Nonrandom Segregation of the Mouse Univalent X Chromosome: Evidence of Spindle-Mediated Meiotic Drive

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ABSTRACT

A fundamental principle of Mendelian inheritance is random segregation of alleles to progeny; however, examples of distorted transmission either of specific alleles or of whole chromosomes have been described in a variety of species. In humans and mice, a distortion in chromosome transmission is often associated with a chromosome abnormality. One such example is the fertile XO female mouse. A transmission distortion effect that results in an excess of XX over XO daughters among the progeny of XO females has been recognized for nearly four decades. Utilizing contemporary methodology that combines immunofluorescence, FISH, and three-dimensional confocal microscopy, we have readdressed the meiotic segregation behavior of the single X chromosome in oocytes from XO females produced on two different inbred backgrounds. Our studies demonstrate that segregation of the univalent X chromosome at the first meiotic division is nonrandom, with preferential retention of the X chromosome in the oocyte in ~60% of cells. We propose that this deviation from Mendelian expectations is facilitated by a spindle-mediated mechanism. This mechanism, which appears to be a general feature of the female meiotic process, has implications for the frequency of nondisjunction in our species.

FIDELITY of chromosome segregation during the meiotic cell divisions is essential to normal reproduction. Thus, the high frequency of chromosome segregation errors during human female meiosis is an enigma. An estimated 10–25% of all human pregnancies are aneuploid as a result of errors during female meiosis (Hassold *et al.* 1996). This incidence of chromosome abnormalities is at least an order of magnitude greater than that observed in the next-best-studied female mammal, the mouse, and orders of magnitude higher than that observed in lower eukaryotes.

Despite the incidence and obvious clinical importance of human aneuploidy, we remain ignorant of the mechanism(s) underlying meiotic nondisjunction. In large part, this reflects the difficulty in obtaining and studying human oocytes, which has hampered attempts to directly analyze female meiotic chromosome segregation. Some cytogenetic data on human oocytes are available; however, virtually all have been derived from analyses of "spare" oocytes retrieved from *in vitro* fertilization procedures, making their relevance to the *in vivo* situation uncertain (reviewed in Hassold *et al.* 1996). Thus, most inferences regarding the incidence and origin of human aneuploidy have been based on studies of clinically recognized fetal wastage, *i.e.*, miscarriages. However, due to the early lethality of autosomal monosomies in our species, these inferences rely on trisomic conceptions, with the implicit assumption that monosomy and trisomy occur in equal frequency. In fact, the validity of this assumption has never been compellingly demonstrated. Direct studies of human oocytes provide little insight, since chromosome loss is an unavoidable technical problem; hence, in these analyses monsomies are typically disregarded.

Is there reason to suspect that the incidence of monosomy and trisomy might be different? Transmission ratio distortion, *i.e.*, significant deviation from Mendelian expectations, has fascinated geneticists for decades. Transmission ratio distortion can result either from genotypic influences that affect gamete function or embryo viability [*e.g.*, the Drosophila *Segregation Distorter* system (reviewed in Ganetzky 1999); the mouse *t* complex (reviewed in Silver 1993), and, most likely, the deviation from Mendelian inheritance observed in interspecific mouse crosses (reviewed in Montagutelli *et al.* 1996)] or from a "true" distortion in meiotic segregation [*e.g.*, knob chromosomes in maize (Rhoades and Dempsey 1966), the homogeneously staining region (HSR) inverted duplication in wild mouse populations (Ruvinsky 1995), the mouse *Om* mutation (Pardo-Manuel de Villena *et al.* 2000), and B chromosome transmission in some species (Jones 1991)]. An additional type of true segregation distortion, chromosome segregation errors during female meiosis leading preferentially to trisomy, has also occasionally been postulated (*e.g.*, Day and

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Taylor 1998). The implication of this suggestion is a chromosome segregation effect in which meiotic nondisjunction would more likely result in the retention of an additional chromosome in the oocyte than in the polar body (Figure 1). However, data to evaluate this suggestion do not exist.

The most widely studied transmission distortion effect in female mammals is a variation on this preferential meiotic segregation, namely, the apparent nonrandom X chromosome segregation observed in the XO mouse. This segregation distortion effect has been recognized since the first breeding studies of XO mice were conducted in the early 1960s (Cattanach 1962). Unlike human 45, X, or Turner syndrome females, XO mice are fertile; however, they produce significantly more XX than X0 daughters (Cattanach 1962). The paucity of XO offspring has been the subject of numerous investigations, with some reports attributing it to reduced viability of XO fetuses and others suggesting that it results from nonrandom segregation of the X chromosome at the first meiotic division (Kaufman 1972; Luthardt 1976; Brook 1983; Hunt 1991; Thornhill and Burgoyne 1993; Sakurada *et al.* 1994).

