Gene dosage effects on transmission ratio distortion and fertility in mice that carry t haplotypes

LEE M. SILVER
Department of Biology, Princeton University, Princeton, NJ 08544-1014
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Summary

Complete t haplotypes can be transmitted at distorted ratios from heterozygous +/t male mice as a consequence of t^-specific alleles at a series of t complex distorter loci (Tcd-1' through Tcd-4') and a t complex responder locus. Partial t haplotypes that lack the Tcd-2' allele cannot be transmitted at the very high ratios characteristic of complete t haplotypes. The breeding studies reported here tested the possibility that the absence of Tcd-2' could be compensated for by the presence of double doses of other Tcd' alleles. The results indicate that a double dose of Tcd-4' alone will not work, but that a double dose of both Tcd-1' and Tcd-4' can promote a very high transmission ratio in the absence of Tcd-2'. These results suggest that the extent to which transmission ratios are distorted is dependent upon the absolute level of expression of the individual Ted genes. Further studies of genotypic effects on transmission ratio distortion, as well as fertility, lead to the suggestion of a fifth t complex distorter (Tcd-5) locus within t haplotypes.

1. Introduction

Mouse t haplotypes are variant forms of chromosome 17 that can be transmitted at non-Mendelian ratios from heterozygous +/- t males (see Silver, 1985 for a review). This transmission ratio distortion (TRD) results from the genetic interaction of a series of t-specific alleles at loci distributed along the 15 cm region present within a complete t haplotype (Lyon & Mason, 1977; Lyon, 1984). Two types of TRD loci have been identified. The t complex responder (Tcr) locus is defined by its role in determining which chromosome homologue of chromosome 17 will be transmitted at a high ratio; Tcr is the only mammalian locus known to have a haploid-specific effect on phenotype. The t complex distorter (Tcd) loci (Tcd-1, Tcd-2, Tcd-3, and Tcd-4) are defined by their ability to control the absolute level at which transmission ratios are distorted; Tcd loci can act in cis or trans configuration to Tcr. If a male carries one complete set of Tcd' alleles and is heterozygous for Tcr' and Tcr*, the chromosome homologue with Tcr' can be transmitted at frequencies of 95% or greater. If less than a full complement of Tcd' alleles are present, this very high transmission of Tcr' is not observed. The situation is somewhat more complicated because the genetic background of an animal (as well as epigenetic phenomena) can influence the level of TRD expressed with certain t-genotypes (Olds-Clarke & McCabe, 1982; Bennett et al. 1983; Gummere et al. 1986; Demin & Safronova, 1979). Nevertheless, with properly controlled breeding experiments, it has been possible to map and characterize the various TRD loci (Styrna & Klein, 1981; Lyon, 1984; Lyon, 1986; Silver & Remis, 1987; Lyon & Zenithon, 1987).

The relative ‘strength’ of the individually-defined Tcd loci can be gauged through a comparison of TRD values expressed by partial t haplotypes, or combinations thereof, that lack only a single one of the Tcd' alleles. If only Tcd-1' or Tcd-3' is absent, transmission ratios are generally observed at a level intermediate between 50% and the very high values associated with complete t haplotypes (Lyon, 1984; Silver & Remis, 1987). However, if Tcd-2' alone is absent, transmission ratios are reduced to approximately 50%. (Currently available partial t haplotypes do not allow the construction of genotypes that lack only Tcd-4'.) These observations would suggest either that Tcd-2' represents a gene with a more powerful effect on TRD than either Tcd-1' or Tcd-3', or that Tcd-2' actually represents two or more independent genes that have yet to be separated by recombination. The latter is a real possibility since the chromosomal region associated with Tcd-2 is much larger than the regions associated with the other Tcd loci (Silver, 1985).
Lyon (1986) has presented compelling evidence that the same loci involved in the expression of TRD in +/t heterozygotes may also be involved in sterility effects on males doubly heterozygous for two t haplotypes (t*/t*). Interestingly, the strongest effect on sterility is expressed by the Tcd-2 locus, with weaker effects expressed by Tcd-1, Tcd-3, and Tcd-4 respectively (see also Silver & Remis, 1984). Therefore, the Tcd loci appear to have the same relative strengths in the expression of both the TRD and sterility phenotypes.

