INTRODUCTION

Proper chromosome segregation during mitotic cell division requires at least two chromosome-associated protein complexes. At the centromere, functional kinetochores must be formed on individual sister chromatids to facilitate the attachment of the chromatids to opposite spindle poles. In addition, cohesion must be established between the centromeres and along the arms of the sister chromatids and maintained until anaphase to prevent their precocious separation.

By comparison with mitotic cell division, the segregation behavior of chromosomes during meiotic cell division is complex, necessitating modification of both the kinetochore and cohesion complexes. Our understanding of these meiotic modifications remains rudimentary. The first meiotic division (MI) involves the segregation of homologs rather than sister chromatids (Fig. 1A). Thus, successful chromosome segregation at MI requires specialized cohesion mechanisms that provide physical connections between homologs (rather than sister chromatids), and impose constraint on the centromeres of sister chromatids so that their kinetochores form attachments to the same rather than opposing spindle poles. The second meiotic division (MII) is similar to a mitotic cell division, involving the segregation of sister chromatids. However, because MI and MII occur without an intervening S phase, successful chromosome segregation at MII requires a mechanism whereby cohesion is released along the chromosome arms at anaphase I but maintained between sister centromeres until anaphase II.

It is commonly assumed that the specialized meiotic cohesion requirements depend upon the unique events of meiotic prophase, e.g. synopsis and recombination. The role of recombination in the disjunction of homologous chromosomes at MI is well established (e.g. Carpenter, 1994; reviewed in Hassold et al., 2000; Hawley, 1988; Koehler et al., 1996; Lamb et al., 1996). The role of synopsis remains less well characterized, but the inferential evidence is compelling: both defects in homolog synopsis and the absence of a homologous partner are associated with an increased frequency of premature separation of sister chromatids at MI in a variety of species (reviewed in Moore and Orr-Weaver, 1998; Wolf, 1993). Moreover studies in corn, yeast and mammals provide evidence for the involvement of components of the
provides a genetic approach to understanding the factors that influence the behavior of sister kinetochores at the first meiotic division. We report here the results of detailed meiotic studies that exclude X-chromosome specific differences and suggest that segregation is influenced by the action of an autosomal gene or genes. We hypothesized that this trans-acting factor(s) would influence the synaptic behavior of the X chromosome, and that failure to undergo self-synapsis involving the centromere of the chromosome would result in the premature separation of X chromatids at MI. Our observations did not fit this expectation, but our studies provide new insight to the complexity of the synaptic process and suggest that inferences about subsequent meiotic events based on pachytene analysis may be misleading. More importantly, however, our efforts to correlate segregation behavior with the retention of synaptonemal complex proteins revealed a surprising difference in centromere-associated proteins between oogenesis and spermatogenesis. Differences in the protein components of the meiotic chromosome cohesion complex may influence the fidelity of meiotic chromosome segregation, thus this intriguing sexual dimorphism may provide a partial explanation for the high chromosome error rate during human female meiosis.

MATERIALS AND METHODS

Production of XO female mice

XO female mice and XX sibling controls were produced on two different inbred strain backgrounds using previously described mating schemes involving males prone to meiotic sex chromosome nondisjunction. Specifically, C57BL/6 females were mated to C57BL/6 males carrying the Y* mutation (Eicher et al., 1982) and C3H females were mated to C3H males carrying the X-linked Paf mutation (Lane and Davisson, 1990). Both crosses produce approximately 20% XO females.

Intercrosses of the two inbred strains were made to generate F1 hybrid females that differed only in the origin of their X chromosome: C57BL/6 females were mated to C3H males carrying the Paf mutation to produce XO females with a C57BL/6 X chromosome and C3H females were mated to C57BL/6 males carrying the Y* mutation to produce XO females with a C3H X chromosome.