Cytogenetic studies conducted in the 1970s provided evidence of preferential retention of the X chromosome in the oocyte at the first meiotic division (Kaufman 1972; Luthardt 1976). The interpretation of these data as evidence of nonrandom segregation, however, was based on the assumption that the single X chromosome segregated intact to one pole at the first meiotic division. Subsequent cytogenetic studies suggested that the segregation pattern of the univalent X chromosome is more complex: Sakurada *et al.* provided evidence of an increased incidence of single chromatids among oocytes that had completed the first meiotic division, although the chromosome(s) involved in the segregation abnormality were not identified (Sakurada *et al.* 1994). By combining fluorescence *in situ* hybridization (FISH), immunofluorescence staining, and three-dimensional microscopy to study intact oocytes, we found that the single X chromosome in oocytes from XO females does indeed segregate intact to one spindle pole in the majority of oocytes. However, in a significant minority of cells, the X chromosome undergoes an equational division, segregating sister chromatids at the first meiotic division (Figure 1). This segregation pattern, which results in one X chromatid in the oocyte and the other in the polar body, was observed in 30% of MII arrested oocytes (LeMaire-Adkins *et al.* 1997). On the basis of this observation, and taking into account the generally poor morphology of chromosome preparations of MII stage oocytes, it seemed likely that the previous reports of nonrandom X chromosome segregation were the result of scoring errors. That is, the scoring of the single X chromatid that results from equational segregation as a whole chromosome would artificially inflate the number of cells in which the X chromosome segregated to the oocyte. To our surprise, a reanalysis of our own data involving only that subset of oocytes in which the univalent X segregated intact at the first division suggested a skewed pattern of segregation, with the X more often remaining in the oocyte (Figure 1). In this article we present the results of additional studies of XO mice produced on two different genetic backgrounds that confirm this segregation distortion effect and a new model, the "dominant pole" hypothesis, to explain this segregation phenomenon. We believe that this simple mechanical explanation provides a plausible explanation not only for the behavior of the univalent X chromosome in oocytes from XO female mice, but also for previously reported instances of apparent segregation distortion described for both human and mouse female carriers of structural rearrangements. Finally, because these observations challenge the assumption that monosomy and trisomy are equally likely events during mammalian female meiosis, the implications of this model to human aneuploidy are discussed.

MATERIALS AND METHODS

Production of XO female mice: Oocytes from XO female mice and XX sibling controls produced on the C57BL/6J and C3H/HeSnJ inbred strain backgrounds were utilized for segregation analysis. The production of XO females on both genetic backgrounds relied on previously described mutations that result in a high frequency of failure of paternal X chromosome transmission, *i.e.*, the presence of the structurally abnormal chromosome, Y*, on the C57BL/6 background (Eicher *et al.* 1991) and the presence of the X-linked mutation, patchy fur (*Paf*), on the C3H background (Lane and Davisson 1990). XO and XX females produced on the C3H background are distinguishable by coat color, whereas those produced on the C57BL/6 background are phenotypically indistinguishable. To determine the karyotype of C57BL/6 females and to confirm the karyotype of C3H females, bone marrow specimens were collected at the time of autopsy and processed for karyotypic analysis (Eicher and Washburn 1978).

Oocyte collection and *in vitro* maturation: The segregation behavior of the X chromosome at the first meiotic division was evaluated in oocytes that had completed MI and were arrested at metaphase of MII. To obtain MII arrested oocytes, germinal vesicle (GV) stage oocytes were collected from the ovaries of 4-week-old female mice and meiotically matured *in vitro*. GV stage oocytes were liberated from the ovary by piercing antral follicles with 26-gauge needles. Oocytes were cultured in 10-μl drops of Waymouth's MB 752/1 medium (GIBCO BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum and 0.23 mm sodium pyruvate, overlaid with Squibb mineral oil, and incubated at 37° in an atmosphere of 5% CO_2 in air. Oocytes exhibiting a first polar body after 16–18 hr in culture were fixed for analysis.