Although candidate clones for a number of the loci involved in TRD have been obtained (Willison et al. 1986; Rappold et al. 1987; Schimenti et al. 1988), no information is available concerning the mechanisms by which these loci might act. In particular, it is not clear whether each of the Tcd1 loci provides an independent and essential factor necessary for complete expression of the TRD phenotype or whether alleles at different Tcd1 loci might be able to substitute for each other. To distinguish between these possibilities, breeding studies have been performed with mice that lack particular Tcd1 alleles, but have two copies of other Tcd1 alleles. The results of a previous study demonstrated that a double dose of Tcd-4 could compensate for the absence of Tcd-1 in the expression of a very high transmission ratio (Silver & Remis, 1987). This suggests that Tcd-1 and Tcd-4 might have a common mode of action within the context of the TRD phenotype. In the present report, these studies have been extended to determine whether the action of the most powerful Tcd locus – Tcd-2 – can be duplicated with extra doses of Tcd-4 and Tcd-1.

2. Materials and Methods

All mice were bred in our colony at Princeton University. Transmission ratios of T[Or]/t[w8] and T[Or]/t1 males were determined by breeding to wild-type (+/+t) females and scoring tail lengths of progeny. [A short tail indicates the transmission of T[Or], and a normal tail indicates the transmission of t1 or t[w8].] Transmission ratios of t[4s]/t[w8] males were determined by breeding to T/t[w8] females. This cross could result in embryos of four different genotypes – t[w8]/t[w8] embryos die in utero; t[4s]/t[w8] animals are born with a normal tail; and both T/t[w8] and T/t[4s] animals are born tailless. The subset of tailless animals that carry t[4s] can be estimated as equivalent to the total number of animals with normal tails. Applying this estimate, it becomes possible to derive the following equation to obtain a value for the transmission of t[w8] from t[4s]/t[w8] males: (number of tailless offspring – number of offspring with normal tails)/(number of tailless offspring). Transmission ratios of +/t[w8] males were determined by breeding to T/+ females – a short tail indicates the transmission of the + chromosome, and taillessness indicates the transmission of the t[w8] chromosome.

All experimental males were maintained with two healthy outbred females for a period of at least 4 months. Fertility was assessed by two parameters. First, if no offspring were produced during this period, males were deemed sterile. Second, if offspring were born to a particular male, a determination was made of the number of young born per female mate per month. The values obtained with all non-sterile males of the same genotype were averaged together for the final number shown in Fig. 1.

3. Results

(i) Tcd genotypes of experimental animals

Transmission ratio distortion and fertility were studied in experimental mice that carry one of three different t complex genotypes (Fig. 1). T[Or]/t[w8] animals carry two doses of both Tcd-1' and Tcd-4', a single dose of Tcd-3' and Tcr, and no copies of Tcd-2'. T[Or]/t3 animals have an identical complement of TRD genes, as they are currently defined. Finally, t[4s]/t[w8] animals have a double dose of only Tcd-4', single doses of Tcd-1', Tcd-3', and Tcr, and no copies of Tcd-2'.

(ii) Transmission ratios

T[Or]/t[w8] males transmitted their Tcr-bearing chromosome (t[w8]) to 96% (109/114) of their offspring. Transmission ratios determined for seven individual males with this genotype ranged from 93% to 100%. Sibling control animals with a genotype of +/t[w8] showed no significant transmission ratio distortion (58% of 50 offspring received the t[w8] chromosome). The difference between our summed experimental (96%) and control (58%) values was determined by a chi-squared calculation to be highly significant (P < 0.0001).

T[Or]/t1 males transmitted their Tcr-bearing chromosome (t1) to 80% (216/270) of their offspring. Transmission ratios determined for seven males with 11 or more offspring ranged from 63% to 87% (63%, 68%, 79%, 79%, 83%, 85%, 87%). Sibling control animals were not tested directly for TRD, however, a survey of past breeding records over an eight-year period in our colony of non-inbred t1-bearing mice indicates no significant variation from a 50% transmission ratio by any individual male. In addition, Dunn & Glocusko-Waelsh (1951) have reported a transmission ratio of 48% in 745 offspring from T/t1 males. The difference between our summed experimental value of 80% and the Mendelian ratio of 50% is highly significant (P < 0.0001).