Oocyte collection, culture and fixation

To assess the segregation of the X chromosome at the first meiotic division, oocytes arrested at metaphase of MI were obtained as follows: meiotically arrested oocytes at the germinal vesicle stage were collected from the ovaries of 3.5-week-old females by piercing the follicles with 26-gauge needles. Oocytes were placed in 10 μl drops of Waymouth’s MB752/1 medium (Gibco BRL) supplemented with 10% fetal bovine serum and 0.23 mM sodium pyruvate, overlaid with mineral oil, and incubated overnight at 37°C in an atmosphere of 5% CO2 in air. After 14-16 hours in culture oocytes were scored for polar body extrusion, indicating completion of the first meiotic division and arrest at MI. Oocytes exhibiting polar body formation were embedded in a fibrin clot attached to a microscope slide as previously described (Hunt et al., 1995), fixed in 2% formaldehyde, 1% Triton X-100, 0.1 mM Pipes, 5 mM MgCl2, and 2.5 mM EGTA for 30 minutes at 37°C, washed in 0.1% normal goat serum (NGS) in PBS for 15 minutes at 37°C, and blocked in 10% NGS containing 0.1% Triton X-100 for 1 hour at 37°C. Fixed oocytes were stored in 10% NGS at 4°C until FISH analysis was performed as described below.

Synaptonemal complex studies

To assess meiotic pairing we used combined immunofluorescence
staining (to visualize the SC) and FISH (to identify the X chromosome and the telomeres). Preparations of oocytes at the pachytene stage were made from ovaries from 16-21 days post coitus (d.p.c.) fetuses and newborns according to the method described by Peters et al. (Peters et al., 1997), and stored at −20°C. To visualize both the SC and the telomeres, indirect immunofluorescence staining was performed with combinations of the following antibodies: (1) SCP2 or SCP3, which recognize two different protein components of the lateral element of the SC, (2) SCP1, which recognizes a protein component of the central element of the SC and (3) CREST serum, which recognizes centromere-associated proteins. For immunostaining, slides were washed in 1% donkey serum in PBS for 1 hour at room temperature, incubated with primary antibody diluted in 1% donkey serum for 2 hours at 37°C, and washed in 1% donkey serum for 1 hour at room temperature. The primary antibodies were detected with an FITC-conjugated donkey anti-goat IgG (Jackson Immunoresearch) for SCP2, Rhodamine-conjugated donkey anti-rabbit IgG (Jackson Immunoresearch) for SCP1 and SCP2, and Rhodamine-conjugated donkey anti-human IgG (Jackson Immunoresearch) for CREST in PBS at 1 hour at 37°C and washed in 1% donkey serum for 1 hour at room temperature. Slides were stored at 4°C in 1% donkey serum until FISH was performed as described below.

Fluorescence in situ hybridization (FISH)

To evaluate X chromosome segregation at the first meiotic division, MII arrested cells were hybridized with the X-linked probe, DXWas70, which recognizes repetitive sequences near the centromere of the mouse X chromosome. The slides were washed in 2× SSC for 5 minutes at room temperature, blotted and covered with 30 μl of Hybrisol VII (Oncor) containing 30 ng of digoxigenin-labeled DXWas70 probe. A coverslip was applied and sealed with rubber cement, the slides were denatured at 85°C for 10 minutes, and hybridized overnight at 37°C in a humid chamber. Following hybridization, slides were washed in 50% formamide/2× SSC at 37°C for 10 minutes followed by a wash of 2× SSC at 37°C for 5 minutes. Hybridized slides were washed in PN buffer for 2 minutes, blocked in PN buffer containing 5% non-fat dry milk and 0.02% sodium azide for 5 minutes at room temperature, detected with an antidigoxigenin-conjugated fluorochrome for 1 hour at 37°C, and washed for 1 hour in PN buffer. Prior to analysis oocytes were counterstained with 100 ng/ml propidium iodide and mounted with 50% glycerol/PBS containing 0.1 μg/ml p-phenylenediamine and a coverslip. Oocytes were visualized on a BioRad MRC600 confocal system.

Hybridization of pachytene preparations was essentially the same as MII arrested oocytes with the following exceptions: (1) in addition to the DXWas70 probe, 10 μl of a biotin-labeled human pan-telomeric probe that recognizes mouse telomeres was applied, (2) slides were denatured for only 5 minutes and (3) an avidin-conjugated fluorochrome was added at the detection step to detect the telomere probe.

