Impact of trisomy on fertility and meiosis in male mice

M.Davisson1, E.Akeson, C.Schmidt, B.Harris, J.Farley and M.A.Handel

The Jackson Laboratory, Bar Harbor, ME, USA

1To whom correspondence should be addressed at: The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA. E-mail: muriel.davisson@jax.org

BACKGROUND: Chromosomal abnormalities frequently are associated with impairment or arrest of spermatogenesis in mammals but are compatible with fertility in female carriers of the same anomaly. In the case of trisomy, mice have extra genomic DNA as well as the chromosomal abnormality, usually present as an extra, unpaired chromosome. Thus, impairment of spermatogenesis in trisomic males could be due to the presence of extra genomic material (i.e. triplicated genes) or to the chromosomal abnormality and presence of an unpaired chromosome in meiosis. METHODS: In this study, fertility and chromosomal pairing configurations during meiotic prophase were analysed in male mice trisomic for different segments of the genome. Four have an extra segmental or tertiary trisomic chromosome—Ts(1716)65Dn, Ts(1016)232Dn, Ts(1217)4Rk and Ts(417)2Lws—and one has the triplicated segment attached to another chromosome—Ts(16C-tel)1Cje. Ts(1716)65Dn and Ts(16C-tel)1Cje have similar gene content triplication and differ primarily in whether the extra DNA is in an extra chromosome or not. RESULTS: The presence of an intact extra chromosome, rather than trisomy per se, is associated with male sterility. Additionally, sterility is correlated with a high frequency of association of the unpaired chromosome with the XY body, which contains the largely unpaired X and Y chromosomes. CONCLUSIONS: Intact extra chromosomes disrupt spermatogenesis, and unpaired chromosomes establish a unique chromatin territory within meiotic nuclei.

Key words: trisomy/male fertility/synaptonemal complex/meiotic pairing/spermatogenesis

Introduction

Chromosomal abnormalities often are associated with sterility in male mice and humans. The laboratory mouse has been a valuable and frequently used model system for studying this phenomenon. Many studies have addressed fertility effects of chromosomal rearrangements, such as reciprocal translocations and Robertsonian chromosomes (e.g. Burgoyne and Baker, 1984; Forejt, 1985; Redi et al., 1985; Tettenborn and Gropp, 1990; Jaafar et al., 1993; Peters et al., 1997; Merico et al., 2003). Less attention has been devoted, however, to determining the gametogenic effects of trisomy (e.g. De Boer and Branje, 1979; Mahadevaiah and Mittwoch, 1986; Setterfield and Mittwoch, 1986; Johansson and Winking, 1998), a chromosomal abnormality in which a single unpaired chromosome is present in a background of normally synapsed autosomal chromosomes during meiosis.

The purpose of this study was to investigate the effects of trisomy on fertility and meiotic prophase dynamics in male mice. We analysed five different segmental trisomies, collectively involving the triplication of segments of five different chromosomes. We examined the fertility effect of trisomic gene content versus the presence of an extra, unpaired trisomic chromosome using male mice segmentally trisomic for similar segments of chromosome 16 (Chr 16), present in one case as an extra chromosome Ts(1716)65Dn (hereafter Ts65Dn) and in the other attached to an unrelated autosomal chromosome Ts(16C-tel)1Cje (hereafter Ts1Cje). The clinical relevance of this study derives from the fact that these trisomies are recognized models for human Down’s syndrome (DS), a complex contiguous gene syndrome resulting from trisomy of Chr 21. These mouse models of DS—Ts65Dn (Davisson, 2005), Ts1Cje (Davisson, 2005) and Ts232Dn (unpublished)—are trisomic for more than half of the orthologues of human Chr 21 genes triplicated in DS. Thus, the results of this study have implications for reproductive health of males with DS. The other trisomies studied involve entirely different segments of the genome. Together, these models provide a unique opportunity to examine the effects of trisomy on male spermatogenesis and male fertility.

