Molecular Evidence for the Rapid Propagation of Mouse t Haplotypes from a Single, Recent, Ancestral Chromosome

Lee M. Silver,* Michael Hammer,* Howard Fox;† James Garrels,† Marija Bucan,‡ Bernhard Herrmann,‡ Anna-Marie Frischau,‡ Hans Lehrach,‡ Heinz Winking,§ Felipe Figueroa,‖ and Jan Klein‖

*Department of Molecular Biology, Princeton University; †Cold Spring Harbor Laboratory; ‡European Molecular Biology Laboratory; §Institut für Biologie, Medizinische Hochschule Luebeck; and ‖Abteilung Immungenetik, Max-Planck Institute für Biologie

Mouse t haplotypes are variant forms of chromosome 17 that exist at high frequencies in worldwide populations of two species of commensal mice. To determine both the relationship of t haplotypes to each other and the species within which they exist, 35 representative t haplotypes were analyzed by means of 10 independent molecular probes, including five DNA clones and five polypeptide spots identified by means of two-dimensional gel electrophoresis. All of the tested haplotypes were found to share restriction fragments and polypeptide spots that are absent in mice carrying wild-type forms of chromosome 17. This observation provides the first direct evidence that all of the known t haplotypes are descendents of a single ancestral chromosome. The absence of variation among t haplotypes could mean that this ancestral chromosome existed relatively recently, in which case it would be necessary to postulate introgressions of t haplotypes across species lines to explain their presence in both Mus domesticus and M. musculus. Alternatively, it is possible that the ancestral chromosome existed prior to the split between M. domesticus and M. musculus and that, by chance, our probes fail to detect polymorphisms that exist among the t haplotypes. A further result of our analysis is the characterization of a partial t haplotype in a wild population of Israeli mice.

Introduction

The proximal portion of mouse chromosome 17 can exist in two distinct forms within natural populations of Mus domesticus and M. musculus (Klein et al. 1984; Nižetić et al. 1984). One form is considered to be wild type (+), and the alternative form is called a t haplotype (see Silver 1985 for a review). The t haplotype is distinguished from the wild type by the presence of two nonoverlapping inversions of genetic material that are responsible for a suppression of recombination along this region in heterozygous mice (see fig. 1). A further property of t haplotypes is the expression of a male-specific transmission-ratio distortion, such that nearly all of the offspring of heterozygous (+/t) males can receive the t form of chromosome 17 from their father. The t-specific properties of recombination suppression and transmission-ratio distortion allow the maintenance of complete t haplotypes as intact genetic entities that are propagated at high frequencies in the wild.

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2. Current address: Department of Medicine-MSTP, University of California at San Francisco, San Francisco, California 94143.

Address for correspondence and reprints: Dr. Lee M. Silver, Department of Molecular Biology, Princeton University, Princeton, New Jersey 08544.

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Wild-type

During the past decade, a large number of new t haplotypes have been isolated directly from wild mice trapped in a variety of locations throughout Europe, northern Africa, South America, and the Middle East (Klein et al. 1984; Nižetić et al. 1984). Klein and his co-workers carried out a series of detailed studies on these chromosomes and demonstrated their derivation from a single ancestral chromosome (Golubic et al. 1984; Nižetić et al. 1984; Figueroa et al. 1985). As a continuation of these earlier studies on the relationship of t haplotypes to one another, we have conducted a comprehensive comparative molecular analysis of a representative group of 28 newly described t haplotypes, in conjunction with a set of six previously defined and well-characterized t haplotypes.