Table 1. X chromosome segregation at MI in oocytes from XO females*

<table>
<thead>
<tr>
<th>Type of segregation</th>
<th>Inbred strains</th>
<th>F1 hybrids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Intact (Type)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>369</td>
<td>286 (76%)</td>
</tr>
<tr>
<td>C3H</td>
<td>427</td>
<td>194 (45%)</td>
</tr>
<tr>
<td>X190</td>
<td>209</td>
<td>102 (49%)</td>
</tr>
<tr>
<td>X193H0</td>
<td>232</td>
<td>122 (53%)</td>
</tr>
</tbody>
</table>

*For inbred strains, data from the present analysis (representing 47 and 205 oocytes from XO females produced on the C57BL/6 and C3H backgrounds, respectively) were combined with previously reported data (LeMaire-Adkins and Hunt, 2000).

All F1 hybrid data comes from the present study.
Chromosome preparations of spermatocytes and oocytes at diakinesis/MI

Chromosome preparations of spermatocytes at diakinesis/MI were prepared from testes from 6-week-old males as previously described (Peters et al., 1997). To obtain comparable preparations in the female, oocytes from 3.5-week-old females were collected and cultured as described above. After 4 hours in culture the zona pellucida was removed by brief incubation in 2% pronase (Calbiochem) in Waymouth’s MB752/1. Zona-free oocytes were placed on a slide dipped in 1% paraformaldehyde (pH to 9.2) and dried in a humid chamber overnight.

RESULTS

At MI, the single X chromosome in XO females can either segregate intact to one spindle pole or undergo PSCS at anaphase I, segregating one chromatid to each pole (Fig. 1 and Hunt et al., 1995). However, previous studies in our laboratory suggested that the segregation frequencies were markedly different in XO females produced on C57BL/6 and C3H inbred strain backgrounds (LeMaire-Adkins and Hunt, 2000). To confirm this observation, we conducted a new analysis. The results were not significantly different from the original study, and the combined data demonstrate a highly significant difference in X chromosome segregation between the two inbred strains ($\chi^2=85.06, P<0.001$), with the incidence of PSCS significantly higher on the C3H inbred background (Table 1).

Differences in X chromosome segregation do not reflect X-chromosome specific differences

To determine if the observed segregation differences were due to X-chromosome specific differences, we crossed the two inbred strains to generate F1 hybrid females that were identical genetically, except that one type of female carried a single X chromosome of C57BL/6 origin (XB6 O females) and the other an X of C3H (X C3H O females) origin. X chromosome segregation in the two types of F1 females was virtually identical (Table 1) and resembled one of the progenitor strains. That is, segregation in both F1 females was similar to that observed for the C3H parental strain, but significantly different from the C57BL/6 strain (e.g. XB6 O F1 females versus C57BL/6 females, $\chi^2=49.82, P<0.001$). The altered segregation pattern of the C57BL/6-derived X chromosome on an F1 background excludes an X-chromosome specific effect, and demonstrates that genetic differences in segregation of the univalent X chromosome are mediated by an autosomal gene or genes.
Coordinated segregation of sister chromatids

2421

Does synaptonemal complex formation during prophase influence segregation?

Coordination behavior of the centromeres of sister chromatids at MI (Fig. 1A) is thought to result from events unique to meiotic prophase (Maguire, 1995). Thus, we hypothesized that strain-specific segregation differences might reflect differences in the synaptic behavior of the X chromosome that influence the deposition or retention of cohesion proteins between sister centromeres.

The absence of a homolog prevents the univalent X from undergoing normal synapsis during meiotic prophase but, in the mouse, the single X chromosome has been reported to exhibit frequent non-homologous self-synapsis (Speed, 1986). To determine if differences in synaptic behavior were correlated with subsequent segregation events, we compared X chromosome synaptic configurations on the two inbred backgrounds. Pachytene cells were examined by combining immunofluorescence to visualize the SC and two-color FISH to identify the X chromosome and distinguish the centromeric and telomeric ends of the chromosome. Fig. 2 shows an example of this methodology and of the different types of X chromosome synaptic configurations observed. These configurations are similar to those reported in previous EM studies of oocytes from XO female mice (Speed, 1986).

