Physical Mapping of Male Fertility and Meiotic Drive Quantitative Trait Loci in the Mouse t Complex Using Chromosome Deficiencies

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Manuscript received November 19, 1999
Accepted for publication March 10, 2000

ABSTRACT

The t complex spans 20 cM of the proximal region of mouse chromosome 17. A variant form, the t haplotype (t), exists at significant frequencies in wild mouse populations and is characterized by the presence of inversions that suppress recombination with wild-type (+) chromosomes. Transmission ratio distortion and sterility are associated with t and affect males only. It is hypothesized that these phenomena are caused by trans-acting distorter/sterility factors that interact with a responder locus (Tcrt) and that the distorter and sterility factors are the same because homozygosity of the distorters causes male sterility. One factor, Tcd1, was previously shown to be amorphic using a chromosome deletion. To overcome limitations imposed by recombination suppression, we used a series of deletions within the t complex in trans to t chromosomes to characterize the Tcd1 region. We find that the distorter activity of Tcd1 is distinct from a linked sterility factor, originally called tcs1. YACs mapped with respect to deletion breakpoints localize tcs1 to a 1.1-Mb interval flanked by D17AUS9 and Tctex1. We present evidence for the existence of multiple proximal t complex regions that exhibit distorter activity. These studies demonstrate the utility of chromosome deletions for complex trait analysis.

The mouse t complex is a 20-cM region that occupies the proximal portion of chromosome 17 (Figure 1). The t haplotype is a variant form of the t complex, differing from wild type by the presence of four nonoverlapping inversions, three of which occurred in the t lineage (Silver 1993). Studies of North American feral mouse populations revealed a high prevalence of t haplotypes in the wild (Ardlie and Silver 1996; Ardlie 1998). Multiple complex systems are affected by mutations present in t haplotypes. The effects include early developmental lethality, male-specific infertility, and male-specific transmission ratio distortion (TRD; Silver 1985). The lethal nature of t is evident in animals that are homozygous for noncomplementing t haplotypes, whereas males bearing complementing t haplotypes are sterile but females are normal. TRD (the non-Mendelian segregation of t haplotypes to as much as 99% of offspring) occurs in animals that are heterozygous for the wild type (+) and t haplotype (t) forms of the t complex.

Although strongly suppressed by the inversions, recombination between + and t chromosomes does occur, and these rare events lead to recombinant chromosomes that are broadly classified as proximal, middle, or distal partial t haplotypes (Fox et al. 1985; Silver 1985). Partial t haplotypes have been invaluable in the identification and mapping of loci that are involved in sterility and TRD. A locus in the middle of the t complex, referred to as the t complex responder (Tcrt), is intrinsic to the manifestation of TRD and sterility (Lyon 1984). Other loci (Figure 1) that genetically interact with the responder in a trans-active fashion have been mapped to different regions of the t complex and are referred to as distorters (Tcd1, Tcd2, etc.; Lyon 1986). Evidence for three to five distorters has been presented (Lyon 1984; Silver and Remis 1987; Silver 1989). While the different distorters act additively to boost transmission of a Tcrt-containing chromosome, some are more potent in this respect than others; Tcd1 and Tcd2 have the greatest ability to effect TRD (Lyon and Zenthon 1987). This, in essence, defines a complex trait.

Males carrying two complete complementing t haplotypes (t haplotypes carrying different lethal mutations) are invariably sterile. Exploiting partial t haplotypes, Lyon (1984) identified three regions of t haplotypes that cause sterility or decreased male fertility when homozygous (Lyon 1984). Each of these t complex sterility loci (tcs1, tcs2, and tcs3) comapped with a distorter locus, leading Lyon to propose that the distorter and sterility factors are the same (Lyon 1986). She related the processes of TRD and sterility in the following hypothesis, which postulates that there are direct interactions between the responder and distorter factors. In +/t males, the wild-type responder allele (Tcrt) is sensitive to deleterious t haplotype distorters whereas the t haplotype responder (Tcrt) allele is relatively refractory (Lyon 1984, 1986). In heterozygotes, where half of all of the distorters are t haplotype alleles, Tcrt is somehow inactivated, causing sperm bearing this allele...
to be incompetent. In contrast, the Tcrt-bearing sperm remain unaffected and fertilize the majority of the eggs resulting in the high transmission observed. However, the refractory nature of the Tcrt is overcome in the germline of males homozygous for the t haplotype, which contains two doses of the distorters. The consequence is complete sterility.