Despite numerous investigations, the causes of infertility in male carriers of chromosomal aberrations are not understood; however, the interruption of spermatogenesis is most frequently observed to be during the meiotic stage. From this finding, the various hypotheses put forward to explain chromosomally induced male sterility are based on meiotic behaviour of chromosomes—pairing, synopsis and disjunction. These posit that sterility can be due to the presence of unpaired chromosomal elements (Miklos, 1974) that activate a meiotic checkpoint (Odorisio et al., 1998) or that aberrant chromosomes interfere with spermatogenic X-Chr inactivation.
(Lifschytz and Lindsay, 1972; Forejt, 1985), although there is no empirical evidence for the latter hypothesis (McKee and Handel, 1993). Alternatively, the disruption of functional nuclear territories that regulate cellular events (Cremer and Cremer, 2001) could be caused by the association of a structurally abnormal autosome with the XY body, which contains the heteromorphic and largely unpaired sex chromosomes (Handel, 2004). For example, recent studies have documented the disruption of typical nuclear spatial territories in spermatocytes of subfertile males carrying chromosomal alterations (Garagna et al., 2001) and modification of unpaired elements (Baarends et al., 2005; Turner et al., 2006).

In this study, we tested the hypothesis that it is the presence of an extra chromosome rather than trisomic gene content that causes male infertility. The analysis of mouse models of DS, involving either an extra chromosome or an extra (trisomic) gene content attached to another chromosome, validated this hypothesis. We also found that extra, unpaired chromosomes associate with the sex chromosomes, suggesting interference with the maintenance of specific nuclear domains. However, an equivalent triplicated segment of chromosome attached to fully paired chromosomes does not preferentially associate with the sex chromosomes and does not cause male infertility. Thus, trisomy-related male infertility is due to the presence of an extra chromosome rather than to altered gene dosage.

Materials and methods

Mice

Male mice used in this study were all trisomic (triplicated) for segments of the mouse genome. Table I and Figure 1 identify the trisomies, their abbreviated symbols and their genetic content, to the extent it is known. Throughout the article, these chromosome aberrations and the mice carrying them are abbreviated by omitting the parentheses and chromosome numbers from the symbols (Table I, column 2). In Ts65Dn, Ts232Dn, Ts2Lws and Ts4Rk mice, the trisomic segments are present as an extra chromosome. In contrast, in Ts1Cje mice, the trisomic segment is translocated to distal Chr 12; no Chr 12 genes are triplicated, although a very small undefined distal telomeric segment of Chr 12 is likely monosomic (Sago et al., 1998). Ts65Dn, Ts232Dn, Ts2Lws and Ts1Cje are maintained on a mixed genetic background, by trisomic segment is translocated to distal Chr 12; no Chr 12 genes are present as an extra chromosome. In contrast, in Ts1Cje mice, the trisomic segments

<table>
<thead>
<tr>
<th>Trisomy symbol/Abbreviation</th>
<th>Location: extent</th>
<th>Gene content (number of genes)</th>
<th>Size (Mb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts(17;16)65Dn</td>
<td>T65Dn</td>
<td>Chr 16: from proximal to Mrpl199 to distal tel&lt;br&gt;Chr 17: from cenproximal to Sod2</td>
<td>−124</td>
<td>−16</td>
</tr>
<tr>
<td>Ts(16C-tel)1Cje</td>
<td>T1Cje</td>
<td>Chr 16: from just distal to Sod1 to distal tel&lt;br&gt;Chr 16: from just proximal to Pit1 to distal tel&lt;br&gt;Chr 10: from cen to 10A2</td>
<td>−71</td>
<td>−10</td>
</tr>
<tr>
<td>Ts(10;4)232Dn</td>
<td>T232Dn</td>
<td>Chr 16: from cen to 12A1&lt;br&gt;Chr 17: from 17E2 to tel</td>
<td>−172</td>
<td>−43</td>
</tr>
<tr>
<td>Ts(12;17)4Rk</td>
<td>T4Rk</td>
<td>Chr 12: from cen to 12A1&lt;br&gt;Chr 17: from 17E2 to tel</td>
<td>−131</td>
<td>−26</td>
</tr>
<tr>
<td>Ts(4;17)2Lws</td>
<td>T2Lws</td>
<td>Chr 4: from cen to 4A4&lt;br&gt;Chr 4: from E4 to tel</td>
<td>−127</td>
<td>−39</td>
</tr>
</tbody>
</table>

cen, centromere; Chr, chromosome; tel, telomere.

Gene content and Mb size are based on NCBI/Ensembl Build 34 and the Mouse Genome Database (MGI, 2005). Gene and Mb numbers for Ts4Rk and Ts2Lws are based on the G-band locations of the chromosomal breakpoints and hence are very approximate.
movement in a forward, linear direction at a speed of 50 μm/s. In addition, the sperm head morphology was analysed from a smear made from 50 μl of sperm suspension on a slide. Two slides were made from each animal, air-dried, fixed in 1% acetic acid in 100% ethanol for 2–3 min, stained for 5–6 min in 0.5% eosin yellow in distilled water, washed three times in 70% ethanol and examined with a 40x objective by counting 100 sperm or the number available per slide.