**Material and Methods**

**Mice**

The $t^{Tuw}$ set of wild-derived mice have been described in several previous publications (Golubic et al. 1984; Klein et al. 1984; Nižetić et al. 1984; Figueroa et al. 1985). A set of 20 recently isolated $t^{Tuw}$ haplotypes used in the present paper, along with their geographical origins, are as follows: (1) $t^{Tuw2}$, Wendelsheim, Germany; (2) $t^{Tuw6}$, Brno, Czechoslovakia; (3) $t^{Tuw7}$, Giza, Egypt; (4) $t^{Tuw8}$, Giza, Egypt; (5) $t^{Tuw10}$, Eday, Great Britain; (6) $t^{Tuw11}$, Buin, Chile; (7) $t^{Tuw12}$, La Roca, Spain; (8) $t^{Tuw15}$, Moya, Spain; (9) $t^{Tuw18}$, Haifa, Israel; (10) $t^{Tuw20}$, Ryazan, USSR; (11) $t^{Tuw21}$, Wendelsheim, Germany; (12) $t^{Tuw23}$, Temuko, Chile; (13) $t^{Tuw24}$, Langenargen, Germany; (14) $t^{Tuw25}$, ObererLindenhof, Germany; (15) $t^{Tuw26}$, Bialowieza, Poland; (16) $t^{Tuw27}$, Dudelhof, Germany; (17) $t^{Tuw28}$, Erpenhausen, Germany; (18) $t^{Tuw29}$, Aulendorf, Germany; (19) $t^{Tuw30}$, Michigan; and (20) $t^{Tuw32}$, Haifa, Israel.

Several members of the $t^{wLub}$ set of wild-derived mice have been described in
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Previous publications (Golubic et al. 1984; Nijetić et al. 1984; Winking and Silver 1984) have shown that a set of eight recently isolated $t^{\text{wLab}}$ haplotypes used in the present study, along with their geographical origins, are as follows: (1) $t^{\text{wLab1}}$, Alpic Drobic, Italy; (2) $t^{\text{wLab3}}$, Ardenno, Italy; (3) $t^{\text{wLab4}}$, Cremona, Italy; (4) $t^{\text{wLab5}}$, Caneto Pavese, Italy; (5) $t^{\text{wLab6}}$, Luino, Italy; (6) $t^{\text{wLab7}}$, Tortona, Italy; (7) $t^{\text{wLab8}}$, Varsi, Italy; and (8) $t^{\text{wLab9}}$, Calcinato, Italy.

All animals from Poland, Russia, and Czechoslovakia are Mus musculus. All others are M. domesticus.

Seven other $t$ haplotypes analyzed in the present study, along with their geographical origins (where known), are as follows: (1) $t^{\text{w2}}$, New York or Philadelphia; (2) $t^{\text{w5}}$, New York; (3) $t^{\text{w12}}$, Marin County, California; (4) $t^{\text{w32}}$, Clinton, Montana; (5) $t^{\text{w75}}$, South Jutland, Denmark; (6) $t^{0}$, of unknown origin, detected in a Paris laboratory; and (7) $t^{\text{Lmb}}$ (described by Silver et al. 1984), detected in a noninbred stock of mice obtained from a British supplier.

DNA Analysis

Five independent genomic clones derived from the $t$-complex region of chromosome 17 were used in the analysis of all recently isolated $t$ haplotypes. The clones Tu66, Tu119, Tu108, and Tu89 represent $t$-complex sequences (fig. 1) obtained by using a method of chromosome microdissection (Fox et al. 1985; Herrmann et al. 1986). A fifth clone contains the alpha-globin pseudogene-4 (Hba-4ps) locus (Fox et al. 1984). Radioactive probes were produced by means of nick-translation according to standard procedures. Preparation of genomic DNA, restriction-enzyme digestions, agarose-gel electrophoresis of digested DNA, blotting, and hybridization were performed as described elsewhere (Fox et al. 1985).

Testicular-Cell Protein Polymorphisms

Partially purified germ-cell populations were prepared and labeled in culture with $^{35}$S-methionine according to a method described elsewhere (Silver et al. 1983). Whole-protein samples were analyzed by using a method of high-resolution two-dimensional gel electrophoresis (2DGE) also described elsewhere (Silver et al. 1983).

Results

2DGE of $t$-Haplotype Polypeptides

In previous studies, we used 2DGE to identify a series of eight $t$-complex polypeptides (called TCP-1 and TCP-3–TCP-9) expressed in the testes uniquely by animals with a $t$ haplotype (Silver et al. 1983). In the present study, we used the 2DGE technique to analyze and compare the expression of five prominent TCP proteins (TCP-1, TCP-3, TCP-4, TCP-5, and TCP-8) by both the 28 recently isolated $t$ haplotypes and the seven previously studied $t$ haplotypes.