Herrmann et al. (1999) have identified the Tcrt as a mutated kinase of the smok1 kinase gene family, resulting from the fusion of smok1 with the ribosome S6 kinase 3 (Rps6ak2) (Zhao et al. 1995). Expression of smok1Tcrt is not evident before 22 days postconception, thus coinciding with spermiogenesis. The phosphorylation activity of Smok1Tcrt is about 10-fold less efficient than Smok1. Smok kinases show similarity to the human kinase, MARK2, in their catalytic domain. MARK2 is a Ser/Thr kinase that phosphorylates MAPs (microtubule-associated proteins). Phosphorylation of the MAPs, tau, MAP2, and MAP4 leads to an increase in microtubule dynamics, whereas overexpression of MARK2 promotes microtubule disruption (Drewes et al. 1997). It has been suggested that the distorter and sterility factors act upstream of Smok and that they possibly regulate the activity of Smok1 (Herrmann et al. 1999). Interestingly, the cytoplasmic bridges that are thought to homogenize the cytoplasm of developing sperm are still present when Smok1Tcrt expression is first detected. Since Smok1Tcrt is a cytoplasmic kinase, it is not known how its diffusion into adjacent spermatids is prevented. Nevertheless, it is proposed that the action of the distorters on Smok1 results in increased Smok1 activity that leads to flagellar instability in +-bearing sperm, whereas the action of the distorters on Smok1Tcrt does not deleteriously increase its kinase activity. Therefore, the t-bearing sperm would develop normally (Herrmann et al. 1999).

High-resolution genetic mapping of the distorter and sterility factors has been impeded by the inversions that prevent normal recombination between + and t chromosomes in the t complex region. Since these loci are currently defined by the breakpoints of partial t haplotypes, which are few in number and clustered in regions that appear to promote exchange across and between inversions (Herrmann et al. 1987; Schimenti et al. 1987), the intervals to which the distorter/sterility loci have been localized are quite large. It is therefore possible that the effects of these individual loci are actually manifestations of multiple separate genes. Although none of the distorters have been cloned, candidates for Tcd1, Tcd2, and Tcd3 have emerged. Tcd1 and Tcd3 were proposed to be dynein light chain components of the inner and outer axonemal dynein arms, respectively (Lader et al. 1989; Huw et al. 1995; O’Neill and Artzt 1995; Inaba et al. 1999). Tcd11, a purported transmembrane protein postulated to be involved in the adenyl cyclase/CAMP signaling pathway (Mazarakis et al. 1991), and Hst6, a complex 1-cM locus affecting oolemma penetration and flagellar curvature (Samant et al. 1999) have been identified as potential candidates for Tcd2. However, the Tcd2 region is larger than 10 cM.

Whatever the nature of distorter/sterility factors may be, it appears that Tcd1 (presumed identical to tcs1) is a null allele based upon work with deletions of the...
TABLE 1

**Haplotypes used in this study and their distorter/sterility loci**

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Distortion/sterility loci</th>
</tr>
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<tbody>
<tr>
<td>t(^{41})</td>
<td>D1/ S1 D4 R D3 D2</td>
</tr>
<tr>
<td>t(^{412})</td>
<td>D1/ S1 D4 R D3 D2</td>
</tr>
<tr>
<td>t(^{43})</td>
<td>+ D4 R D3 D2</td>
</tr>
<tr>
<td>t(^{45})</td>
<td>+ D4 R D3 D2</td>
</tr>
<tr>
<td>Tt(^{46})</td>
<td>+ D4 R D3 D2</td>
</tr>
<tr>
<td>t(^{60})</td>
<td>+ D4 R D3 +</td>
</tr>
</tbody>
</table>

The t-haplotypes of the TRD and sterility factors are shown as symbols. D1/ S1 refers to Tcd1/ tcs1 whereas D2, D3, and D4 refer to the combined distortion/sterility factors Tcd2, Tcd3, and Tcd4, respectively. R corresponds to the t-allele of the responder. +, the wild-type allele of the corresponding factor.

proximal region of the t complex (Bennett and Artzt 1990; Lyon 1992). This information on the nature of these alleles affords the ability to use an alternative approach to standard recombination mapping of these loci: deletion mapping. This article describes genetic experiments that employed a series of targeted, nested deletions within the proximal region of the t complex to refine the map location of Tcd1. While a sterility activity could be localized clearly to a small, well-defined interval, the distorter activities associated with Tcd1 appear to be more complicated and genetically separable from the sterility locus. We discuss these results with respect to the hypothesis that the Tcd1 and tcs1 are identical. These experiments demonstrate the power of deletions as tools for dissecting the components of complex traits.

**MATERIALS AND METHODS**

**Mice:** All mice used in this study were maintained at The Jackson Laboratory. Four of the deletions used, D17Aus9\(^{9}\), D17Aus9\(^{10}\), D17Aus9\(^{11}\), and D17Aus9\(^{12}\), were derived from \(\gamma\)-irradiated hybrid (129/ SvJae x BALB/C)F, embryonic stem (ES) cells (Yoo et al. 1997a). At the time of the experiments, mice bearing these deletions had been crossed for one or two generations to C57BL/ 6J (B6). The deletion Del(17)T\(^{11}\) arose spontaneously in B6 (Bilinski et al. 1997), whereas Del(17)T\(^{21}\) was generated by X-ray irradiation of male mice (Lyon 1992). For the purpose of brevity, the deletions will be referred to as S1, 10J, 12J, 13J, 7J, and T\(^{21}\), respectively. The t haplotypes used in this study were maintained in various strain backgrounds. t\(^{41}\) was congenic in B6; t\(^{43}\) was maintained in a mixed background stock into which contributions from strains 129/ SvJ and B6 were introduced. Tt\(^{46}\) and t\(^{60}\) were maintained congenic in the 129/ SvJ background, but crossed one or two generations into B6 at the time they were used in this study. The t stock contained a mixture of B6 and TTF strain backgrounds. Table 1 summarizes the genetic properties of the t haplotypes whereas Figure 2 illustrates the genetic location of the distorters and the deletion breakpoints in relation to a map of the t complex.