Fixation, immunofluorescence, and *in situ* hybridization of oocytes: Prior to fixation, oocytes were embedded in a fibrin clot (bovine fibrinogen type IV, Calbiochem, La Jolla, CA; bovine thrombin, Sigma, St. Louis) attached to a microscope slide as previously described (Hunt *et al.* 1995). To control for hybridization efficiency, oocytes from XO and XX females were placed in separate clots on the same slide. Immediately after clotting, oocytes were fixed in 2% formaldehyde, 1% Triton X-100, 0.1 mm Pipes, 5 mm $MgCl_2$, and 2.5 mm EGTA

for 30 min at 37°. Following fixation, oocytes were washed in 0.1% normal goat serum (NGS; GIBCO BRL/phosphate buffered saline (PBS) for 10 min, blocked in a PBS wash solution containing 10% NGS, 0.02% sodium azide and 0.1% Triton X-100 for a minimum of 1 hr at 37°, and stored in the blocking solution at 4°.

To visualize the meiotic spindle for confirmation that all oocytes were arrested at MII metaphase, oocytes were incubated in a 1:2000 dilution of a primary mouse monoclonal antibody to acetylated tubulin (Sigma) for 1 hr at 37°, washed in 5% NGS/PBS for 15 min at 37°, blocked in 10% NGS/PBS for 45 min at 37°, and incubated in a 1:100 dilution of a Rhodamine- or Cy5-conjugated goat anti-mouse IgG (Accurate Chemical, Westbury, NY) for 1 hr at 37°. Following detection with the secondary antibody, oocytes were washed for 15 min at 37° and stored in 10% NGS/PBS until analysis. Immediately prior to analysis, oocytes were stained with 100 ng/ml propidium iodide and a coverslip applied with mounting medium (50% glycerol/4× SSC containing 0.1 μg/ml *p*-phenylenediamine) and sealed with rubber cement. The MII spindle and associated chromosomes and the chromosomes in the first polar body were visualized on a Zeiss (Thornwood, NY) Axioplan microscope or a Bio-Rad (Hercules, CA) MRC 600 confocal microscope.

Following immunofluorescence staining and analysis, oocytes were hybridized with the X chromosome-specific probe, DXWas 70 (American Type Culture Collection, Rockville, MD) as described previously (Hunt *et al.* 1995). DXWas70, which recognizes repetitive pericentromeric sequences on the mouse X chromosome (Disteche *et al.* 1987), was labeled with digoxigenin (Boehringer Mannheim, Indianapolis) and detected with FITC-conjugated antidigoxigenin (Boehringer Mannheim). To analyze the segregation behavior of the single X chromosome at the first meiotic division, hybridized oocytes were analyzed on a Bio-Rad MRC 600 confocal microscope using three-dimensional optical sectioning to determine the position of the fluorescently labeled X chromosome or of the two X chromatids in individual oocytes.

Activation of oocytes for segregation analysis during MII: To determine if nonrandom segregation of single X chromatids occurred at the second meiotic division, MII arrested oocytes were collected from the oviducts of superovulated females and artificially activated to induce completion of MII. Four-week-old XO and XX females produced on the C57BL/6 background were injected with 2.5 IU of pregnant mare serum gonadotropin (Sigma), followed 44–48 hr later by 5 IU of human chorionic gonadotropin (Sigma). MII arrested oocytes were recovered from the oviducts ~16 hr after the second injection, denuded of adherent cumulus cells by a brief exposure to 200 μg/ml hyaluronidase (Sigma) in culture medium, washed through two changes of fresh medium, and incubated at 37° in 5% CO_2 in air for 2–3 hr. Oocytes were artifically activated by placing them in 7% ethanol in PBS for 5 min. Following ethanol exposure, oocytes were washed in fresh medium, incubated, and monitored at 30-min intervals for evidence of polar body formation. Oocytes were fixed at the first sign of polar body extrusion to capture cells at telophase, thus ensuring that the group of chromosomes being extruded in the second polar body was distinguishable from those extruded in the first polar body. Oocytes were fixed and immunostained as described above and only chromosomes positioned within the telophase spindle apparatus were scored as products of the second meiotic division.