**Testicular histological and synaptonemal complex analyses**

For each mouse analysed, both tests were removed and weighed; the left testis from each animal was used for the analysis of pachytene spermatocytes and the right testis for histological analysis. For histological analysis, the testis was fixed in JB4 fixative (20 ml of 10% glutaraldehyde, 10 ml of 10% paraformaldehyde, 10 ml of cacodylate buffer, 60 ml of distilled water; cacodylate buffer: 42.8 g of cacodylate acid sodium salt dissolved in 100 ml of distilled water, pH 7.8, brought to 200 ml with distilled water) provided by The Jackson Laboratory Histology Service and embedded in paraffin. Tissues were sectioned at 2–3 μm and stained with haematoxylin and eosin or periodic acid–Schiff (PAS). For synaptonemal complex (SC) analysis, chromatin from a testicular cell suspension was surface spread on polycline-coated slides for light microscopy and film-coated slides for subsequent transfer to grids for electron microscopic examination (Moses, 1977, 1980; Dresser and Moses, 1980). These preparations were stained with 50% silver nitrate and a protective colloidal developer for visualization by light and electron microscopy (Howell and Black, 1980). Cells were scored at the light microscope level for SC, XY bivalent and XY body positioning and pachytene staging. Cells representative of what was observed at the light microscope level then were photographed at the electron microscopic level.

**Statistical analysis**

Tests weights, sperm counts and motility measurements were treated as continuous variables, and both parametric and non-parametric versions of two sample t-tests were applied to test the equality of two means. Because the measurements did not present a normal distribution and the sample sizes were rather small, the P-values that are obtained from non-parametric tests (the Wilcoxon rank sum test) were considered to be more reliable than the parametric test values. One-sided tests were done for all comparisons except those involving Ts1Cje where we had no previous experience (two-sided test). For the number of plugs, the Fisher exact test was applied. For pairwise comparisons between strains, multiple comparison adjustments were applied using Hochberg’s step-up Bonferroni method to control overall type I error rate. The type I error rate was set at 0.05 for all of the analyses; thus, comparison with P-values >0.5 is not significant. All of the analyses where we had previous experience were tested using the Fisher exact test performed in SAS 9.1.3 and R 2.0. The multiple testing problem arises when we perform many pairwise comparison hypothesis tests on the same data set, because multiple testing greatly increases the probability of declaring false significances. To control the family-wise error rate (the error rate for all the tests run combined) at or below the declared level of 0.05, we used Hochberg’s step-up Bonferroni method implemented in SASH9.1.3 PROC MULTTEST to adjust the P-values from a family of hypothesis tests.

**Results**

**Breeding studies**

None of the males carrying any one of the four trisomies as an extra chromosome produced progeny. Vaginal plugs were observed from only one of these males. Failure of most of these males to produce a vaginal plug in their mates suggested either failure to mate or insufficient seminal fluid to produce a plug. Although one of the six Ts65Dn males produced a vaginal plug twice in the female with which he was mated, no offspring resulted. These observations suggest infertility of males with an extra chromosome, which was confirmed by sperm and histological analyses below. On the contrary, Ts1Cje males carrying a translocated trisomic segment had reduced fertility, but five of the six males produced plugs and three sired pups, as did all diploid control males. The average litter size for Ts1Cje males was 4, whereas the diploid control males produced an average litter size of 6.25.

**Sperm analysis**

Sperm concentration was significantly reduced below controls in all the trisomic males except for Ts2Lws (Table II); although in Ts1Cje males carrying the trisomic segment translocated to Chr 12, it was not as low as in the extra-chromosome trisomic mice. Considerable variability in sperm concentration was observed among males of each trisomic type except Ts1Cje. Although the motility of the sperm, as defined by head movement, was not significantly different between trisomic and control males (except for Ts4Rk) or between the extra-chromosome trisomies and Ts1Cje, the extra-chromosome trisomies had significantly reduced frequencies of sperm with progressive motility, defined as forward movement. All extra-chromosome trisomies had significantly more sperm with head abnormalities (Figure 2A and B) than did control (Figure 2C) or Ts1Cje mice (Figure 2D). In the one trisomic translocation heterozygous (T4Rk+/-,Ts4Rk) male, the frequency of sperm head abnormalities was not different from diploid controls. Some extra-chromosome trisomic males had too few sperm to score morphology on the standard 100 sperm per male on the two slides made.

