Sex-dependent frequency and type of autosomal univalency at the first meiotic metaphase in mouse germ cells

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Univalents at the first meiotic metaphase in mouse spermatocytes occur mainly in the XY pair, making it difficult to compare the amounts of univalency in males and females. In this study, the amounts of autosomal univalency in male and female meiosis were compared using the model strain CBA-T6, in which univalency of the small marker autosome pair T6 has been shown to occur very frequently in spermatocytes. Mice from inbred CBA and DBA strains were also analysed. The total frequencies of univalency (sex chromosomes plus autosomes) in metaphase I spermatocytes were 45.6% in CBA, 36.9% in CBA-T6, and 37.3% in DBA males. The aneuploidy in metaphase II spermatocytes ranged from 1.4 to 3% in these strains, which was in agreement with previous findings that most primary spermatocytes with abnormal chromosome configurations are arrested in their development before metaphase II. In the CBA-T6 strain, autosomal univalency at metaphase I mostly involved chromosome pair T6; however, its frequency differed significantly between the sexes, amounting to 18.9% in spermatocytes and 4.3% in oocytes. In the CBA strain, autosomal univalents at metaphase I were seen in 7.7% of the spermatocytes and 1.4% of the oocytes and, in DBA mice, in 4.9% of the spermatocytes and 3.8% of the oocytes. However, in DBA oocytes, when univalency occurred it usually concerned a greater number of bivalents in one cell (range: 2–19 disjoined bivalents), a phenomenon very rare in males of this strain. This study shows that univalent formation differs between the male and female types of meiosis.

Introduction

At the prophase stage of the first meiotic division, the homologous chromosomes undergo pairing to form bivalents. Such a configuration of chromosomes enables genetic recombination, one of the basic functions of meiosis, to take place. However, the bivalent structure has to be maintained at later stages of the first meiotic division to ensure proper segregation of homologous chromosomes at the first meiotic anaphase (Polanski and Kubiak, 1999). The occurrence of homologous chromosomes in an unpaired state, as univalents at the first meiotic metaphase, affects the subsequent anaphase in two ways: (1) the homologues segregate randomly; and (2) the chromatids of the homologue may separate (an event that takes place usually at the second meiotic anaphase). Either effect may produce aneuploid gametes and eventually give rise to an aneuploid embryo (Griffin, 1996).

Studies on primary spermatocytes in mice have found that univalency occurs occasionally and that its frequency is determined genetically (Rapp et al., 1977; Matsuda et al., 1982; Biddle et al., 1985; Krzanowska, 1989). In some inbred strains, univalency occurs frequently: 33% in CBA (Krzanowska, 1989) or 37% in DBA/2J mice (Biddle et al., 1985). In all these studies, the majority of cases of univalent formation involve the XY chromosome pair. The amount of autosomal univalency is much lower: for example, among four inbred strains studied by Krzanowska (1989) it ranges from 1.3 to 4.6%. An exceptional case has been described for male CBA-T6 mice, in which the overall frequency of univalents is typical of CBA mice, but the frequency of autosomal univalency varies from 4.5% in CBA to 15.6% in CBA-T6 males, owing to the high susceptibility of the small marker chromosome pair T6 to this anomaly (Krzanowska and Wabik-Sliz, 1994).

Oocytes are more difficult material for this type of study because the methodology is more complicated, but there are some reports on univalency in metaphase I oocytes. The frequency of univalents in different substrains of C57 mice ranges from 0.2% (Luthardt et al., 1973) to 3% (Speed, 1977) and, in very old females (12–15 months), reaches 5% (Sugawara and Mikamo, 1986). In the CBA strain, this frequency seems even lower, since no univalents were found in the oocytes of young or old female mice (U. Eichenlaub-Ritter and I. Boll, unpublished, cited in Eichenlaub-Ritter, 1996). The extent to which the formation of univalents depends on sex remains an open question. The XY pair comprising most cases of univalency in males does not exist
in females, implying that comparisons between the sexes should consider only autosomal univalents. However, the low incidence of autosomal univalents makes it difficult to detect any statistically significant difference between the rates of autosomal univalency in spermatocytes and oocytes of the same strain. Thus, the CBA-T6 strain, with an increased number of autosomal univalents in males, provides a model for reliable comparison of autosomal univalency frequencies in males and females. This article describes such an analysis.