The $t$-specific forms of TCP-1, TCP-5, and TCP-8 are expressed by all mice that carry any one of the 35 $t$ haplotypes analyzed (see fig. 2). The $t$ form of TCP-3 is expressed by all except $t^{\text{Taw32}}$, and the $t$ form of TCP-4 is expressed by all except $t^{\text{Taw11}}$. Mice homozygous for the $t^{\text{Taw32}}$ haplotype express only the wild-type form of the TCP-3 protein. Therefore, the $t^{\text{Taw32}}$ chromosome is associated with a wild-type allele at the $TcP-3$ locus, which encodes the TCP-3 protein. It is possible to analyze homozygous $t^{\text{Taw11}}$ animals because this haplotype is associated with a recessive lethal mutation. However, mice heterozygous for the $t^{\text{Taw11}}$ haplotype and a wild-type chromosome continue to express the wild-type TCP-4 at a level consistent with that found...
in animals heterozygous for other complete \(t\) haplotypes. Therefore, it is most likely that the \(t^{\text{Taw}11}\) chromosome carries either a null allele or a novel mutant allele at the \(TcP-4\) locus that produces a polypeptide not readily detectable in the 2DGE pattern.

**Molecular Analysis of \(t\)-Haplotype Restriction Fragments**

In previous studies, we used a series of independent DNA clones derived from different regions of the \(t\) complex to identify a set of \(t\)-specific, restriction-fragment-length polymorphisms (RFLPs) (Fox et al. 1984, 1985; Herrmann et al. 1986). The clones Tu119 and alpha-globin pseudogene-4 (\(Hba-4ps\)) each detect single \(t\)-specific \(BglII\) and \(TaqI\) restriction fragments present in genomic DNA from animals that carry a \(t\) haplotype. The clones Tu66, Tu89, and Tu108 each detect multiple restriction fragments located within \(t\)-haplotype DNA; some of these restriction fragments appear to be \(t\)-specific, and others are shared by both the \(t\) and the wild-type forms of chromosome 17 (Fox et al. 1985; M. Bucan, L. M. Silver, A.-M. Frischau, and H. Lehrach, unpublished observations; see fig. 1 for locations of all markers used in the present study). Two \(t\)-specific \(TaqI\) fragments were detected with Tu66; two \(t\)-specific \(BglII\) fragments were detected with Tu89; and four \(t\)-specific \(PvuII\) fragments were detected with Tu108.

DNA obtained from animals that carry one each of the 28 newly characterized \(t\) haplotypes was digested with restriction enzymes and hybridized with each of the five \(t\)-complex DNA clones. For analysis with each clone, one restriction enzyme that was known to produce one or more \(t\)-specific restriction fragments was chosen, as
described above. On analysis with each of the five probes (see figs. 3, 4), 27 of the new \( t \) haplotypes appeared identical both to each other and to six previously analyzed \( t \) haplotypes. The exception, \( t^{Tun32} \), carried \( t \)-specific restriction fragments detected with Tu119, Tu66, and the \( Hba-4ps \) probe (fig. 3, lane 3); however, this haplotype was not associated with any of the \( t \)-specific restriction fragments detected by means of either Tu89 or Tu108. In total, the two \( t \)-specific Tu89 fragments, the four \( t \)-specific Tu108 fragments, and one of the \( t \)-specific Tu66 fragments were not detected in mice that carried the \( t^{Tun32} \) haplotype.

The \( t^{Tun32} \) Haplotype Is a Partial \( t \) Haplotype

The \( t^{Tun32} \) haplotype is different from all of the other naturally occurring \( t \) haplotypes examined in either the present study or in other studies, in that it is not associated with a number of \( t \)-specific markers that map to the distal region of complete \( t \)-haplotypes (see fig. 1). A number of genetic experiments to investigate further the structure and functional properties of the \( t^{Tun32} \) haplotype gave the following results:

First, the \( t^{Tun32} \) haplotype is transmitted from heterozygous \((+/t^{Tun32})\) males at a normal ratio of 50%, whereas complete \( t \) haplotypes are transmitted at ratios of \( \geq 95\% \). Second,

![Diagram](image_url)