<table>
<thead>
<tr>
<th>Chr 16</th>
<th>Ts65DN</th>
<th>Ts232DN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts1Cje</td>
<td>10</td>
<td>13.7</td>
</tr>
<tr>
<td>Other Chr Mb</td>
<td>0</td>
<td>2.3</td>
</tr>
<tr>
<td>Total Mb</td>
<td>10</td>
<td>16.0</td>
</tr>
</tbody>
</table>

**Figure 1.** Diagram showing comparative sizes of the three chromosome 16 (Chr 16) trisomic regions. The trisomic segments ( broad bars) are shown to scale based on megabases (Mb). The centromeres are indicated by closed circles. The origins of the centromere ends are (from left to right) Chrs 12, 17 and 10.
Table II. Fertility parameters (mean ± SD)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of mice</th>
<th>Average testis wta</th>
<th>Total sperm (number)</th>
<th>Sperm concentration (million/ml)</th>
<th>Motile sperm (number)</th>
<th>Progressive sperm (number)</th>
<th>Sperm slide countb</th>
<th>Abnormal sperm frequencyc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts65Dn</td>
<td>6</td>
<td>0.041 ± 0.004*</td>
<td>1250 ± 971</td>
<td>9.80 ± 5.12*</td>
<td>693 ± 860</td>
<td>154 ± 181*</td>
<td>163 ± 95*</td>
<td>0.44 ± 0.12***</td>
</tr>
<tr>
<td>Ts232Dn</td>
<td>6</td>
<td>0.038 ± 0.003*</td>
<td>921 ± 633</td>
<td>3.42 ± 1.68*</td>
<td>511 ± 476</td>
<td>74 ± 76*</td>
<td>192 ± 55*</td>
<td>0.42 ± 0.14***</td>
</tr>
<tr>
<td>TsDn_control</td>
<td>2</td>
<td>0.12 ± 0.01</td>
<td>1450 ± 424</td>
<td>37.95 ± 7.28</td>
<td>1278 ± 295</td>
<td>835 ± 183</td>
<td>340 ± 18</td>
<td>0.006 ± 0.004</td>
</tr>
<tr>
<td>Ts4Rk</td>
<td>6</td>
<td>0.04 ± 0.01*</td>
<td>1069 ± 515</td>
<td>6.00 ± 3.36*</td>
<td>565 ± 280*</td>
<td>95 ± 103</td>
<td>145 ± 77</td>
<td>0.48 ± 0.06***</td>
</tr>
<tr>
<td>Ts4Rk_control</td>
<td>2</td>
<td>0.11 ± 0.03</td>
<td>2065 ± 472</td>
<td>73.95 ± 16.90</td>
<td>1904 ± 476</td>
<td>1126 ± 264</td>
<td>305 ± 66</td>
<td>0.07 ± 0.09</td>
</tr>
<tr>
<td>Ts2Lws</td>
<td>6</td>
<td>0.053 ± 0.006*</td>
<td>985 ± 621</td>
<td>13.48 ± 10.93</td>
<td>447 ± 484</td>
<td>34 ± 32</td>
<td>164 ± 117</td>
<td>0.32 ± 0.20***</td>
</tr>
<tr>
<td>Ts2Lws_control</td>
<td>2</td>
<td>0.10 ± 0.01</td>
<td>1621 ± 243</td>
<td>28.95 ± 4.31</td>
<td>1076 ± 136</td>
<td>428 ± 32</td>
<td>272 ± 40</td>
<td>0.09 ± 0.04</td>
</tr>
<tr>
<td>Ts1Cje</td>
<td>6</td>
<td>0.08 ± 0.01*</td>
<td>872 ± 233</td>
<td>20.20 ± 4.57**</td>
<td>430 ± 208</td>
<td>220 ± 128</td>
<td>228 ± 58</td>
<td>0.06 ± 0.02</td>
</tr>
<tr>
<td>Ts1Cje_control</td>
<td>6</td>
<td>0.10 ± 0.01</td>
<td>1395 ± 426</td>
<td>31.17 ± 3.02</td>
<td>828 ± 331</td>
<td>399 ± 165</td>
<td>223 ± 30</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>

*Testis weights for both testes of all males of each genotype were averaged because they were not significantly different.

*bNumber of sperm scored on a slide preparation for abnormal sperm.