RESULTS

Segregation of the univalent X chromosome during the first meiotic division: We recently reported the results of MI segregation analysis of the univalent X chromosome from studies of intact MII arrested oocytes from XO females (LeMaire-Adkins *et al.* 1997). These studies demonstrated a complex segregation pattern, with the X chromosome segregating intact to one pole in approximately two-thirds of oocytes and undergoing premature equational segregation of X chromatids in the remaining one-third (Figure 1). To determine if there was any evidence of nonrandom segregation among the subset of oocytes in which the X chromosome segregated intact at MI, we evaluated the direction of segregation (*e.g.*, to the oocyte or to the polar body) of the intact X chromosome in the previously published data set. Of 148 oocytes collected from XO females produced on the C57BL/6 background, the univalent X chromosome (hereafter referred to as X^{B6}) segregated intact at MI in 103 (Table 1a); in 69 of the 103 oocytes (67%) the univalent X chromosome remained in the oocyte (Figure 2, a and b) and in the remaining 34 cells (33.1%) the X chromosome segregated to the polar body (Table 1a). This distribution is highly significantly different from Mendelian expectations ($\chi^2_1 = 11.9$; $P < 0.005$).

Nonrandom segregation at MI is a feature of univalent X chromosomes on two different inbred genetic backgrounds: To confirm the apparent nonrandom segregation behavior of the univalent X chromosome, we conducted a second, independent analysis of XO oocytes produced on the C57BL/6 inbred background. A total of 323 oocytes from XO females and 415 from XX sibling controls were analyzed (Table 1b). Intact segregation of the univalent X^{B6} chromosome (Figure 1) at MI was observed in 249 (77.1%) of the 323 oocytes from XO females; of these, the X chromosome was retained in the oocyte in 150 (60.2%) oocytes and segregated to the polar body in the remaining 99 (39.8%). This distribution is not significantly different from the previous data set ($\chi^2_1 = 2.08$; $P > 0.10$) but is significantly different from Mendelian expectations ($\chi^2_1 = 10.4$; $P < 0.005$). In control oocytes, one X chromosome signal was present in the oocyte and one was present in the polar body in 406 (97.8%) cells (Table 1b), indicating normal segregation of X homologs at MI. In the remaining 9 (2.2%) oocytes, only one X chromosome signal was evident, indicating hybridization failure rather than X chromosome nondisjunction and suggesting a hybridization efficiency of >97%.

To determine whether genetic background diminished or eliminated the segregation distortion effect, we conducted segregation studies of XO oocytes produced on a different inbred genetic background. A total of 222 oocytes from XO females and 104 from XX sibling controls produced on the C3H inbred genetic background were analyzed (Table 1b). Unlike the univalent X^{B6} chromosome, intact segregation of the univalent X chromosome on the C3H background (X^{C3H}) was not observed in the majority of oocytes. Of the 222 oocytes