To compare synapsis on the two inbred backgrounds, configurations were categorized as detailed in Fig. 2. Data from the study of 678 pachytene cells from 17-20 d.p.c. C57BL/6 fetuses and 623 cells from 17-19 d.p.c. C3H fetuses are shown in Table 2. In addition, a total of 361 control oocytes from XX females produced on either the C57BL/6 or C3H strain were scored, and no synaptic defects involving the X bivalent were observed (data not shown). Inspection of the data from the two types of XO females revealed no striking difference in the synaptic behavior of the univalent X chromosome on the two genetic backgrounds (Table 2). However, to specifically assess self-synapsis involving the centromeric region of the chromosome (e.g. to test the hypothesis that PSCS is a consequence of failure of the X to undergo self-synapsis involving the centromeric region of the chromosome), we compared the proportion of cells in which synapsis included the centromere (e.g. ‘fully self-synapsed’ and ‘partially self-synapsed including the centromere’; Fig. 2C,D). Contrary to expectation, the background showing the highest incidence of centromeric self-synapsis (C3H) was also the background with the highest incidence of PSCS. Thus, the hypothesis that nonhomologous synapsis involving the centromeric region of the X chromosome in some way prevents PSCS at MI was not supported by the synaptic profiles of the two different inbred strains.

Because SCP3 only recognizes one component of the SC (the lateral element), we could not conclude that all cases scored as self-synapsed actually involved the formation of a fully mature tripartite SC. To examine this further, we repeated the analysis using the SCP3 antibody in combination with SCP1, an antibody that recognizes a component of the central

---

**Fig. 4.** Percentage of pachytene oocytes with an asynapsed X chromosome on successive days of gestation. The two strains used in this study, C57Bl/6 (squares) and C3H (triangles), exhibit an initial decline in such cells followed by a slight increase while the Swiss Albino strain (diamonds) (used by Speed et al., 1986) exhibits a steady decline.

**Fig. 5.** SCP2/SCP3 localization at diakinesis/metaphase I in spermatocytes and oocytes. (A,C) Chromosomes (blue) immunostained with CREST antiserum, which localizes to the centromeres (green). (B,D) Chromosomes (blue) and SCP3 localization (red). (A,B) Two spermatocytes at diakinesis/MI exhibiting SCP3 localization to all centromeres. (C,D) A comparably fixed oocyte showing CREST staining but no SCP3 localization. (Note: the localization pattern of SCP2 is identical.)
element of the fully formed SC. SCP1 staining was evident on all self-synapsed X chromosomes (Fig. 3D). However, unexpectedly, a significant proportion of cells with an asynapsed X chromosome exhibited SCP1 staining along the length of the X chromosome lateral element (Fig. 3F).

Is there a pachytene checkpoint difference between backgrounds?

It is generally thought that a checkpoint mechanism operates to cull cells with synaptic abnormalities (Odorisio et al., 1998). Perinatal germ cell loss is dramatically increased in XO females by comparison with XX siblings (Burgoyne and Baker, 1985), suggesting that aberrations in X chromosome synapsis increase germ cell loss at this stage. Because self-synapsis of the X chromosome has been suggested to be necessary for progression beyond pachytene (Speed, 1986), we reasoned that differences in the stringency of the checkpoint mechanism on the two genetic backgrounds might account for the observed segregation differences. That is, the increased incidence of PSCS in the C3H strain might reflect a strain-specific relaxation in cell cycle control that allowed a greater number of cells with an asynapsed X chromosome to bypass the pachytene checkpoint mechanism.

The rapidity of germ cell loss in the perinatal ovary precludes the analysis of synaptic behavior among apoptotic cells (e.g. combined tunel assay and immunostaining with antibodies to SC proteins). Thus, to test the hypothesis that the survival of cells with an asynapsed X differed on the two genetic backgrounds, we compared the proportion of cells with an asynapsed X among late pachytene cells. For this analysis, we scored pachytene cells in the perinatal ovary at a time at which, for both strains, greater than 60% of prophase cells had progressed beyond pachytene (e.g. 20 d.p.c. for C57BL/6 and 19 d.p.c. for C3H). Our expectation was that the accumulation of pachytene cells with an asynapsed X chromosome (e.g. pachytene arrest followed by apoptosis) or their rapid elimination would skew the distribution of synaptic configurations. However, although temporal fluctuation in the number of cells with an asynapsed X chromosome was observed on both backgrounds (see Fig. 4, and Discussion)