**Molecular genotyping:** Southern blotting of restriction enzyme-digested mouse DNA was performed by standard procedures, using alkaline transfer onto nylon membranes (MSI). The probes used in this study were C5-1, a 700-bp EcoRI fragment corresponding to most of the Td1 gene (Lader et al. 1989) and p119A-A, a 3.2-kb EcoRI fragment that hybridizes to both D17Lh11911 and II (Herrmann et al. 1987). Proximal and distal partial t haplotypes were genotyped by PCR fragment length variants at the Tc1 (Morita et al. 1993) and Hba-ps4 (Schimenti and Hammner 1990) loci, respectively. In the former case, an improved primer pair (5'-gacaatcagctctg ttccag-3' and 5'-gacgtgttatcttctgag-3'; Ann Baker, personal communication) was used that yielded a specific product of 600 bp compared to 425 bp in wild type.

**Fertility assay:** Males of the genotype Del/ tcs1 (where “Del” refers to a deletion chromosome) were mated with two B6 females each. Fecundity was expressed as offspring/ female/ month (O/ F/M), asper Lyon (1986) with the following modification. The mean of means for all males, expressed as O/ F/ M, was computed to avoid biasing the result in favor of sterile males. Sterile males were assigned a mean of zero O/ F/ M. Males for the fertility study were selected on the basis of the absence of a tail (5J and 7J) or by PCR analysis of the Ha-ps4 locus (10J, 12J, and 13J). Animals bearing a deletion of Brachyury (T) in trans to wild type typically have a short or kinky tail whereas those with a deletion of T in trans to the t haplotype tct (t complex tail) interaction factor lack a tail. We observed that in the case of 5J, the severity of the tail phenotype was such that a few S1/+ males were tailless. Some of these males were probably included in the fertility assay thus artificially raising the O/ F/ M value. Their contribution cannot be confidently subtracted from the results because tissue was not retained for genotypic verification.

Of the deletions that do not span T, 10J/tcs1 males were created by crossing t\(^{41}\) + (or t\(^{41}\)/ t\(^{43}\)) females to T\(^{46}\)/10J males. Normal-tailed male progeny were tested at the Hba-ps4 locus for the presence of t\(^{41}\) and the appropriate males were selected for the assay. However, the possibility existed that recombination within the male parent would generate normal-tailed siblings—used in the study—which would actually be a recombinant “wild type” in trans to t\(^{41}\). Given the proximity of the deletion to the centromere, we reasoned that this would be an infrequent event. It appears from genetic analysis of males used in the TRD assay that our assumption was wrong and that recombination within this region of the chromosome is sufficiently frequent to have warranted further characterization of the males used in this facet of the fertility assay. Again, this effect may have also skewed the O/ F/ M result upward from its true value.

**TRD assays:** Males were bred to two B6 females, and the transmission of relevant chromosomes was scored in one of two ways. In the case of males bearing deletions of 5J, 7J, and T\(^{21}\), offspring were scored by tail phenotype (T + animals have a short or kinked tail). Alternatively, offspring were genotyped by PCR analysis of the Tc1 or Hba-ps4 loci to detect inheritance of proximal or distal partial t haplotypes, respectively.

**Yeast artificial chromosome analysis:** The Whitehead/MIT820 mouse yeast artificial chromosome (YAC) library (Halidi et al. 1996; Research Genetics, Huntsville, AL) was screened by PCR to identify clones that were positive for D17Tu1, D17Aus9, and Td1. Primer pairs were used to amplify regions that contain simple sequence repeats (D17Tu1, D17Aus9, and D17Lh48) or to amplify nonrepetitive intron sequences (Td1). The primer sequences were Tu1F (5'-gggacagctaaagtcgc-3'), Tu1R (5'-tcctctctcaaggtctgca-3'), Au9F (5'-cagcgtggttcttctgacg-3'), Au9R (5'-ctcctctctgattggcaaat-3'), Tu48F (5'-atcacaagctctgctgca-3'), Tu48R (5'-gctcgtgattctcagcctgca-3'), and 14tex1F (5'-cgcacagctaggtttcaaggtc-3'), and 14tex1R (5'-gacacagcagctagggaaq-3').
Figure 2.—Map of the t-complex deletions and complete/partial t-haplotypes used in this study in relation to the t-complex. The thick, solid bars used to define the deletions signify the known extent of the deletion and the dashed lines indicate the uncertainty in the breakpoints. t-DNA in the complete and partial t-haplotypes is shown as a solid bar, whereas the + DNA is shown as a thin, solid line. In the absence of the Brachury mutation (T), the partial t-haplotype Tt6 is referred to as +Tt6. The dashed line connecting the partial t-haplotypes (Tt6) and T'6 signifies a duplication composed of + and t DNA in that region. Marker abbreviations follow the same convention as in Figure 1. Markers 172 and 195 are abbreviated by dropping “D17Mit.”
Statistical analysis: $\chi^2$ and $P$-values were computed according to the method of Fisher’s exact probability test using the program Fisher2 (written by Kaz Matsuki and freely distributed). The one-tailed probability value was used in all cases.