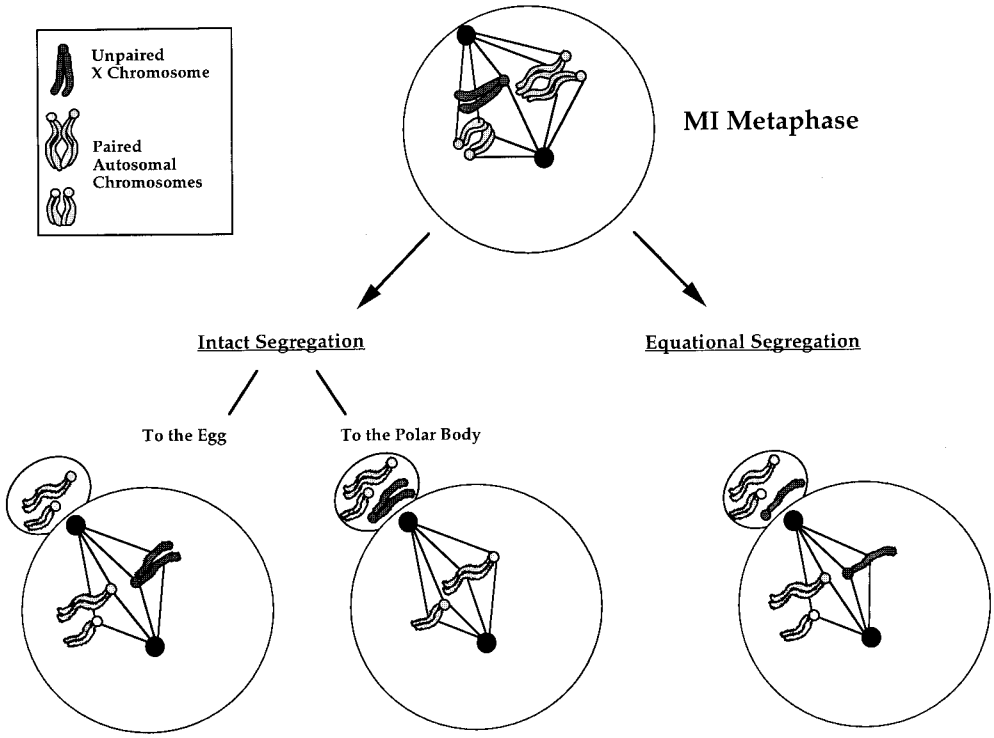

FIGURE 1.—MI segregation of the univalent X chromosome in oocytes from XO females. Schematic of oocyte containing a univalent X chromosome at metaphase I (top), illustrating the two types of segregation that can occur (bottom). Intact segregation: Both X chromatids segregate intact to one spindle pole and, at MII metaphase, both X chromatids are either aligned on the MII spindle (bottom left) or in the polar body (bottom center). Equational segregation: Sister chromatids segregate to opposite poles and, at MII metaphase, one chromatid is present in the oocyte and one is in the polar body.

analyzed, intact segregation was observed in 97 (43.7%) oocytes and equational segregation in the remaining 125 (Table 1b). The difference in the frequency of intact and equational segregants on the two inbred strains was highly significant ($\chi^2_1 = 64.5$; $P < 0.005$). Nevertheless, among oocytes in which the X chromosome segregated intact to one pole at MI, a segregation distortion affect similar to that observed on the C57BL/6 background was evident on the C3H background; the X chromosome was retained in the oocyte in 58 (59.8%) cells and segregated to the polar body in the remaining 39 (40.2%). The deviation from random segregation approached significance on the C3H background ($\chi^2_1 = 3.7$; $P < 0.1$) and the pattern of intact segregants was

TABLE 1

Segregation of the univalent X chromosome at MI

		Segregation pattern		Segregation of intact X	
		Equational (%)	Intact (%)	Egg (%)	Polar body (%)
a. X chromosome segregation in oocytes from XO females produced on the C57BL/6 background					
C57BL/6	XO females	45	103	69	34
	$n = 148$[a]	(30.4)	(69.6)	(66.9)	(33.1)
b. X chromosome segregation in oocytes from XO and XX sibling controls produced on the C57BL/6 and C3H inbred backgrounds					
C57BL/6	XO females	73	249	150	99
	$n = 323$[b]	(22.6)	(77.1)	(60.2)	(39.8)
	XX females	—	406	—	—
	$n = 415$[c]		(97.8)		
C3H	XO females	125	97	58	39
	$n = 222$	(56.3)	(43.7)	(59.8)	(40.2)
	XX females	—	104	—	—
	$n = 104$		(100)		

[a] Data from LEMAIRE-ADKINS *et al.* (1997).
[b] One oocyte failed to hybridize.
[c] In 9 (2.2%) control oocytes only one X chromosome signal was observed. Assuming that this reflects hybridization failure rather than X chromosome aneuploidy, hybridization efficiency for this study was >97%.

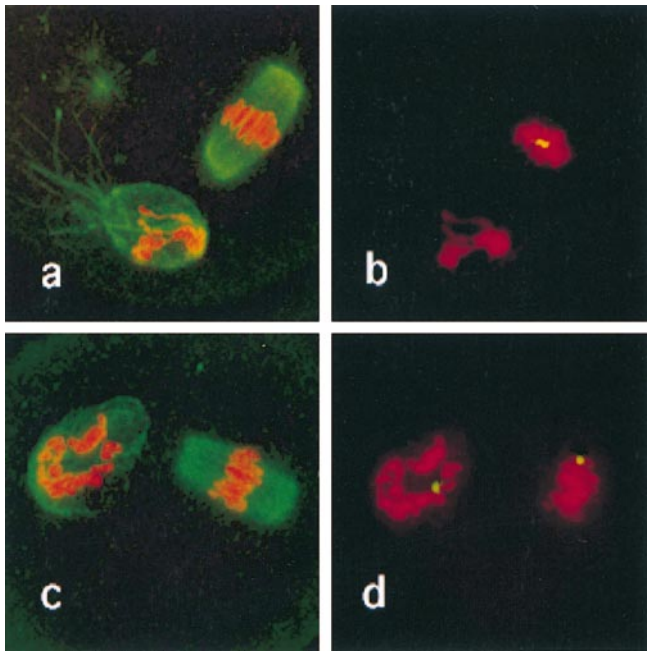

FIGURE 2.—Combined immunofluorescence and FISH analysis of oocytes from XO females. Confocal images of oocytes that have completed the first meiotic division and are arrested at MII metaphase. (a, c) Immunofluorescence staining with an antibody to β-tubulin (green) and chromatin staining with propidium iodide (red) allow visualization of the MII spindle and aligned chromosomes as well as the group of chromosomes that segregated to the polar body (left-most structure in both images). (b, d) Images of the chromatin (red) from the same oocytes following FISH with an X chromosome-specific probe (yellow). (b) The result of intact segregation at MI, showing signals for both X chromatids among chromosomes aligned at the MII spindle equator. (d) The result of equational segregation at MI, showing one X chromatid signal in the oocyte (right) and the other in the polar body (left).

not different for the univalent X^{B6} and X^{C3H} chromosomes ($\chi^2_1 = 0.19$; $P > 0.8$). All 104 oocytes from control females exhibited normal segregation of homologous X chromosomes.