Table 2. Frequency of different X chromosome synaptic configurations among pachytene cells from XO females produced on two different inbred backgrounds

<table>
<thead>
<tr>
<th>Fetus</th>
<th>d.p.c.</th>
<th>n</th>
<th>Asynapsed</th>
<th>Fully self-synapsed</th>
<th>Partially self-synapsed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>including centromere</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>17</td>
<td>161</td>
<td>107 (66%)</td>
<td>14 (9%)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>223</td>
<td>97 (43%)</td>
<td>66 (30%)</td>
<td>23 (10%)</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>126</td>
<td>60 (48%)</td>
<td>34 (27%)</td>
<td>8 (6%)</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>168</td>
<td>87 (52%)</td>
<td>35 (21%)</td>
<td>21 (13%)</td>
</tr>
<tr>
<td></td>
<td>17</td>
<td>233</td>
<td>126 (54%)</td>
<td>49 (21%)</td>
<td>10 (4%)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>182</td>
<td>78 (43%)</td>
<td>58 (32%)</td>
<td>7 (4%)</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>208</td>
<td>94 (45%)</td>
<td>81 (39%)</td>
<td>14 (7%)</td>
</tr>
</tbody>
</table>
Coordinated segregation of sister chromatids

Under normal meiotic conditions, where bivalents rather than univalents are segregated at MI, premature separation of sister

<table>
<thead>
<tr>
<th>Background</th>
<th>Stage (d.p.c.)</th>
<th>n</th>
<th>SCP1 staining</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Present</td>
<td>Absent*</td>
</tr>
<tr>
<td>C3H</td>
<td>Early pachytene (17)</td>
<td>46</td>
<td>5 (11%)</td>
<td>41 (89%)</td>
</tr>
<tr>
<td></td>
<td>Late pachytene (19)</td>
<td>41</td>
<td>19 (46%)</td>
<td>22 (54%)</td>
</tr>
<tr>
<td>C57BL/6</td>
<td>Late pachytene</td>
<td>30</td>
<td>12 (40%)</td>
<td>18 (60%)</td>
</tr>
</tbody>
</table>

*Includes oocytes with no or only very weak SCP1 staining.
chromatids results in the production of aneuploid gametes. Indeed, our interest in the factors that predispose to this type of segregation error is motivated by the fact that PSCS has been postulated to be the major mechanism of age-related nondisjunction in our species (Angell, 1997; Angell et al., 1994; Wolstenholme and Angell, 2000). This aberrant MI segregation behavior requires two events that normally do not occur until MI: (1) the differentiation of functionally distinct kinetochores at the centromeres of sister chromatids and (2) the premature release of cohesion between sister centromeres at anaphase. The coordinated segregation of sister chromatids during mammalian female meiosis remains poorly understood. Hence differences in the propensity for premature segregation of X chromatids in oocytes from XO females produced on two different inbred strains provide a genetic tool for understanding the factors that influence sister chromatid behavior at MI.

**An autosomal, trans-acting factor influences sister chromatid segregation at MI**

Based on meiotic studies in lower eukaryotes, it seemed likely that X chromosome segregation differences would reflect subtle differences between the X chromosomes on the two inbred strains. That is, detailed studies of univalent chromosomes in the grasshopper suggest that the patterns of meiotic behavior and segregation of univalent chromosomes reflect chromosome-specific features (Rebollo et al., 1998). Similarly, in yeast, mutants with a single division meiotic phenotype exhibit a mixed segregation pattern, with some chromosomes exhibiting a proclivity for intact and others for equational segregation. Experiments in which centromeric sequences were transferred between chromosomes with different segregation patterns demonstrated that the segregation phenotype is a property of the centromere (reviewed in Simchen and Hugerat, 1993).