On average, t[4s]/t[w8] males transmitted their t[w8] chromosome at less than Mendelian ratios (31%). The indirectly-calculated transmission ratios for 10
males ranged from 0% to 64%. (A total of 124 tailless and 85 normal-tailed animals were born from the cross described in the Materials and Methods.) Clearly, a double dose of Tcd-4' alone cannot compensate for the absence of Tcd-2'.

(iii) Fertility

The fertility of Tt\textsuperscript{Or1}/t\textsuperscript{w8} males is significantly less than that of the two other experimental genotypes studied in this report. Four of the 11 animals tested appeared to be completely sterile, and the other seven produced an average of 1.4 offspring per female mate per month. Both Hammerberg (1981) and Bennett et al. (1983) have reported reduced fertility in Tt\textsuperscript{Or1}/t\textsuperscript{w8} males, and Lyon (1986) has obtained the same result with t\textsuperscript{10}/t\textsuperscript{w8} males which appear to have a Tcd genotype identical to that of Tt\textsuperscript{Or1}/t\textsuperscript{w8}. In contrast, both Tt\textsuperscript{Or1}/t\textsuperscript{1} and Tt\textsuperscript{45}/t\textsuperscript{w8} males showed normal levels of fertility.

4. Discussion

(i) Compensation relationships among Tcd loci

TRD values for Tt\textsuperscript{Or1}/t\textsuperscript{w8} males have been reported in two previous publications. In one case, t\textsuperscript{w8} was transmitted at a ratio of 65% in a sample size of only 20 offspring (Hammerberg, 1981). In the second case, the observed transmission ratio was 87% in a sample size of 46 (Bennett et al. 1983). Although these values are not as high as reported here, in both cases, they were found to be greater than TRD values obtained with control +/+t\textsuperscript{w8} animals. [In the first case, the sample size was too small to provide significant information; in the second case, the calculated difference between experimental and control values as highly significant (P < 0.0001).]

The accumulated data indicate that a double dose of both Tcd-1' and Tcd-4' alleles can compensate for the absence of Tcd-2', and within at least one genetic background (e.g. that present in the mice tested here), Tt\textsuperscript{Or1}/t\textsuperscript{w8} mice can express very high transmission ratios characteristic of heterozygotes for complete t haplotypes. Although a double dose of Tcd-4' alone cannot compensate for the absence of Tcd-2', it can compensate for the absence of Tcd-1' (Silver & Remis, 1987). These data lend support to the view of a Tcd-2 locus that is approximately twice as strong as Tcd-1 or Tcd-4 alone in effecting the TRD phenotype.

One interpretation of the accumulated results is that at least three of the four Tcd\textsuperscript{1} loci characterized to date have a common mode of action in the expression of the TRD phenotype. (Analogous information on Tcd-3 cannot be obtained with the currently available partial t haplotypes.) Alternatively, it is possible that each Tcd locus has a distinct mode of action, and that the underlying cause of TRD is different in animals with different t-genotypes. This latter interpretation is supported by comparative physiological studies on sperm from +/+t mice (Olds-Clarke, 1989; Brown et al. 1989). In either case, the results strongly suggest that the extent to which transmission ratios are distorted is dependent upon the absolute level of expression of the individual Tcd genes.

(ii) The effect of the T\textsuperscript{45} haplotype on TRD

Unexpectedly, we observed that T\textsuperscript{45}/t\textsuperscript{w8} males transmitted the t\textsuperscript{w8} haplotype at a very low ratio of 31%. In fact, Moser & Gluecksohn-Waelsch (1967) also obtained a value of 31% for the transmission of a t\textsuperscript{w8}-like haplotype from +/+t males. Therefore, one interpretation of the result reported here is that the T\textsuperscript{45} haplotype is acting identically to the wild-type chromosome in this particular genotype. However, we have not observed a ratio this low with +/+t\textsuperscript{w8} mice in our colony. An alternative explanation is based on the results of molecular studies which imply that the T\textsuperscript{45} chromosome carries a duplication of the Tcd-4 locus, with one Tcd-4' allele and one Tcd-4* allele (Herrmann et al. 1986). It is possible that this duplication actually reduces the transmission of t\textsuperscript{w8} relative to that observed with +/+t\textsuperscript{w8} animals.