RESULTS

Deletion mapping of the t-complex sterility 1 (tcs1) locus: The use of deletions as tools to genetically characterize the proximal distorter/sterility locus (Tcd1/tcs1) was first reported by Bennett and Artzt (1990), who suggested that the distorter and sterility activities were genetically separable. Soon afterwards, Lyon discovered that a deletion (T22h) spanning the proximal t complex of wild-type mimicked the t haplotype effects of both Tcd1 and tcs1 (Lyon 1992). These results suggested that a deletion-based strategy could be used to refine their map positions in wild-type chromosomes. Additionally, the same strategy could be a useful genetic test in establishing whether or not TRD and sterility are due to the same locus (Lyon 1986), or to two distinct but closely linked loci. Accordingly, we performed a series of breeding experiments designed to measure whether a series of chromosomal deletions in the proximal t complex elicit distorter or sterility factor activity. Males bearing a complete t haplotype in trans to a deletion of tcs1 are expected to be sterile or subfertile. Such mice would be entirely deficient for tcs1 (assuming the t allele of tcs1 is a true null) and heterozygous for the remaining t haplotype distortion/sterility loci. Except for 7J, the deletions used in this study were derived by irradiation of ES cells, and their breakpoints have been characterized with a number of molecular markers (see Figure 2) (Bilinski et al. 1997; You et al. 1997a; Bergstrom et al. 1998).

Five deletions were placed in trans to the complete t haplotype $t^{e2}$ or $t^{a2}$ (one animal), and several males representing each deletion were tested for fertility. The results of these assays are summarized in Table 2. All 10 13J/t$^{e2}$ males produced offspring with an overall high fecundity (5.2 O/F/M), indicating 13J does not delete tcs1. In contrast, the remaining deletions in trans to $t^{e2}$ resulted in males that, for the most part, were sterile or subfertile as reflected by the average O/F/M for each deletion examined (Table 2, rows one through four). Of those in this group, two 5J/t$^{e2}$ males and one 10J/t$^{e2}$ male had normal fertility. As noted in materials and methods, the fertile 5J males were probably in trans to wild type and not $t^{e2}$. Although taillessness is used as the criterion for whether or not an animal has the T/t genotype, our experience indicates that this assumption must be approached cautiously; occasional tailless animals presumed to be T/t may in fact be T/+ as a consequence of severe manifestation of the Brachyury (short tail) phenotype in T/+ mice (see materials and methods). The fertile 10J males were likely not 10J but wild type due to low-frequency recombination in the 10J/T$^{e2}$ parents (described in materials and methods). From these data we conclude that the deletions 5J, 7J, 10J, and 12J remove tcs1.

By comparing the deletion breakpoints with the results from the fertility tests reported here, the data are consistent with tcs1 residing between D17Aus9 and D17Leh119I (Figure 3). This conclusion is based upon the following analysis. The distal breakpoint of 13J, which does not induce sterility, lies between D17Aus9 and D17Leh48. tcs1 must be distal, rather than proximal to this interval, since 5J and 7J do not extend as far proximally as 13J. Since 10J and 12J remove tcs1, and their distal breakpoints extend past the proximal breakpoint of 7J, we concluded that the distal breakpoints of 10J and 12J would delimit the distalmost boundary of tcs1. These endpoints were refined by analysis of restriction fragment length polymorphisms associated with the D17Leh119I loci of wild-type chromosomes. Using the p119A-R probe described in Herrmann et al. (1987), which recognizes a 2.7-kb band corresponding to D17Leh119I and a doublet at 1.5/1.2 kb corresponding to D17Leh119II, we found that 10J removes D17Leh119I, but 12J does not (data not shown). This indicates that tcs1 is proximal to the D17Leh119I locus.