To test for X-chromosome specific differences, we generated F1 hybrid females carrying either a single X chromosome derived from the parental C57BL/6 or the C3H inbred strain. With the exception of the X chromosome, the two types of F1 females were genetically identical. Meiotic studies revealed no difference in the segregation pattern of the univalent X chromosome between the F1 females. Thus, contrary to expectation, X-chromosome specific differences are not a plausible explanation for the observed segregation differences. We conclude that the actions of an autosomal gene or genes influence X chromosome segregation. Further, since the segregation phenotypes of both F1 females and one of the parental strains were identical, this trans-acting genetic effect appears to be dominant.

**Synaptic behavior at pachytene is not a reliable predictor of segregation**

Based on studies of maize mutants, Maguire postulated that the proteins of the synaptonemal complex confer the specialized meiotic centromere cohesion requirements necessary for proper chromosome segregation (reviewed in Maguire, 1995). Support for this hypothesis can be drawn from a variety of sources, including (1) mutations with precocious separation of sister chromatids indicative of complete loss of sister chromatid cohesion (e.g. in Drosophila, S. pombe and Sordaria macrospora), which also exhibit defects in synopsis and/or recombination (reviewed in Moore and Orr-Weaver, 1998), (2) mutations in the SC components of S. cerevisiae (e.g. red1, zip1 and hop1), which exhibit a slight increase in PSCS at MI (Hollingsworth and Byers, 1989; Smith and Roeder, 1997; Sym and Roeder, 1994), (3) immunolocalization studies in male mammals, which demonstrate that remnants of the lateral element of the SC are retained at the centromere until anaphase II (Dobson et al., 1994; Offenberg et al., 1998) and (4) recent observations suggesting that the SC components SCP2 and SCP3 interact with the cohesion proteins SMC1 and SMC3 (Eijpe et al., 2000).

We hypothesized that genetic differences in MI segregation behavior of the univalent X chromosome might reflect differences in the synaptic behavior of the X chromosome that influence the deposition or retention of cohesion proteins at the centromere. The behavior of the univalent X during prophase is intriguing; although the X has no homolog, previous studies have demonstrated that the univalent chromosome frequently exhibits a fold-back, self-synaptic behavior at pachytene (Speed, 1986). Indeed, self-synapsis has been suggested to be essential for germ cell survival, with the high incidence of cells with an asynapsed X providing an explanation for the increased perinatal germ cell loss observed in the XO mouse (Burgoyne and Baker, 1985; Speed, 1986).

Detailed analysis of pachytene stage oocytes revealed no striking difference in the self-synaptic behavior of the X chromosome on the two genetic backgrounds. Our expectation was that the strain with the highest incidence of PSCS would have more cells in which self-synapsis excluded the centromeric region of the chromosome. Although we did observe a small difference between the two genetic backgrounds, it was the opposite of our prediction; the C3H background, which showed a higher frequency of PSCS, had a slightly increased frequency of cells in which the X chromosome centromeric region was self-synapsed.

The analysis of X chromosome synopsis is complicated by the fact that cell selection is occurring, and the assumption that all pachytene configurations are equally likely to survive is almost certainly not valid. Although the exact timing of the so called ‘pachytene’ checkpoint control mechanism is not known, a previous study of XO mice by Speed suggested a decline in cells with an asynapsed X with advancing developmental age (Speed, 1986). Thus, we attempted to enrich for late pachytene cells to test the hypothesis that selection against cells with an asynapsed X chromosome might differ on the two backgrounds, and that enhanced survival of such cells might be associated with an increased frequency of PSCS. We observed a slight but non-significant difference in the frequency of pachytene cells with an asynapsed X chromosome; however, the background with the higher level of PSCS (C3H) had the lowest frequency of such cells. Thus, our studies provided no evidence of relaxed cell selection.

The observation by Speed of a decline in cells with an asynapsed X chromosome with advancing developmental age (Speed, 1986), is consistent with selection against this category of cells. In contrast, we observed an initial decline followed by a slight increase at the most advanced developmental ages (Fig. 4). Although the difference between Speed’s study and our own may be due to strain differences, our subsequent studies suggested that the apparent increase we observed reflected a change over time in the conformation of the X chromosome.
That is, in immunolocalization studies using the SCP1 antibody to assess formation of mature SC, we observed SCP1 staining on asynapsed X chromosomes in some cells. This may simply reflect a pattern of binding similar to that observed on the unsynapsed portion of the X as occurs during male meiosis (P. Moens, personal communication). However, a comparison of early and late pachytene cells demonstrated a significant increase in such cells with advancing developmental age. Hence, we suggest that the presence of SCP1 is a remnant of self-synthesis, and that the apparent asynapsed configuration is analogous to the synaptic adjustment described in tandem duplications (Moses and Poorman, 1981), or the residual SCP1 observed on separated cores of autosomal chromosomes at diplotene (Moens and Spyropoulos, 1995). However, there was no difference between strains in the frequency of such cells, hence it does not provide insight to the genetic background effect on segregation.