**Fig. 3.—Hybridization analysis of the \( Hba-4ps \) locus by means of \( Taql \)-digested DNA. Lanes 1–13 and 15 contain DNA samples from animals that carry independent \( t \) haplotypes. The \( t \) haplotypes represented are \( t^{Tun22} \) (1), \( t^{Tun27} \) (2), \( t^{Tun32} \) (3), \( t^{Tun3} \) (4), \( t^{Tun15} \) (5), \( t^{Tun22} \) (6), \( t^{Tun12} \) (7), \( t^{Tun1} \) (8), \( t^{Ta} \) (9), \( t^{Ta} \) (10), \( t^{Ta} \) (11), \( t^{Ta} \) (12), \( t^{Ta} \) (13), and \( t^{Ta} \) (15). Lane 14 contains DNA from an inbred 129/SvJ mouse, and lane 15 contains DNA from a congenic 129-\( +/t^{a} \) N20 animal. Animals represented in lanes 3, 4, and 13 were homozygous for \( t \) haplotypes that did not carry lethal mutations. All other \( t \)-carrying animals carried recessive lethal mutations and could only be bred as heterozygotes with a wild-type form of chromosome 17. A 5.2-kb restriction fragment (t) is associated with all \( t \) haplotypes. The wild-type form of \( Hba-4ps \) is polymorphic. The \( Hba-4ps^{a} \) allele is detected as a 1.4-kb (a2) and a 3.8-kb (a1) \( Taql \) fragment. The \( Hba-4ps^{b} \) allele (b) is detected as a 3.4-kb \( Taql \) fragment. All minor bands represent cross-hybridization of the \( Hba-4ps \) probe with other alpha-globin genes.
FIG. 4.—Hybridization analysis of loci defined by means of microdissection-derived probes. A, Alleles of the T119 locus detected in BgIII-digested DNA. The genotypes represented in each lane are +/T<sup>Tow27</sup> (1), T<sup>Tow32</sup>/T<sup>Tow32</sup> (2), T<sup>Tow19</sup>/T<sup>Tow18</sup> (3), +/T<sup>Tow24</sup> (4), +/T<sup>Tow20</sup> (5), +/T<sup>Tow29</sup> (6), T<sup>Tow17</sup>/T<sup>Tow27</sup> (7), and +/+ of the inbred 129/SvJ strain (8). The wild-type band (+) and the t-specific bands (t) are shown. B, Alleles of the T89 locus detected in BgIII-digested DNA. The genotypes represented in each lane are +/T<sup>Tow11</sup> (1), +/T<sup>Tow20</sup> (2), +/T<sup>Tow24</sup> (3), +/T<sup>Tow15</sup> (4), +/T<sup>Tow28</sup> (5), +/T<sup>Tow20</sup> (6), and +/+ (7). The two nonallelic wild-type fragments detected with the Tu89 probe (x, y) and the two t-specific bands (ta, tb), are shown. C, Alleles of the T66 loci detected in TaqI-digested DNA. The wild-type band (+) is shown. The genotypes represented in each lane are T<sup>Tow6</sup>/T<sup>Tow6</sup> (1), +/T<sup>Tow26</sup> (2), T<sup>Tow16</sup>/T<sup>Tow16</sup> (3), +/T<sup>Tow15</sup> (4), +/T<sup>Tow28</sup> (5), +/T<sup>Tow21</sup> (6), T<sup>Tow10</sup>/T<sup>Tow19</sup> (7), +/T<sup>Tow29</sup> (8), +/+ (9) of the inbred C57BL/6 strain, and +/+ (10). The two t-specific bands actually represent three loci detected by means of the Tu66 probe (J. Schimenti and L. M. Silver, unpublished observations). The upper band represents the T66A-a locus (ta), and the lower band is a doublet representing the T66B-a (tb) and T66C-a (tc) loci. The extra band present in lane 9 is another wild-type band in C57 DNA. This band does not comigrate with the nearby t-specific band.

homzygous T<sup>Tow32</sup> males are fertile, whereas males homozygous for a complete t haplotype are sterile. Third, mapping data obtained from a six-point cross (with the markers T48, T, if, Hba-4ps, Acry-1, and H-2; shown in fig. 1) indicate that the T<sup>Tow32</sup> haplotype is not associated with the distal inversion characteristic of complete t hap-
lotypes (see fig. 1); and, as a consequence, normal recombination occurs within this region of the genome in heterozygous (+/t\textsuperscript{Tuw32}) mice. These results indicate that \textit{t}\textsuperscript{Tuw32} is a partial \textit{t} haplotype associated only with the proximal portion of \textit{t} DNA present in complete \textit{t} haplotypes.