The location of tcs1 can be further narrowed by considering published observations with respect to the spontaneous deletion T$^{0}$ (Bennett and Artzt 1990). The proximal breakpoint of T$^{0}$ lies in the interval flanked by Ттц1 and D17Leh48, a region that is deleted in 5J, 7J, 10J, and 12J but not 13J. However, when in trans to a complete t haplotype, T$^{0}$ is fertile (Bennett and Artzt 1990). This result constrains the distal endpoint of the tcs1-critical region to between Ттц1 and D17Leh48, wherein lies the proximal breakpoint of T$^{0}$. Therefore, we conclude that tcs1 is bounded proximally by D17Aus9 and distally by Ттц1 (Figure 3).

Physical map of the tcs1-critical region: We screened the Whitehead/MIT820 mouse YAC library to isolate clones that could be used to determine the physical size of the interval flanked by D17Aus9 and Ттц1. Using primers described in materials and methods, we isolated two independent YACs, AP-PTC1 and AP-PTC2 (Figure 3). AP-PTC1 was assayed by pulsed-field gel elec-
Figure 3.—Map position of tcs1 and of the multiple loci that exhibit some distorter activity. The solid bars indicate the extent of each deletion whereas the dashed lines indicate the uncertainty in the location of the deletion breakpoints. Below the deletions the stippled arrows indicate regions with potential distorter activity (see text). tcs1 is shown between Aus9 and tctex1. AP-PTC1 and AP-PTC2 are YACs that are described in the text. The left arm of AP-PTC2 was rescued. From the sequence thus obtained, primers were designed (D17AP1F/R) that were used to screen AP-PTC2 and a third YAC that contains D17Bu1 and D17Aus9, but not D17Leh48 or tctex1. The expected product (155 bp) was observed in both of these YACs (A. Planchart and J. C. Schimenti, unpublished observations). We therefore conclude that the left arm of AP-PTC2 is not chimeric. Marker abbreviations follow the same convention as in Figure 1. Markers 164, 18, 171, (48, 57, 195), 156, and 112 are abbreviated by dropping “D17Mit.” Marker 122 is abbreviated by dropping “D17Leh.”

phoresis (on a Bio-Rad CHEF apparatus) at 0.9 Mb and it includes D17Bu1, D17Aus9, and D17Leh48. AP-PTC2 was similarly sized at 1.1 Mb and includes the same markers. In addition, we observed a weak hybridization signal on a Southern blot of AP-PTC2 with the Tctex1-specific probe C5-1; thus it also appears to contain part of the Tctex1 gene family (data not shown).

Southern analysis of EcoRI-restricted 5J, 7J, 10J, and 12J genomic DNA with C5-1 revealed that the Tctex1 gene complex is removed in all four deletions. However, a similar analysis of 13J detected an intact Tctex1 gene complex (data not shown).

Effect of the deletions on TRD: TRD of t haplotypes is strictly dependent upon the t complex responder (Tcrt) being present in the heterozygous state. The various distorters act additively to raise transmission of Tcrt from 15% to nearly 100% as their dosage increases from none to all three to five genes (Lyon 1984; Silver and Remis 1987). In experiments with partial t haplotypes, Tcd1 was defined as a locus that significantly boosts transmission of a Tcrt-bearing chromosome (Lyon and Mason 1964). Lyon found that a deletion of the Tcd1 region in wild-type chromosomes had a similar effect (Lyon 1992). We therefore surveyed our collection of deletions in the proximal t complex to determine what interval(s) would boost Tcrt transmission when deleted and thus refine the Tcd1-critical region on the basis of the location of deletion breakpoints. The deletions were bred in trans to three different Tcrt-containing, Tcd1-lacking partial t haplotypes: t6, t6, and t6. Transmission of the partial t haplotypes in these compound heterozygous males was then evaluated in crosses to wild-type
mice. The results of these studies are summarized in Table 3.

In the first assay, the deletions 5J and 7J were capable of significantly increasing the transmission of t₆ from 73% (in +/+ t₆ males) to over 97% (χ² = 11.9, P ≤ 0.01) and 99% (χ² = 34.5, P ≤ 0.01), respectively. These results suggest that Tcd1 is located distal to D17Aus9, a marker that is centromeric to the 7J proximal breakpoint (Figure 3).

The second assay yielded conflicting results. In this experiment, transmission of the distal partial haplotype t₄₆, which contains Tcrt and the distorters Tcd2, Tcd3, and Tcd4, was expected to increase substantially when placed in trans to a deletion spanning Tcd1. 7J and 10J significantly boosted transmission of t₄₆ from 56 to 66% (χ² = 5.9, P ≤ 0.02) and 71% (χ² = 19.9, P ≤ 0.02), respectively, whereas 5J, 12J, and 13J did not elevate transmission of t₄₆ [in fact, 13J significantly decreased transmission of t₄₆ (χ² = 3.7, P ≤ 0.05)]. The failure of 5J to demonstrate Tcd1-like activity in this assay was surprising in light of the finding that it clearly enhanced the transmission of t₆. Since the region of deletion overlap in 7J and 10J is also shared by 5J, it is possible that other loci within the deletions (both proximal and distal to the 5J breakpoints) or genetic background effects are responsible (see discussion). In the absence of the 5J results with t₆, the data would indicate that Tcd1 resides between the distal breakpoints of 12J and 10J, defined by the markers D17Mt172 and D17Lh119I. This is adjacent to the interval to which tcs1 maps.