**Materials and Methods**

Female (2–5 months old) and male (3–5 months old) mice from the inbred strains CBA/H, CBA/H-T6 carrying the T(14,5)6 Ca translocation (Festing, 1993), and DBA/2 were used. Hereafter, these strains are designated CBA, CBA-T6 and DBA, respectively.

Chromosome preparations from testis were obtained according to the method described by Meredith (1969). For most males, the slides were processed for C-banding according to the method of Sumner (1972). Briefly, slides were incubated in 0.2 mol HCl l–1 for 1 h, in 2.5% (w/v) Ba(OH)2 (supersaturated solution) for 60–75 s, and in 2 × SSC (17.53 g sodium chloride and 8.82 g sodium citrate 1–1 distilled water) at 62.5°C for 1 h.

Oocytes at the germinal vesicle stage were isolated and cultured in M2 culture medium (Fulton and Whittingham, 1978) as described by Polanski (1997a), except that dibutyryl cyclic AMP was not used during isolation. After 5.5–6.5 h of in vitro maturation, these oocytes were processed for chromosome preparations according to Tarkowski’s technique (1966). Since extrusion of the first polar body in both CBA and DBA mice starts 7–8 h after the start of culture (Polanski, 1997a,b), the time chosen ensured that relatively advanced metaphase I oocytes were analysed, without the risk of scoring anaphases.

The preparations from both male and female germ cells were stained with 2% (w/v) Giemsa in phosphate buffer, pH 6.8, for 30 min.

The metaphase I plates were scored for the presence of univalents. In general, except for the T6 pair, autosomal univalents were formed by small chromosome pairs. Chromosome preparations from testis were obtained according to the method described by Meredith (1969). For most males, the slides were processed for C-banding according to the method of Sumner (1972). Briefly, slides were incubated in 0.2 mol HCl l–1 for 1 h, in 2.5% (w/v) Ba(OH)2 (supersaturated solution) for 60–75 s, and in 2 × SSC (17.53 g sodium chloride and 8.82 g sodium citrate 1–1 distilled water) at 62.5°C for 1 h.

**Table 1. Frequency of univalents at the first meiotic metaphase and aneuploidy at the second meiotic metaphase in spermatocytes from inbred mouse strains CBA, CBA-T6 and DBA**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of cells examined</th>
<th>XY</th>
<th>Autosomal</th>
<th>Total</th>
<th>Number of cells examined</th>
<th>Hyperhaploid</th>
<th>Others</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA</td>
<td>520</td>
<td>39.4 ± 3.6</td>
<td>7.7 ± 2.1</td>
<td>45.6 ± 3.4</td>
<td>132</td>
<td>2 (1.5)</td>
<td>0</td>
<td>1.5%</td>
</tr>
<tr>
<td>CBA-T6</td>
<td>683</td>
<td>21.0 ± 3.0</td>
<td>18.9 ± 2.7</td>
<td>36.9 ± 4.4</td>
<td>142</td>
<td>1 (0.7)</td>
<td>0</td>
<td>0.7%</td>
</tr>
<tr>
<td>DBA</td>
<td>475</td>
<td>32.3 ± 4.6</td>
<td>5.4 ± 1.7</td>
<td>37.3 ± 4.9</td>
<td>201</td>
<td>2 (1.0)</td>
<td>1 (0.5)</td>
<td>1.5%</td>
</tr>
</tbody>
</table>

n: number of males.

Metaphase II chromosomes were analysed only from C-banded slides.

* Most of these were spermatocytes with T6 univalents (13.5% ± 1.9).