The percentages for each mouse were calculated as the number of events divided by sperm slide count.

*dControl for both Ts65Dn and Ts232Dn (see Materials and methods)

One Ts2Lws mouse had no sperm detected in our experiment, which has caused large SDs.

*Significantly different from controls at P < 0.05.

**Significantly different from controls at P < 0.01.

***Significantly different from controls at P < 0.0001.

Figure 2. Example of sperm head abnormalities seen in extra chromosome trisomies. Semen samples were diluted by the same amount. (A) Control, (B and C) Ts65Dn and (D) Ts1Cje. Note the concentration of sperm in the same-sized aliquot is much less in samples from trisomic males than from controls. In panels (B–D), arrows indicate examples of abnormal sperm heads, and arrowheads indicate examples of normal sperm heads. Bar = 100 μm.
**Testicular histological analysis**

All four types of extra-chromosome trisomic males had significantly smaller testes than either controls or Ts1Cje males (Table II). Ts1Cje males had smaller testes as well, but they were closer in size to the controls; all extra-chromosome trisomic males had significantly smaller testes than Ts1Cje males.

Histological assays of adult, but not aged, males (10–14 weeks of age) clearly showed the basis for infertility observed in the breeding studies. These histological analyses of testes revealed that males carrying any one of the extra-chromosome trisomies showed striking evidence of spermatogenic failure (Figure 3B). Spermatogenesis was arrested at meiosis (prophase or metaphase I) or round spermatid stages in many tubule cross-sections, although some tubule cross-sections exhibited apparently normal spermatogenesis. The stage and the extent of spermatogenic arrest varied from mouse to mouse, consistent with the variability in sperm concentration among trisomic males. In contrast, tubule cross-sections from Ts1Cje males were relatively normal, as would be predicted from their more normal sperm counts and testicular weights (Figure 3C).

**SC analysis**

Because chromosomal sterility often is associated with anomalous interactions between the abnormal chromosome and the XY bivalent in pachytene spermatocytes, we analysed silver-stained preparations of pachytene spermatocytes by light microscopy to determine relative positions of the extra trisomic chromosome and the XY bivalent within the XY body. At least 50 cells from each mouse were analysed. The criteria for scoring the association of the axial element (AE) of the extra trisomic chromosome with the AEs or SC of the XY pair were if (i) the trisomy AE was included within the XY body chromatin domain or (ii) the distance of the trisomy AE from the XY AE/SC was ≤1/2 the length of the X axial core. Each trisomic male with an extra chromosome displayed a high frequency of association of a small, ‘unpaired’ AE, presumed to be that of the extra chromosome, with the XY SC/AEs (Table III; Figure 4A–C). Sometimes, the presumed trisomy AE showed a close association with the unpaired AE of the X chromosome, often within the XY body chromatin (Table III). The small, unpaired AE appeared thicker and more densely silver-stained than the paired autosomal AEs, resembling the unpaired AEs of the X and Y (Figure 4A–C). At the light microscope level, we cannot distinguish unpaired AE thickening from possible self-synapsis. The pachytene stage in cells in which the trisomic chromosome was not associated with the XY or the sex body was not consistent for all trisomies. Such cells in Ts65Dn and Ts232Dn were mostly mid-to-late pachytene; in Ts2Lws and T4Rk, they were early-to-late pachytene with a slight preponderance in early pachytene.

In Ts1Cje males, where the trisomic segment is attached to Chr 12, no unusual association of an autosomal SC with the XY SC/AEs or XY body chromatin was observed (Table III). In the one translocation heterozygote trisomic (T4Rk+/Ts4Rk) male, the association of the trisomy AE with the XY SC was seen in only 7 of 50 (14%) cells, whereas in 46% of the cells it was associated with the translocation SC quadrivalent configuration (Figure 4C). T4Rk+/Ts4Rk mice have two copies of the small extra chromosome, and this observation suggests that the two small autosomal translocation chromosomes may pair with each other rather than associate with the unpaired XY domain.
A minority of spermatocytes in extra-chromosome trisomies showed non-homologous pairing of the trisomy AE with an autosome (Table III).

### Discussion

Chromosome abnormalities that lead to unpaired regions during meiotic pachynema almost always are associated with male infertility (De Boer and Branje, 1979; Peters et al., 1997; Merico et al., 2003); however, data for the effect of an extra chromosome are limited. In this study, we took advantage of five mouse models of trisomy to address two questions: How does chromosomal trisomy impact male fertility in the mouse? Are the effects due to gene dosage or presence of an extra, unpaired chromosome?