The final assay measured the ability of deletions to elevate the transmission of t₆[1], a middle partial haplotype that is transmitted at low levels in t₆[1]/+ males (17.8%). Again, the assay yielded conflicting results. Although 7J raised transmission of t₆[1] significantly (to 39%, χ² = 63.4, P ≤ 0.01), none of the other deletions tested did so (5J: 23.5%, χ² = 2.0, P = 0.1; 10J: 22%, χ² = 0.4, P ≥ 0.1; 13J: 17%, χ² = 0.1, P ≥ 0.1, and T²₆H: 18%, χ² = 0.0, P ≥ 0.1). The results obtained with T²₆H, a deletion previously shown to increase transmission of t₆ and t₃ (a low-ratio partial t haplotype similar in structure to t₆[1]), were particularly surprising. These results, however, are consistent with the observations of Lyon et al. (2000), who observed that three deletions markedly elevate transmission of t₆ but fail to boost transmission of t₃. Thus, it is possible to interpret these results from the standpoint that low-ratio partial t haplotypes, for some unknown reason, appear not to be sensitive tools for measuring Tcd1 loss of function. Complicating the issue is that the effect can be variable; here we show that 7J boosted t₆[1] transmission, whereas our results with T²₆H did not replicate the earlier studies showing the ability of this chromosome to elevate t₃ transmission. As discussed later, potential explanations for these incongruous results include genetic background effects, other t complex loci affected by the various deletions, and erroneous predictions of TRD from studies of the ability of Tcd1-containing partial t haplotypes to distort transmission of a Tcrt-containing chromosome.

Given the variability of TRD assays on deletions involving partial t haplotypes other than t₆, a judgement as to whether a deletion removes Tcd1 is somewhat subjective. One way to interpret the results is to consider any deletion that is positive for Tcd1 “activity,” in any one of the three assays, to have removed Tcd1. Using this criterion, 5J, 7J, and 10J are deemed to be deleted for Tcd1, whereas 12J and 13J are not. These conclusions are tentative in that 12J and 13J were not evaluated in trans to t₆; 12J excluded, this places Tcd1 in a region commonly deleted by 5J, 7J, and 10J, defined on the proximal end by D17Aus9 and on the distal end by D17Lh66E(I). If further tests confirm the lack of distorter activity in 12J, this would greatly refine the location of Tcd1 to the interval between D17Mt172 and D17Lh66E(I).

DISCUSSION

Physical boundaries defining tcs1: Lyon’s analysis of the T²₆H deletion established that tcs1 (thought to be
identical to Tcd1) was a loss-of-function mutation. At that time, T<sup>2H</sup> was known to delete D17L<sub>eh48</sub>. In conjunction with t<sup>ε</sup>, which genetically lacks Tcd1/tcs1, this deletion tentatively assigned Tcd1/tcs1 to the interval flanked proximally by D17L<sub>eh48</sub> and distally by the Tctex1 gene family (Lyon 1992). However, later work unrelated to Tcd1/tcs1 mapping (deletion analysis of the hot locus) refined the proximal breakpoint of T<sup>2H</sup> to include D17Mit19 (Bergerstrom et al. 1998), resulting in a drastic decrease in the resolution of the map position of Tcd1/tcs1. Our results, however, demonstrate that the interval of tcs1 activity can be confidently defined by taking into account only the region of DNA that is commonly deleted by 5J, 7J, 10J, and 12J. Consequently, the tcs1 locus is flanked proximally by D17Aus9 and distally by the Tctex1 gene family.

The order of D17Aus9 and D17L<sub>eh48</sub> was established previously (Bilinski et al. 1997). Tctex1 was mapped between D17Aus9 and D17Tu1 by genetic means (Himmelbauer and Silver 1993) but this is not consistent with our observations. For example, 13J, which is the smallest and most proximal deletion we examined, deletes D17Tu1 and D17Aus9 but does not delete Tctex1 (see results), thereby establishing that Tctex1 is telomeric to both of these markers. The order of the markers that define the tcs1 interval becomes D17Aus9/D17L<sub>eh48</sub>-Tctex1. The physical size of this region is not greater than 1.1 Mbp as defined by YAC AP-PTC2 (Figure 3). In addition, YAC AP-PTC1, which contains D17Tu1, D17Aus9, and D17L<sub>eh48</sub> but not Tctex1, places an upper limit of 200 kb on the interval flanked by D17L<sub>eh48</sub> and Tctex1 (Figure 3).