**Results**

The males from all three strains analysed showed high frequencies of univalents in the spermatocytes (Table 1). The percentages obtained for CBA mice were even higher than those reported by Krzanowska and Wabik-Śliz (1994) (33% for both CBA and CBA-T6 males) whereas, in DBA males, the frequencies were identical to those found by Biddle et al. (1985). In agreement with the report of Krzanowska and Wabik-Śliz (1994), univalency of the T6 pair was often observed in CBA-T6 males (Fig. 1b), giving a 18.9% total frequency of autosomal univalents. In general, except for the T6 pair, autosomal univalents were formed by small chromosome pairs.

Univalent chromosomes may segregate randomly or splice to single chromatids: if they segregate randomly, the metaphase II may contain an aneuploid number of chromosomes; if they splice to single chromatids, the separate chromatids are present at the metaphase II plate. The numbers of chromosomes in metaphase II spermatocytes were also analysed to determine whether there was a relationship between univalency at metaphase I and numerical aneuploidy at metaphase II (Figs. 1c,d). As usual, hypohaploid plates were excluded from the analysis, since in many cases these result from artefact chromosome loss during treatment. The frequencies of numerical aberrations scored (hyperhaploid plates plus plates containing single chromatids; Fig. 1d) ranged from 0.7 to 1.5%, depending on the strain (Table 1), giving frequencies of aneuploidy (doubling of the hyperhaploid plates plus plates with single chromatids) of 1.4, 2.5 and 3% for the CBA-T6, DBA and CBA strains, respectively.

In CBA oocytes, the frequency of univalency was 1.4%, whereas in CBA-T6 oocytes it was 4.3% (Table 2). As was the case in CBA-T6 males, in females, most cases of univalent formation involved the T6 pair (Fig. 2b). The frequency of
univalent formation for chromosome pairs other than T6 were the same in CBA-T6 and CBA females and, in both strains, concerned smaller chromosomes (for this reason all univalents are recognized as autosomal). Although there was a clear relationship between the presence of T6 chromosomes and the rate of autosomal univalents in both sexes, the total number of autosomal univalents scored was still lower by a factor of almost five in oocytes (compare Tables 1 and 2). In CBA-T6 mice, the rate of formation of autosomal univalents in oocytes was significantly lower than it was in spermatocytes ($P < 0.01$); the difference between the sexes within the CBA strain was also significant ($P < 0.02$). In DBA females, 6 of 159 metaphases tested contained univalents, giving a total frequency of 3.8%, not significantly different from the total frequency of autosomal univalent formation in spermatocytes of this strain. However, among these six plates, five contained multiple univalents (Fig. 2c, Table 2), with the following numbers of involved homologues in the plates: 19, 16, 14, 3 and 2. Multiple univalents were not observed in the oocytes of other strains. Sometimes two

**Fig. 1.** Chromosomes of the first (a,b) and second meiotic metaphase (c,d) in mouse spermatocytes. (a) Normal chromosome configuration with 20 bivalents, CBA male; (b) CBA-T6 male, T6 univalents visible on two opposite sides of the metaphase plate; (c) normal configuration with 20 chromosomes, CBA-T6 male; and (d) hyperhaploid plate (21 chromosomes), CBA-T6 male. Scale bar represents 5 µm.
univalents on one plate were observed in spermatocytes, with frequencies of 1.6, 3.3 and 0.9% in males of the CBA, CBA-T6, and DBA strains, respectively. These low percentages indicate that the occurrence of such plates in spermatocytes was a simple product of the probabilities of univalency comprising two homologues in one cell, rather than a specific susceptibility to multiple univalency, as was the case in DBA oocytes. The DBA spermatocytes and oocytes were compared with respect to the proportion of multiple univalency among all cells containing univalents by the chi-squared test ($2 \times 2$ table with Yates correction) and the difference was found to be significant ($P < 0.0001$).