(iii) Evidence for the existence of a fifth Tcd locus

Highly significant differences in the expression of both the TRD and fertility phenotypes were observed in a comparison of Tt\textsuperscript{Or1}/t\textsuperscript{1} males with Tt\textsuperscript{Or1}/t\textsuperscript{w8} males, even though t\textsuperscript{w8} and t\textsuperscript{1} are thought to carry an equivalent set of TRD loci (Fig. 1). Possible explanations for these differences include: (1) genetic background effects; (2) haplotype-specific differences in the Tcr\textsuperscript{1} region, of the type described by Lyon (1988); and (3) an additional distorter/sterility (Tcd-5) locus in the region of t-DNA present in t\textsuperscript{w8} and not in t\textsuperscript{1} (Fig. 1).

Two independent lines of evidence argue in favour of this third hypothesis. First, all haplotypes of the t\textsuperscript{w8} class appear to act differently from haplotypes of the t\textsuperscript{1} class within the context of simple +/+t heterozygotes. To date, five independent examples of t\textsuperscript{w8}-like chromosomes - t\textsuperscript{45}, t\textsuperscript{1}, t\textsuperscript{w8}, t\textsuperscript{452}, and t\textsuperscript{xl} - have been characterized in various experiments (Moser & Gluecksohn-Waelsch, 1967; Bennett et al. 1976; Bennett & Dunn, 1960). Three of these chromosomes - t\textsuperscript{45}, t\textsuperscript{w8}, and t\textsuperscript{452} - have been characterized in molecular studies and appear indistinguishable from each other (Bucan et al. 1987). Four (all except t\textsuperscript{45}) have been characterized in breeding studies which have demonstrated a high degree of variability in transmission ratios expressed by +/+t mice from different colonies as well as individual males within a colony, with values as low as 31%, in a sample size of 1239 offspring, and as high as 63%, in a sample size of 580.
Fig. 1. Dosage effects of Tcd loci on transmission ratio distortion and fertility. The extent of t-DNA associated with each homologue in six different genotypes is shown (Silver & Remis, 1987). The presence of a particular region of t-DNA is indicated by a patterned box — one pattern is used for Tcd loci, a second pattern is used for Tcr, and a third pattern is used for the D17Leh89 region with the proposed Tcd-5 locus. The actual size of genomic regions associated with each locus varies greatly, and is not indicated in this figure. The transmission frequency of the Tcr-bearing chromosome is indicated at the right of each genotype. In addition, for the three compound genotypes, a box is presented with fertility figures: the top fraction represents (number of fertile males)/(total males tested); the lower number represents the average offspring born per female mate per month for non-sterile animals, followed by the standard deviation in this number.

offspring (Moser & Gluecksohn-Waelsch, 1967; Bennett et al. 1976). In contrast, significant departures from Mendelian ratios have not been observed with heterozygotes for either of the two t haplotypes known to be members of the t1 class (Dunn & Gluecksohn-Waelsch, 1951; Styrna & Klein, 1981; unpublished data).

Second, in studies from four different laboratories, reduced fertility was observed with mice that carry p18 in a genotype with either Tcr+ or t1 (both of which carry Tcd-1' and Tcd-4'); Hammerberg, 1981; Bennett et al. 1983; Lyon, 1986; this report). In contrast, normal levels of fertility are observed with Tcr+/t1 males (Fig. 1). Furthermore, in our colony, 91% (42/46) of males homozygous for the t1 haplotype were found to be fertile, and these males generally sired frequent, large litters (unpublished data). Therefore, homozygosity for Tcd-1', Tcd-3' and Tcd-4' is not sufficient significantly to reduce fertility levels.

The simplest explanation for the accumulated observations is that (1) a fifth t complex distorter locus (Tcd-5) exists in the region defined by the D17Leh89 marker (Fig. 1); (2) haplotypes of the t18 class are more susceptible than t1 to genetic background effects on TRD as a consequence of a t-allele at this locus; (3) the Tcd-5' allele is absolutely required for the expression of a very high transmission ratio (over 90%), and (4) the Tcd-5' allele acts within animals homozygous for Tcd-1' and Tcd-4' to reduce fertility. However, further experiments will be necessary to substantiate the existence of Tcd-5. If the data presented here are confirmed, six independent genes will have been defined that play a role in t haplotype effects on sperm function.

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