#### Trisomy and fertility parameters

Male fertility is exquisitely sensitive to the presence of chromosome anomalies. In this study, all males with an extra chromosome were functionally sterile, failing to produce progeny. These results confirm our observations from historical colony-breeding data for these stocks. Consistent with the infertility, all four extra-chromosome trisomies were associated with significantly lower testis weight and lower sperm counts, although there was a considerable variability in specific reproductive parameters among trisomic males. Among Ts65Dn males, especially, sperm count ranged from none to nearly normal levels. Indeed, we have been able to produce progeny from some Ts65Dn males using IVF. Testes of the infertile trisomic males exhibited dramatic depletion of germ cells and impairment of spermatogenesis, although both qualitative and quantitative aspects of this phenotype varied among males and among tubule cross-sections from a single male. This observation is consistent with findings in oligozoospermic human males where an inverse relationship between sperm concentration and aneuploidy has been demonstrated (Martin et al., 2003). However, there was not, as might be expected (Odorisio et al., 1998), a global arrest either at pachynema when unpaired autosomal axes might be recognized or at metaphase I in the trisomic male mice. This observation argues against a single checkpoint-mediated arrest of gametogenesis in trisomic males.

### Table III. Synaptonemal complex (SC) analysis (mean ± SD)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Number of mice</th>
<th>% Ts associated with XY</th>
<th>% Ts associated with autosome</th>
<th>% Ts unassociated with autosome</th>
<th>% Ts paired with autosome</th>
<th>% Autosome associated with XY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ts65Dn</td>
<td>6</td>
<td>0.88 ± 0.02</td>
<td>0.22 ± 0.05</td>
<td>0.06 ± 0.04</td>
<td>0.03 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>Ts232Dn</td>
<td>6</td>
<td>0.80 ± 0.06</td>
<td>0.20 ± 0.08</td>
<td>0.07 ± 0.05</td>
<td>0.09 ± 0.07</td>
<td>–</td>
</tr>
<tr>
<td>TsDn_control</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.39 ± 0.13</td>
</tr>
<tr>
<td>Ts4Rk</td>
<td>6</td>
<td>0.74 ± 0.07</td>
<td>0.34 ± 0.08</td>
<td>0.06 ± 0.03</td>
<td>0.20 ± 0.11</td>
<td>–</td>
</tr>
<tr>
<td>Ts4Rk_control</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.34 ± 0.00</td>
<td>–</td>
</tr>
<tr>
<td>Ts2Lws</td>
<td>2</td>
<td>0.83 ± 0.05</td>
<td>0.14 ± 0.06</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.02</td>
<td>–</td>
</tr>
<tr>
<td>Ts2Lws_control</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.28 ± 0.03</td>
</tr>
<tr>
<td>Ts1Cje</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.44 ± 0.06</td>
</tr>
<tr>
<td>Ts1Cje_control</td>
<td>2</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.45 ± 0.16</td>
</tr>
</tbody>
</table>

*Note: A total of 50 cells were examined for each mouse, and the percentages were calculated as the number of events divided by 50.

*Includes the few cells in which the trisomic SC was actually paired with the XY SC.*

Figure 4. Synaptonemal complexes (SCs) from trisomic mice: (A) Ts65Dn and (B) Ts232Dn showing the association of the trisomy axial element (arrows) with the sex chromosomes (labelled X and Y); (C) SC from trisomic translocation heterozygote T4Rk, Ts4Rk showing pairing between the small extra chromosome and the translocation tetravalent, 12 and 17 indicate Chrs 12 and 17, 1712 is the large T4Rk translocation product, and 1217 is the small translocation product, which is present in two copies (reprinted with the permission of Elsevier, Inc., from *Mouse in Biomedical Research*, 2nd edn, 2006.)
In addition to the reduction of sperm number in extra-chromosome trisomic males, a high percentage of the sperm present had abnormal head morphology. Although it has been proposed that such abnormalities could be diagnostic for chromosomally unbalanced gametes, Wyrobek et al. (1975) failed to show a correlation between chromosomal abnormalities and sperm head abnormalities in mice. This study indirectly supports this conclusion, because the frequency of abnormal sperm is higher than the frequency likely to be carrying the extra chromosome. Thus, we hypothesize that the high frequency of abnormal sperm reflects the disturbed gametogenesis observed in the extra-chromosome trisomic males.