**Table 2.** Frequencies of univalency at the first meiotic metaphase in mouse oocytes from inbred strains CBA, CBA-T6 and DBA

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of females</th>
<th>Number of cells examined</th>
<th>Number (%) of oocytes with univalents involving</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>One bivalent</td>
</tr>
<tr>
<td>CBA</td>
<td>6</td>
<td>143</td>
<td>2 (1.4)</td>
</tr>
<tr>
<td>CBA-T6</td>
<td>8</td>
<td>140</td>
<td>6 (4.3)$^a$</td>
</tr>
<tr>
<td>DBA</td>
<td>6</td>
<td>159</td>
<td>1 (0.6)</td>
</tr>
</tbody>
</table>

$^a$In four of these oocytes, univalents appeared to comprise the T6 homologues.

$^b$Significantly lower than in spermatocytes of corresponding strains (compared with data from Table 1); ANOVA; $^bP < 0.02$, $^cP < 0.01$.

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**Fig. 2.** Chromosomes of the first meiotic metaphase in mouse oocytes. (a) Normal configuration with 20 bivalents, CBA-T6 female; (b) CBA-T6 female, T6 univalents (arrows); and (c) multiple univalents (three pairs) in DBA female. Scale bar represents 5 µm.
Discussion

The present study found a reasonable proportion of univalents in meiotic metaphase I spermatocytes of DBA strain and both CBA substrain male mice, in agreement with previous reports relating to these strains. Univalency is associated with pairing abnormalities and reduced recombination between the involved homologues (Eichenlaub-Ritter, 1996; Ferguson et al., 1996). No data were available on the pairing and recombination in spermatocytes of the mice strains used in the present study. However, analysis of XY pairing in spermatocytes of pubertal Swiss mice showed incidences of unpaired sex homologues between 0 and 7.7% (Goetz et al., 1984). Krzanowska (1989) studied the frequency of univalency at meiotic metaphase I in pubertal males from four inbred strains and found incidences of sex chromosome univalents ranging from 9.5 to 37.3%, depending on the strain. Thus, the males from these four strains display greater univalency at metaphase than do Swiss males at pachytene (Goetz et al., 1984). Moreover, in C57/Bl spermatocytes, the incidence of univalents at late pachytene was 1.6% (Tepperberg et al., 1997), much lower than the 8.5% univalents found in spermatocytes of the same strain at metaphase (Krzanowska 1989). These findings indicate that the rate of univalency observed at metaphase may be affected not only by lack of pairing but also by precocious separation of homologues.

The possible link between the occurrence of univalents at metaphase I and aneuploidy at metaphase II was analysed on the theoretical grounds that univalents at metaphase I should give rise to abnormal numbers of chromosomes at metaphase II as a result of random segregation of homologous chromosomes or their premature splitting into separate chromatids (Griffin, 1996). If the abnormal numbers of chromosomes at metaphase II are the result of random segregation of homologous chromosomes, then 25% of the resulting daughter secondary spermatocytes should have hyperhaploid chromosome count. If the abnormal numbers of chromosomes at metaphase II are the result of random segregation of homologous chromosomes or their premature splitting into separate chromatids (Griffin, 1996). Mouse spermatocytes with abnormal chromosome configuration at meiotic metaphase I arrest their subsequent development before reaching meiotic metaphase II (Burgoyne and Mahadevaiah, 1993). For most extreme anomalies, when the frequency of univalents is nearly 100%, it may produce male sterility, as in interspecific hybrids (Matsuda et al., 1991, 1992) or in mice lacking the Mlh1 gene (Baker et al., 1996; Edelmann et al., 1996). CBA males, although fully fertile, have low testis mass (Krzanowska, 1989), and analysis of the chromosome complement does not reveal increased aneuploidy in

Table 3. Lack of the relationship between univalency at first meiotic metaphase and aneuploidy at the second meiotic metaphase in spermatocytes from inbred mouse strains CBA, CBA-T6 and DBA