Unexpectedly, the original \textit{t}\textsuperscript{Tuw32} chromosome was found in association with "\textit{t}-like" alleles at several loci within the freely recombining distal \textit{t}-complex region (including \textit{Hba-4ps}; [fig. 3, lane 3], \textit{H-2K} [Nižetić et al. 1984], and the \textit{E-alpha} gene of the MHC [Dembic et al. 1984]). To further investigate the relationship between the \textit{Hba-4ps} locus associated with the original \textit{t}\textsuperscript{Tuw32} chromosome and that characteristic of complete \textit{t} haplotypes, we used a frequently cutting restriction enzyme to detect and compare multiple restriction sites within this locus. A total of 14 \textit{BstNI} restriction fragments were identified within a 1.6-kb region that has been subcloned and used as a probe of genomic DNA from different animals (details in fig. 5). Five \textit{t} haplotypes were analyzed and found to be associated with an identical restriction pattern (fig. 5, lanes 4, 5, 7, and 8). DNA samples from six inbred strains were also analyzed at the \textit{Hba-4ps} locus and found to be identical to each other (fig. 5, lanes 1–3). However, the wild-type pattern differs from the \textit{t}-haplotype pattern by the loss and gain of three separate fragments (fragments \textit{h}, \textit{k}, and \textit{n} are \textit{t} specific, whereas fragments \textit{a}, \textit{e}, and \textit{i} are wild-type specific). The data indicate that at least three base substitutions must have occurred to cause the divergence of the wild-type and \textit{t}-haplotype restriction patterns. DNA from a homozygous \textit{t}\textsuperscript{Tuw32} animal associated with a \textit{t}-specific \textit{Hba-4ps TaqI} fragment was analyzed after digestion with \textit{BstNI}. As shown in figure 5, lane 6, the \textit{BstNI} restriction pattern observed with \textit{t}\textsuperscript{Tuw32} DNA is identical to that observed with wild-type DNA.

**Discussion**

In the present paper we have described a comprehensive molecular analysis of 35 independent \textit{t} haplotypes that are direct descendents of wild mice of two species—\textit{Mus domesticus}, trapped in North America (New York, Michigan, and Montana), South America (Chile), Western Europe (Great Britain, France, Spain, Germany, and Italy), Africa (Egypt), and the Middle East (Israel), and \textit{M. musculus}, trapped in Eastern Europe (Poland, the USSR, and Czechoslovakia). The expression of at least five distinct \textit{t}-haplotype-encoded polypeptides and the presence of at least 10 distinct restriction fragments (with 20 restriction sites representing 112 nucleotides) was determined for each \textit{t} haplotype analyzed.

The most significant finding of the present study is the failure to detect any polymorphisms within a minimum of 112 nucleotides from each of 33 complete \textit{t} haplotypes. This continues to hold true in our ongoing studies of these \textit{t} haplotypes by means of additional restriction enzymes. This finding is surprising because direct sequence comparisons of wild-type and \textit{t} DNA have demonstrated a divergence on the order of 1%–1.4%, indicating that \textit{t} haplotypes and wild-type forms of chromosome 17 diverged from a common ancestor ≥1–2 Myr ago, prior to the wild-type split into \textit{M. domesticus} and \textit{M. musculus} lines (Frischauf 1985; Willison et al. 1986). Furthermore, the probes used in the present study allow detection of many polymorphisms among different wild-type forms of chromosome 17 (Fox et al. 1985; Herrmann et al. 1986). If \textit{t} haplotypes diverged from each other at the same time that they diverged from the wild-type chromosome, we would expect to find on the order of 37–52
polymorphisms among the 3,696 nucleotides (112 nucleotides $\times$ 33 chromosomes) that we examined.