Sexual dichotomy in fertility is seen in mice and humans with chromosomal aberrations (Burgoyne et al., 1985; Hunt and Hassold, 2002), and, notably, in humans with DS, for which the Ts65Dn mouse is a model. Females with DS vary in reproductive capacity but have been documented to produce offspring (Jagiello, 1981). Likewise, we find during colony maintenance that female carriers of the extra Ts65Dn chromosome are fertile, even though litter size varies among females and the reproductive life span appears to be shortened. Consistent with results of this study, there is evidence that human males with DS produce offspring only rarely, if at all. We have been able to find only one published report (Zuhlke et al., 1994). In young adult males with DS, the impairment of spermatogenesis has been reported to be variable, and in biopsies from one 18-year-old male, the extra 21 chromosome was often not visible at pachynema and hypothesized to be within the XY body (Johannisson et al., 1983), although the possibility of a small population of normal diploid germ cells due to mitotic loss of the extra chromosome was not excluded.

**Extra chromosome versus gene dosage**

The effect on fertility of gene triplication per se versus the presence of an extra chromosome was assessed by comparing the extra-chromosome trisomy Ts65Dn and Ts232Dn males with Ts1Cje males. The fertility and relatively normal sperm parameters of Ts1Cje males provide an important clue to the nature of the disruption in other trisomic males. Ts1Cje individuals are trisomic for a gene content quite similar to the extra gene content in Ts65Dn males; the two trisomies are distinguished by the fact that the extra gene content is present in an extra chromosome in Ts65Dn individuals (Table I; Figure 1). Because the Ts1Cje males are fertile with acceptable sperm parameters, this study shows that it is likely the presence of an extra chromosome, rather than extra genetic material per se, that causes abnormalities of male gametogenesis and interferes with the normal production of spermatozoa. Information from the most recent NCBI Build of the mouse genome sequence (via Ensembl) and a more precise definition of the proximal boundary of the Ts65Dn segment (Kahlem et al., 2004) suggest that the Ts65Dn trisomic segment could have up to 50 more genes triplicated than in the trisomic segment in Ts1Cje. The difference in gene content between Ts232Dn and Ts1Cje is even greater (Table I). Thus, these three models cannot definitively rule out the possibility that the difference in gene content between Ts1Cje and Ts65Dn crosses a threshold of toleration of gene triplication compatible with male fertility. However, the genes and predicted coding sequences present in Ts65Dn but not in Ts1Cje are ones expressed ubiquitously, and none are expressed specifically in testes (GNF SymAtlas, http://wombat.gnf.org/SymAtlas/).

Two other pieces of evidence (unpublished data from colony-breeding records) support our hypothesis that sterility in segmentally trisomic males is caused by the extra chromosome. First, males carrying a chromosomal rearrangement involving fusion of the entire Ts65Dn chromosome to the centromere of Chr 12 [Rb(12.Ts1765Dn)2Cje] (Villar et al., 2005) are fertile, supporting the conclusion that it is not the extra gene content of the Ts65Dn chromosome, but its presence as an extra chromosome, that leads to the loss of fertility and poor sperm parameters. Twenty-one of 27 males mated produced progeny with an average litter size of 7.3. These numbers are not statistically different from trisomic females in the same colony; 20 of 33 were fertile with an average litter size of 6.0. Second, females heterozygous for the T4Rk translocation produce trisomic T4Rk progeny that carry the small translocation chromosome as an extra chromosome. These trisomic mice may be either heterozygous (T4Rk+/+) or wild type (+/+ ) for the translocation. Six +/-, T4Rk males that were mated were all sterile. Five T4Rk/+, T4Rk males mated during the same time period were all fertile and produced an average litter size ranging from 2.2 to 4.0, a litter size that did not differ from that of T4Rk/+ males. In pachytenic cells from the T4Rk, T4Rk male in the current study, the trisomic T4Rk extra chromosome was associated with the translocation quadrivalent more frequently than with the XY bivalent.

