14: 335-358.

Snow, M.H.L. and Bennett, D. (1979). Gastrulation in the mouse: assessment of cell populations in the epiblast of t^{w18}/t^{w18} embryos. J. Embryol. Exp. Morphol. 47: 39-52.

Spiegelman, M. and Bennett, D. (1974). Fine structural study of cell migration in the early mesoderm of normal and mutant mouse embryos (T-locus: t^9/t^9). J. Embryol. Exp. Morphol. 32: 723-738.

Statts, J. (1981). Inbred and segregating mouse strains. In The Mouse in Biomedical Research, Vol. 1, H.L. Foster, J.D. Small, and J.G. Fox, eds. (New York: Academic Press) pp. 170-213.

Steinmetz, M., Frelinger, J.G., Fisher, D., Hunkapiller, T., Pereira, D., Weissman, S.M., Uehara, H., Nathenson, S., and Hood, L. (1981). Three cDNA clones encoding mouse transplantation antigens: homology to immunoglobulin genes. Cell 24: 125-134.

Steinmetz, M., Winoto, A., Minard, K., and Hood, L. (1982a). Clusters of genes encoding mouse transplantation antigens. Cell 26: 484-498.

Steinmetz, M., Minold, K., Horvath, S., McNicholas, J., Frelinger, J., Wake, C., Long, E., Mach, B., and Hood, L. (1982b). A molecular map of the immune responce region from the major histocompatibility complex of the mouse. Nature 300: 35-42.

Strum, S., Figueroa, F., and Klein, J. (1982). The relationship between t and H-2 complexes in wild mice. I. The H-2 haplotypes of 20 t-bearing strains. Genet. Res. 40: 73-88.

Sturtevant, A.H. (1926). A crossover reducer in Drosophila melanogaster due to inversion of a section of the third chromosome. Biologisches Zentralblatt 46: 697-702.

Styrna, J. and Klein, J. (1981). Evidence for two regions in the mouse t complex controlling transmission ratios. Genet. Res. 38: 315-325.

Tessler, S., Carey, J.E., and Olds-Clarke, P. (1981). Mouse sperm motility affected by factors in the T/t complex. J. Exp. Zool. 217: 277-285.

Theiler, K. and Gluecksohn-Waelsch, S. (1956). The morphological effects and the development of the fused mutation in the mouse. Anat. Rec. 125: 83-104.

Tres, L.L. and Erickson, R.P. (1982). Electron microscopy of t-allele synaptonemal complexes discloses no inversions. Nature 299: 752-754.

Tucker, M.J. (1980). Explanation of sterility in t^X/t^Y male mice. Nature 288: 367-368.

Tykocindki, M.L. and Max, E.C. (1984). CG clusters in MHC genes and 5' demethylated genes. Nucl. Acids Res. 12: 4385-4396.

Vitetta, E.S., Artzt, K., Bennett, D., Boyse, E.A., and Jacob, F. (1975). Structural similarities between a product of the T/t-locus isolated from sperm and teratoma cells, and H-2 antigens isolated from splenocytes. Proc. Natl. Acad. Sci. USA 72: 3215-3219.

Vojtiskova, M., Viklicky, V., Voracova, B., Lewis, S.E., and Gluecksohn-Waelsch, S. (1976). The effects of a t-allele (t^{AE5}) in the mouse on the lymphoid system and reproduction. J. Embryol. Exp. Morphol. 36: 443-451.

Washburn, L.L. and Eicher, E.M. (1983). Sex reversal in XY mice caused by dominant mutation on chromosome 17. Nature 303: 338-340.

Weiss, E.H., Gordon, L., Fahrner, K., Meller, A.C., Devlin, J.J., Bullman, H., Tiddus, H., Bud, H., and Flavell, R.A. (1984). Organization and evolution of the class I gene family in the major histocompatability complex of the C57BL/10 mouse. Nature 310: 650-655.

Winking, H., Mouse News Letter 60: 56 (1979).

Winking, H. and Silver, L.M. (1984). Characterization of a recombinant mouse t haplotype that expresses a dominant lethal maternal effect. Genetics 108: 1013-1020.

Winoto, A., Steinmetz, M., and Hood, L. (1983). Genetic mapping in the major histocompatibility complex by restriction enzyme site polymorphisms: Most mouse class I genes map to the Tla complex. Proc. Natl. Acad. Sci. USA 80: 3425-3429.

Womack, J.E. and Roderick, T.H. (1974). T-alleles in the mouse are probably not inversions. J. Hered. 65: 308-310.