One interpretation of these results is that all complete $t$ haplotypes are descendents of a single ancestral chromosome that existed at a much more recent time in evolution, subsequent to the divergence of $M.\ domesticus$ and $M.\ musculus$, an event believed to have occurred $\geq$1 Myr ago. To explain the presence of $t$ haplotypes in both $M.\ domesticus$ and $M.\ musculus$ according to this interpretation, one would have to postulate one or more recent introgressions of the chromosome from $M.\ domesticus$ to
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*M. musculus*, as first suggested by Dunn and his colleagues (Dunn and Bennett 1971; Dunn et al. 1973). This direction of postulated introgression is based on the observation that \( t \) haplotypes are much more diversified in *M. domesticus* than in *M. musculus* (Klein et al. 1984). However, the flow of nuclear genes across this hybrid zone is normally retarded. Thus, the introgression of \( t \) haplotypes would represent the only example of nuclear gene flow between species of mice.

An alternative interpretation is that the observed invariability of restriction sites and polypeptides is not an indication of recent divergence but rather an effect of the small number of DNA sites actually examined within each chromosome, coupled with the possibility that many of the \( t \) haplotypes studied can be placed into a smaller number of groups of more closely related chromosomes. This interpretation is supported by the observation that certain other DNA probes differentiate among the \( t \) haplotypes and that some of the "\( t \)-specific" fragments identified by probes used in the present paper are also found in some wild mouse populations free of complete \( t \) haplotypes (M. Kasahara, F. Figueroa, and J. Klein, unpublished data). It is also possible, however, that for unknown reasons some of the \( t \)-specific alleles studied in the present paper are selectively conserved among \( t \) haplotypes. Whatever the explanation, the described invariability, in two species of mice, provides the first direct evidence for the origin of all known \( t \) haplotypes from a single ancestral form. Previous studies provided evidence for groups of related \( t \) haplotypes but did not prove a common origin for all \( t \) haplotypes.

We identified one wild-mouse-derived \( t \) haplotype that is distinct from all others analyzed to date. The \( t^{Tuw32} \) haplotype is a partial \( t \) haplotype discovered in an Israeli population of mice. Partial \( t \) haplotypes are recovered, at a rate of 1 in 500 offspring, as products of rare recombination events in complete \( t \) haplotypes maintained in the laboratory. Their presence is not expected at a high frequency in wild mouse populations since they no longer express the transmission-ratio-distortion phenotype that provides the \( t \)-bearing chromosome with a selective advantage in its own propagation. Further analysis of Israeli mice will be necessary to determine whether (1) \( t^{Tuw32} \) represents the one partial \( t \) haplotype expected in every 500 wild-type \( t \) samples or (2) partial \( t \) haplotypes are unexpectedly present at a higher frequency in this population.

Although the \( t^{Tuw32} \) haplotype allows free recombination in the \( tf-MHC \) region, it was originally derived in association with a number of \( t \)-specific alleles within this region, at the \( Hba-4ps \) locus and several MHC-associated loci. This observation can be explained by a unique situation that appears to exist in Israel, where a number of "\( t \)-specific" alleles are found in association with some non-\( t \) chromosomes present within wild Israeli mice (M. Kasahara, F. Figueroa, and J. Klein, unpublished data). Such alleles at loci within the \( tf-MHC \) region could easily become associated with the partial \( t^{Tuw32} \) haplotype through normal recombination events in nature. These results can be interpreted as evidence for the preservation, in both wild-type Israeli chromosomes and \( t \) haplotypes, of polymorphisms that existed within the ancient population from which \( t \) haplotypes and wild-type forms of chromosome 17 must have diverged.

We performed a higher-resolution comparative analysis of restriction sites present within the \( Hba-4ps \) locus associated with different forms of chromosome 17 and found that the \( t^{Tuw32} \)-associated \( Hba-4ps \) locus appears to be wild type except for a single "\( t \)-specific" *TaqI* restriction fragment. Therefore, in at least this one case, we are able to detect divergence between the Israeli \( t \)-like allele and the allele associated with present-day \( t \) haplotypes.
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