**Trisomy and nuclear domain structure**

The finding that it is an extra chromosome rather than extra gene content that causes trisomic males to be infertile led us to investigate the effects of trisomy on nuclear domains in trisomic spermatocytes. Previous studies (Garagna et al., 2001) have reported alterations in spermatocyte nuclear architecture in subfertile mice with heterozygosity for Robertsonian chromosomes, and indeed, chromosomal anomalies in general have been reported to lead to associations of aberrant chromosomes with the unpaired domains of the sex bivalent in spermatocytes (e.g. Forejt et al., 1981; Mahadeviah and Mittwoch, 1986; Setterfield and Mittwoch, 1986). In spermatocytes, during the pachytenic stage of meiosis, the XY pair forms a unique nuclear domain, the XY body (Handel, 2004). This domain is transcriptionally inactive and exhibits characteristic protein modifications associated with transcriptional silencing of chromatin, such as histone modification (Khalil et al., 2004; Baarends et al., 2005), and unpaired autosomal axes similarly modified are silenced (Baarends et al., 2005; Turner et al., 2006). Our analysis of SCs in spread spermatocyte chromatin showed a high correlation between infertility and association of the small, unpaired AE (presumably of the trisomic chromosome) with the unpaired axes of the sex chromosomes (Table III). In contrast, among the fertile Ts1Cje males, trisomic in gene content but not bearing an extra chromosome, no such skewed
association was detected. Additionally, the axes of the Ts65Dn chromosome, whether separate from or contiguous to or part of the XY body, are modified with proteins such as γH2AX that are characteristic of the XY chromatin domain (data not shown). Together, these observations suggest that chromosomal axes attached to unpaired centromeres may cluster in a common domain during meiotic prophase, and, although it has not yet been examined experimentally, this domain is likely associated with transcriptional silencing, as has been shown for other unpaired autosomal domains (Turner et al., 2006). If the trisomic chromatin is inactivated, this presents a conundrum: inactivation would restore gene dosage to a diploid gene dosage, which should be advantageous to the germ cells; yet the trisomic males experience disruption to spermatogenesis and infertility. While it is possible that, as is the case for meiotic silencing of unpaired DNA in Neurospora crassa (Shiu and Metzenberg, 2002), inactivation could spread in trans to the normal Chr 16, recent data examining the translocation of an autosomal sequence onto the X chromosome, where it is meiotically unpaired, suggest that this is not the case (Turner et al., 2006). These considerations also are consistent with the concept that it is the presence of extra chromosomal axes per se, rather than gene dosage, which leads to infertility.

Another postulated function of protein modifications and transcriptional silencing of XY chromatin is that it masks the unpaired status from checkpoint surveillance, allowing spermatogenesis to proceed. Our observations suggest that this is unlikely. This hypothesis would predict that spermatocytes with unpaired trisomic chromosomes in the same domain as the sex chromosomes might be rescued from synopsis surveillance. To the contrary, the frequency of association of unpaired trisomic chromosomes with the sex chromosomes is positively correlated with spermatogenic failure. Jaafar et al. (1993) postulated that the association of autosomal elements with the XY chromatin inactivates the autosomal chromatin by a spreading effect and that reduced gene dosage, perhaps of genes critical for spermatogenesis, accounts for the failure of spermatogenesis. However, as discussed above, the inactivation of a trisomic chromosome by a spreading effect from proximity to the sex chromosomes would restore the normal gene dosage, presumably advantageous to the germ cells; yet the trisomic males are sterile. And, although Forejt (1985), Speed (1986) and others have postulated that unpaired elements may interfere with the inactivation of the sex chromosomes, we observed no abnormalities in the morphology (or protein modification, data not shown) of the XY body heterochromatic nuclear domain in trisomic males.

In conclusion, the infertility parameters of trisomic males are consistent with an effect from the presence of an extra chromosome and not from the extra trisomic gene content. Additionally, in pachytene spermatocytes, unpaired autosomal chromosomes show unusual associations with unpaired elements of the X and Y chromosomes, whereas there is no evidence that trisomic gene regions attached to paired centromeres do. Unpaired chromosomes may establish aberrant chromatin territories within meiotic nuclei that impact progression through spermatogenesis.

Acknowledgements
The authors thank Susan A. Cook for providing T4Rk mice, Suzanne Taylor and Lesley Bechtold for assistance with electron microscopy, The Jackson Laboratory’s Histology Service, Jin Szatkiewicz in The Jackson Laboratory’s Computational Biology Resource for statistical analysis, Dr. Charles Epstein for providing Ts1Cje mice for these studies before publication and Jennifer Torrance for assistance preparing figures. We thank Drs John Eppig and Laura Reinholdt for thoughtful comments on the manuscript. This work was supported by National Institutes of Health grants HD73265 (MTD), HD33816 (MAH) and Cancer Core grant CA34916 for partial support of the Bioimaging Service (electron microscopy and histology).

References