Candidate genes for tcs1: Several reports have suggested that Tctex1 is a candidate for Tcd1/tcs1 on the basis of three observations. The first is its eightfold overexpression at the level of the mRNA in the testis of t<sup>t</sup>/<t<sup>t</sup> males (Lader et al. 1989). Second, mutations within the A<sup>α</sup> and B<sup>α</sup> forms have been found (the family consists of four genes per haploid genome that are grouped into two classes, A and B). A<sup>α</sup> members have the T to A transversion in the start codon that is assumed to abolish translation, whereas B<sup>ε</sup> members have multiple amino acid substitutions, some of which are postulated to be drastic (O’Neill and Artzt 1995). Finally, the localization of the Tctex1 protein to the flagellum of mouse sperm (O’Neill and Artzt 1995) and its identification as a light chain of dynein (King et al. 1996) that functions as a component of the Chlamydomonas flagellum inner arm I (Harrison et al. 1998) are consistent with its potential role in sperm function. When we examined 5J, 7J, 10J, 12J, and 13J, we observed a correspondence between a deletion’s ability to elicit male sterility in trans to a complete t haplotype and the loss of the gene family, thus seeming to further implicate Tctex1. However, this argument is not supported by previous work of Bennett and Artzt (1990). They reported that T<sup>ε</sup>, a deletion that removes Tctex1<sup>+</sup> (Figure 3) in trans to a complete t haplotype, did not result in male sterility. Taken with our data, this places tcs1 between—but not including—D17L<sub>eh48</sub> and Tctex1.

The apparent loss of function of Tctex1 in haplotypes is thought to be due to abolished expression of Tctex1<sup>α</sup> (A<sup>α</sup>) and to expression of a mutant form of Tctex1<sup>ε</sup> (B<sup>ε</sup>) that results in a protein with an aberrant function (O’Neill and Artzt 1995). However, the T<sup>ε</sup> results and the fact that Tctex1 is expressed at relatively high levels in many other tissues—thus implying a role in other systems not related to sperm development—suggest that the role of Tctex1 in t haplotype-mediated male sterility remains unresolved (Lader et al. 1989; Bennett and Artzt 1990; King et al. 1996). A gene knock-out approach would be a conclusive test of Tctex1’s involvement in tcs1-mediated male sterility. Since Tctex1 has been reported to be a four-gene family per haploid genome, multiple gene disruption events or a Cre-lox approach would be required.

Intracytoplasmic sperm injection studies have demonstrated that the sperm from a sterile complete t haplotype male (t<sup>ε</sup>/t<sup>ε</sup>) are capable of fertilizing the egg (Kuret et al. 1996). Thus, there appear to be no genetic abnormalities that would prevent t-bearing sperm from contributing to the formation of a zygote except the inability to swim to, find, bind to, or fuse with the egg. In essence, whatever the mutation is, it must by necessity fall into one of four categories: motility, chemotaxis, binding/penetration, or fusion. Alternatively, proteins that regulate the expression of genes encoding necessary components of these four systems could also be affected. Only the first, motility, would be directly compromised by flagellar abnormalities. Our examination of several of the 5J/t<sup>ε</sup> sterile males did not reveal any visual abnormalities in the structure of the sperm or any departure from normal motility and/or progression (J. Farley, J. Sztain and A. Planchart, unpublished observations). This suggests that the effect of the deletion is to disrupt a component of one of the other three mechanisms.

Physical boundaries defining Tcd1: The ability to assign the location of tcs1 to a narrow interval of the t complex is contrasted by the ambiguity with which the deletions define a region affecting the transmission ratio of several tester partial t haplotypes. The effect of 5J on the transmission of t<sup>ε</sup>, t<sup>0</sup>, and t<sup>ε</sup> and the effect of T<sup>2H</sup> on the transmission of t<sup>ε</sup> demonstrate this point. Whereas 5J, 7J, and T<sup>2H</sup> overlap extensively and all three distort the transmission of t<sup>ε</sup>, only 7J showed the ability to boost the transmission of the other partial t haplotypes (T<sup>2H</sup> was not tested in trans to t<sup>ε</sup>). One possible explanation for this observation is the genetic background of the deletion-bearing animals. Multiple studies have shown that background factors on the homologous chromosome 17, as well as on nonhomologous chromosomes, can affect the transmission of tester partial t haplotypes (Bennett et al. 1983; Gummer et al. 1984).
1986). If background is a cause of the observed effects, it is interesting that TRD appears to be much more sensitive to its effects than the male sterility-inducing counterpart. This may be an important clue in validating the hypothesis that the distorter and sterility factors are the same when candidate genes are uncovered and characterized.