<table>
<thead>
<tr>
<th>Strain</th>
<th>Number of males</th>
<th>Proportion of univalents a</th>
<th>Expected b</th>
<th>Found</th>
<th>( p^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBA</td>
<td>3</td>
<td>226/520</td>
<td>14/132</td>
<td>2/132</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>CBA-T6</td>
<td>3</td>
<td>158/511</td>
<td>11/142</td>
<td>1/142</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>DBA</td>
<td>5</td>
<td>172/475</td>
<td>18/201</td>
<td>3/201</td>
<td>&lt; 0.002</td>
</tr>
</tbody>
</table>

All data were from C-banded slides.

a Number of cells containing univalent(s)/ total number of cells examined.

b (Number of cells with hyperhaploid chromosome count + cells containing separate chromatids)/total number of cells examined.

c Calculated as 1/4 of secondary spermatocytes originating from the primary spermatocytes bearing univalent (see text).

d Calculated by chi -squared test (2 \times 2 table) with Yates’ correction for low quantities.
spermatocytes bearing univalents.

Whereas the spindle-assembly checkpoint efficiently operates in spermatocytes, its functioning in mammalian oocytes is unclear. Oocytes with multiple univalents from Mlh1-deficient mice do not enter anaphase (Woods et al., 1999). Moreover, in the mouse oocyte, the first anaphase is executed only after each bivalent establishes bipolar attachment with microtubule fibres (Brunet et al., 1999). These two observations indicate spindle-assembly checkpoint functioning in oocyte meiosis. Contrasting results come from studies on meiosis in X0 female mice, which contain only one X chromosome, occurring as a univalent at meiotic metaphase I; this anomaly neither blocks nor delays meiotic anaphase I (LeMaire-Adkins et al., 1997). In the course of the first meiotic division, two thirds of the X0 oocytes segregate the intact X chromosome to one spindle pole; in the remaining oocytes, the X undergoes premature equational division, segregating a single chromatid to each spindle pole (Hunt et al., 1995; LeMaire-Adkins et al., 1997). These data indicate that the spindle-assembly checkpoint in female meiosis is less efficient than the checkpoint operating in males.

The results from DBA males in the present study contradict the results of Leotard et al. (1987) in which the univalency in DBA/2J males (41%) was comparable with the present data but the frequency of hyperhaploid secondary spermatocytes in DBA males was much higher, reaching 11%. Leotard et al. (1987) tested other strains and their crosses (for example 5.3 versus 11.2%, respectively, for the cross between C57/Bl and Swiss mice). The counts given for C-bands, an important requirement for reliable analysis of MII chromosomes (Mailhes, 1987). The main aim of the present study was to compare the frequencies of autosomal univalents at meiotic metaphase I in males and females of the same genotype. It was found, as a general feature in both sexes, that autosomal univalency involves the smaller homologues. This finding indicates that the smaller the magnitude of the pairing region, the higher the risk of univalency and applies equally to the T6 mutant miniature autosome and to the male sex chromosome bivalent in which the pairing area is limited to the ‘pseudoautosomal’ region (Graves, 1998). However, in the present study, the frequencies of autosomal univalency were higher in males than they were in females in the analysed strains. In this context, it is useful to note the different levels of recombination between the sexes, since reduced recombination is linked with univalency (Ferguson et al., 1995). In mice, the number of chiasmata, reflecting the level of recombination, is higher in females (Speed, 1977; Lawrie et al., 1995). This difference may even be as high as seven chiasmata per cell in the random-bred Q strain (Speed, 1977).

These findings suggest that males are more prone to univalency, supporting the main finding of the present study.

Krzanowska (1989) compared two congenic strains differing in the source of the Y chromosome and showed that the Y chromosome has no direct effect on the frequency of XY univalents in spermatocytes. How then would the presence of the Y chromosome increase the frequency of autosomal univalents in males in comparison with females having the same genetic constitution? The Y chromosome may act indirectly through its primary function: determination of the germ cells of the given sex. After switching on the male programme of development, the effect of the Y chromosome would no longer be exerted, and sex differences in the frequency or type of univalents formed should be ascribed to differences in the physiology of male and female meiosis.

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