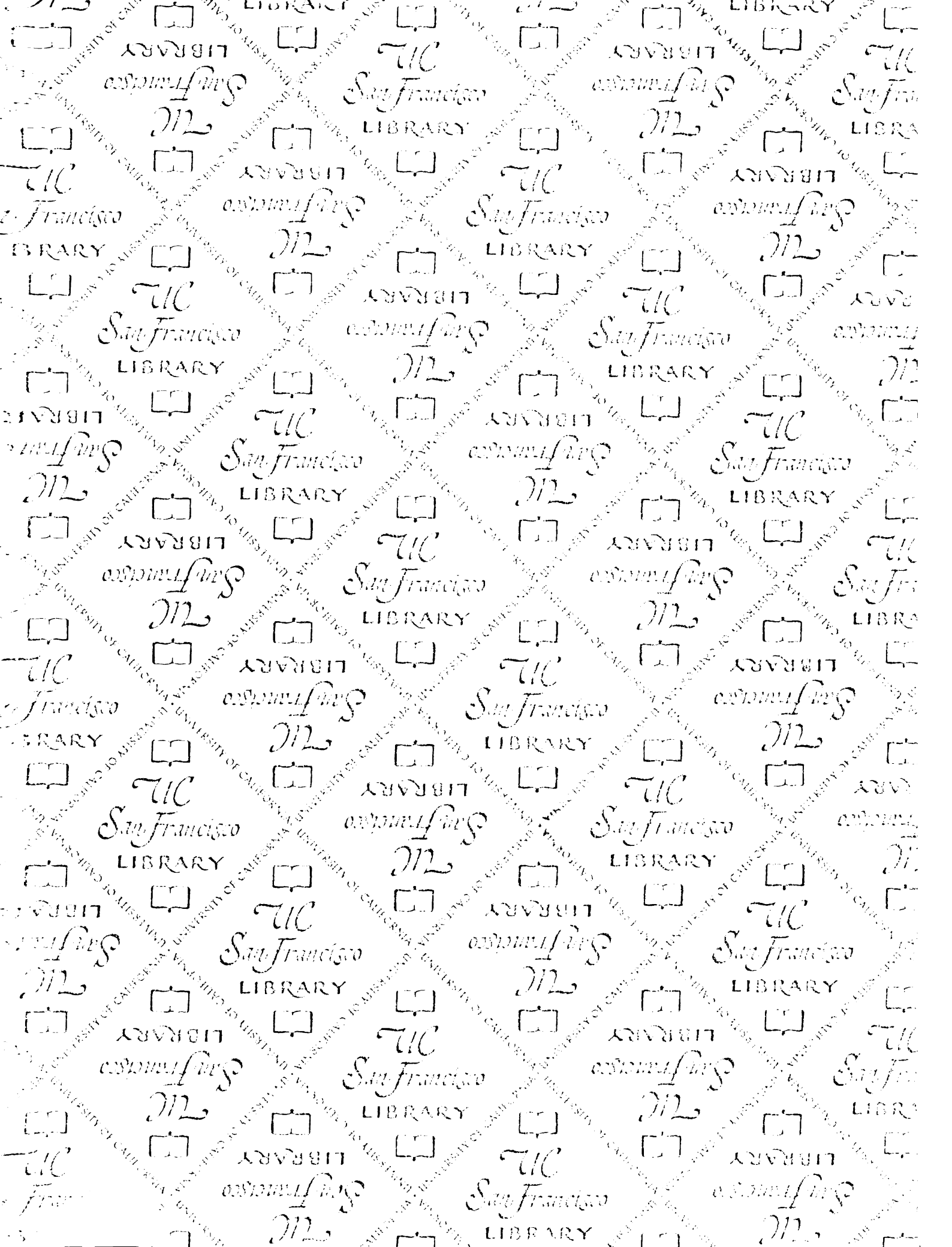
Wudl, L.R. and Sherman, M.I. (1976). In vitro studies of mouse embryos bearing mutations at the T locus: t^{w5} and t^{12}. Cell 9: 523-531.

Wudl, L.R. and Sherman, M.I. (1978). In vitro studies of mouse embryos bearing mutations at the T locus: t^6. J. Embryol. Exp. Morphol. 48: 127-151.

Wudl, L.R., Sherman, M.I., and Hillman, N. (1977). Nature of lethality of t mutations in embryos. Nature 270: 137-140.

Yanagisawa, K., Bennett, D., Boyse, E.A., Dunn, L.C. and Dimeo, A. (1974). Serological identification of sperm antigens specified by lethal t-alleles in the mouse. Immunogenetics 1: 57-67.

Young, M.W. (1979). Middle repetitive DNA: a fluid component of the Drosophila genome. Proc. Natl. Acad. Sci. USA 76: 6274-6278.


UC
San Francisco
LIBRARY

FOR REFERENCE

NOT TO BE TAKEN FROM THE ROOM