An alternative explanation for the seemingly contradictory results obtained with t$^{D61}$ (and t$^d$) is suggested by the differences in the distal breakpoints of 5J, 7J, and T$^{12H}$ and a previous report on the effect of T$^{10b}$ on the transmission of t$^c$ (Lyon 1992). T$^{10b}$, a deletion that spans the Tme locus and is therefore more distal in extent than any of the other three deletions, boosts the transmission of the partial t haplotype t$^{6J}$ (which is very similar to t$^{D61}$ with respect to the distorters present within it; see Table 1). Significantly, T$^{10b}$ does not cause sterility in trans to a complete t haplotype (Lyon 1992) and thus it appears not to overlap with 10J or 12J. Since 10J significantly boosts the transmission of t$^c$, it is reasonable to suggest that T$^{10b}$ boosts the transmission of t$^{6J}$ by a mechanism that involves a distal factor not deleted by 10J. However, T$^{12H}$ in one study distorted the transmission of t$^{6J}$ to a greater extent than was observed with T$^{10b}$ (Lyon 1992). One possibility may be that there are multiple loci in the distal region (defined by D17M it48 and qk) capable of exerting a distortion effect and that these distal distortion loci differentially interact with the two partial t haplotypes t$^{D61}$ and t$^d$. Tcd4, previously defined by Silver and Remis (1987) and shown by partial t haplotype mapping to reside close to the region of the distal breakpoints of T$^{10b}$ and 7J, is a candidate for one of the postulated distal distortion loci observed with these deletions. D17M it48 and the qk locus grossly define this region. It is possible that rather than being a single locus, Tcd4 is actually several loci. Accordingly, the interaction between these postulated distal loci and the partial t haplotypes t$^{D61}$ and t$^d$ would be such that if more than one of the distorter loci is affected (such as would be the case with 7J and T$^{10b}$), distortion of t$^{D61}$ and t$^d$ would be observed. But if only one is affected (5J and T$^{12H}$) only the transmission of t$^{6J}$ would be distorted.

It is possible that 10J boosts the transmission of t$^c$ via a factor that is located in the proximal t complex in a region flanked by D17M it245 and D17M it19 (Figure 3). This hypothesis is supported by Lyon et al. (2000) in which they report the observation of a distortion effect that maps between D17M it164 and D17L it19, a region that appears to overlap with the interval flanked by D17M it245 and D17M it19. If indeed there is distortion activity that maps between D17M it245 and D17M it19 this would extend the proximal boundary of the t complex, currently defined up to D17Tu1 (Howard et al. 1990).

If there are distal and proximal loci that affect the transmission of t$^{D61}$ and t$^c$ by 7J and 10J, respectively, then the transmission of t$^c$ by 5J would have to be due to elements commonly deleted by 5J and 7J but not 10J or T$^{10b}$. D17L fh66EI and D17L fh66EII define such an interval. These loci, which have been previously characterized as part of a large (650 kb) duplicated element in wild type (Herrmann et al. 1987), define the distal breakpoint of 10J and the proximal breakpoint of T$^{10b}$ (Figure 3). Interestingly, Lyon et al. (2000) similarly define a region of distortion activity flanked by D17L fh66E and T (Figure 3).

Another question that is raised by our results is whether or not partial t haplotypes that are not of the long distal type (such as t$^d$) are good reporters of distortion activity when used in conjunction with deletions. One manner in which this could be resolved would be to place every deletion in trans to t$^6$ and determine the transmission of t$^6$ as a function of the deletion.

Are tcs1 and Tcd1 the same factor? Central to the hypothesis of TRD and male-specific sterility is the proposal that the distorter and sterility factors are the same. Accordingly, whether a male mouse exhibits TRD or is sterile depends upon the number of distorters and their consequent action upon the responder locus. We propose that in the case of tcs1 and Tcd1, the two appear to be different on the basis of the following set of observations. First, 12J did not distort but did induce male sterility. Second, 13J did not affect male fertility but did appear to have an effect on the transmission of t$^c$. Third, T$^{10a}$ and T$^{10b}$ have been reported to have no effect on male fertility but to affect distortion of partial t haplotypes. Neglecting a potential effect of genetic background on the activity of 12J with regard to TRD, the simplest explanation for these observations is that there are no distortion-related loci within the region defined by the 12J deletion. This reduces sterility and distortion effects to separate regions of the chromosome and places tcs1 between the distal breakpoint of 13J and the proximal breakpoint of T$^{10a}$, a 1.1-Mb region of the genome that appears to be devoid of distortion activity.

The sterility and TRD associated with t haplotypes are classic complex traits. Up to six TRD (Tcd1, Tcd1a, Tcd2-5) and three sterility loci (tcs1-3) have now been implicated in these phenomena. They can be considered quantitative trait loci (QTL), since each contributes in an additive way to complete manifestation of the phenotypes. Mapping of QTL in mice is a notoriously difficult task, generally requiring the construction of congenic lines for each QTL involved in a trait. The ability to generate deletions at preselected loci within the t complex has expanded the repertoire of tools useful in addressing the complexities of such t-associated phenomena as male sterility and TRD. The deletions described in this work have been successfully employed as “probes” for measuring the impact of limited regions of the proximal t complex in these two phenomena. Accordingly, these deletions have significantly narrowed the genomic interval where tcs1 resides. Whereas it is eminently clear that further analysis of the t complex
LITERATURE CITED


Lyon, M. F., 1986 Male sterility of the mouse t complex is due to homozygosity of the distorer genes. Cell 44: 357–363.