Evidence that meiotic centromeric cohesion is sexually dimorphic

Given the complications of cell selection and the apparent changes in the synaptic configuration of the X over time, further studies of meiotic prophase seemed unlikely to provide insight to the segregation behavior of the X chromosome. However, previous immunolocalization studies in the male demonstrated persistence of the lateral element proteins SCP2 and SCP3 at the centromere until anaphase II (Dobson et al., 1994; Offenberg et al., 1998). Thus we reasoned that PSCS segregation might be explained by the premature disappearance of these proteins from the X chromosome centromere. However, to our surprise, immunolocalization studies of MI prometaphase cells failed to detect either SCP2 or SCP3 in oocytes from XO or control females. Although initially we assumed that this was a technical artifact, subsequent studies demonstrated that this was not the case. First, using a modified fixation protocol, we were able to demonstrate SCP2 and SCP3 localization at the centromeres in spermatocytes but not oocytes at the diakinesis/MI stage. Second, the SCP3 protein was not detectable in germline vesicle stage oocytes. Third, an analysis of fetal oocytes that had progressed beyond pachytene and were entering dictyate arrest suggested that, as the SC disassembles, foci of SCP2 and SCP3 proteins remain briefly associated with the centromere but do not persist throughout the period of meiotic arrest. Clearly, these studies do not rule out the possibility that a small amount of these proteins persists but is below the level of detection by immunostaining and western analysis. Nevertheless, it seems more likely that the role of these SC proteins is limited to meiotic prophase in the female. Thus, the analysis of other meiosis-specific centromere-associated proteins – for example, the recently identified cohesin component, Rec8 (Watanabe and Nurse, 1999) or a homolog of yeast monopolin (Toth et al., 2000), a kinetochore protein required for normal MI segregation – will be necessary to understand the segregation behavior of the univalent X chromosome.

Our observations suggest an important sexual dimorphism in centromere associated proteins during the first meiotic division. Disappearance at diplotene of the protein now known as SCP3 was reported in an early localization study of rat oocytes (Dietrich et al., 1992). However, this report appears to have gone unnoticed, as persistence of SCP3 at the centromere throughout MI is frequently mentioned as though it is a universal feature of mammalian meiosis. The disappearance of the two lateral element components, SCP2 and SCP3, coincident with entry into meiotic arrest suggests that, at least in the female, these proteins are not essential components of the cohesion complex that mediates the specialized MI behavior of sister centromeres. This difference between male and female meiosis may have functional consequences, e.g. the disappearance of these proteins prior to the first division may contribute to the vulnerability of the female meiotic process by increasing the likelihood of PSCS.

Interestingly, the phenotype of the recently reported SCP3 knockout mouse suggests that sex-specific differences in the meiotic role of this protein may not be limited to the meiotic divisions: the SCP3 null mutant male is sterile, exhibiting synaptic failure and meiotic arrest at the zygotene stage (Yuan et al., 2000). In contrast, the SCP3 null female is apparently fertile. Detailed meiotic studies of the mutant female have yet to be conducted. It will not only be interesting to learn how homolog synapsis and recombination proceed in the absence of this protein, but also whether chromosome segregation is affected. Thus, the SCP3 protein and other SC components may provide long-sought insight to sex-specific differences in meiotic cell division in mammals.

We are grateful to T. Hassold and H. F. Willard for helpful discussions and comments on the manuscript. In addition, we thank T. Ashley, C. Heyting, R. Jessberger and P. Moens for generous gifts of the synaptonemal complex antibodies used in these studies. This work was supported by National Institutes of Health grant R01 HD31866 to P.A.H.

REFERENCES