Segregation of individual X chromatids at MII: To determine if the single X chromatid resulting from equational segregation of the univalent X chromosome at MI exhibited nonrandom segregation behavior at MII, we analyzed the behavior of single X^{B6} chromatids at MII anaphase. A total of 216 MII arrested oocytes from XO females were treated with ethanol to induce completion of the second meiotic division (Table 2). A total of 124 (57.4%) oocytes exhibited signs of second polar body extrusion and, of these, 71 were at telophase at the time of fixation and exhibited clearly distinguishable oocyte and polar body chromosomes. Sixteen (22.5%) of the 71 oocytes were the product of an equational division at MI and exhibited a single X chromatid signal either in the oocyte or the forming second polar body. This is consistent with the frequency of equational segregation observed in studies of MII arrested oocytes on the C57BL/6 background (Table 1b). Of the 16 cells in which a single chromatid was segregating at MII, 7 exhibited segregation to the oocyte (Figure 3, a and b) and 9 to the second polar body.

X chromosome segregation was evaluated in 227 ethanol-treated control oocytes. Of the 113 oocytes that exhibited signs of second polar body extrusion, 69 (60%) cells were at telophase and in all 69 cells segregation appeared normal, with one chromatid segregating to the oocyte and one to the second polar body (data not shown).

DISCUSSION

Nonrandom meiotic segregation of the univalent X chromosome was first postulated by Cattanach as the mechanism responsible for the excess of XX daughters among the offspring of XO female mice (CATTANACH 1962). Subsequently, several studies have provided support for the hypothesis, but others have suggested that the reduced number of XO daughters reflects *in utero* selection (KAUFMAN 1972; LUTHARDT 1976; BROOK 1983; HUNT 1991; THORNHILL and BURGOYNE 1993; SAKURADA *et al.* 1994). Recent meiotic studies of oocytes from XO females in our laboratory demonstrated that segregation of the single X chromosome during the first meiotic division is more complex than previously thought, with the X chromosome segregating "intact" in the majority of cells but exhibiting a pattern typical of MII (*i.e.*, the segregation of sister chromatids) in a significant minority (HUNT *et al.* 1995). This unexpected segregation behavior suggests that, in the early cytogenetic studies of MII oocytes from XO females, the observations may have been misinterpreted. That is, using conventional cytogenetics, it is likely that an MII oocyte con-

TABLE 2

Analysis of segregation at MII in ETOH-activated oocytes

Total MII arrested oocytes activated with ETOH	216
Oocytes captured at telophase	71
Inferred[a] MI segregation	
Intact	55 (77.5%)
Equational	16 (22.5%)
Segregation of single X chromatid	
Chromatid in egg	7 (43.8%)
Chromatid in p.b.	9 (56.3%)

[a] Fifty-five oocytes were classified as "intact segregation" at MI: In 45 (82%) an X chromosome signal was present in both groups of telophase chromosomes, indicating equational MII segregation of an intact X. In the remaining 10 (18%) oocytes no signal was present in the telophase chromosomes, reflecting intact segregation of the X to the polar body at MI. Sixteen oocytes were classified as "equational segregation" at MI. In these oocytes a single X signal (representing a single X chromatid resulting from equational segregation at MI) was observed.

taining a single X chromatid (as a result of sister chromatid segregation at MI) would have been scored as retaining the intact X chromosome; thus, the apparent proportion of MII oocytes containing an X chromosome would have been artifactually inflated.

Unlike the previous cytogenetic studies, our analyses were conducted using molecular cytogenetic methods to study intact oocytes. This approach has several advantages over conventional cytogenetic techniques. First, it eliminates the technical artifact of chromosome loss. Second, the use of a FISH probe to repetitive sequences on the proximal part of the X chromosome allows us to distinguish a single X chromatid from an intact X chromosome and thus to differentiate the two patterns of MI segregation. Third, since both products of the MI division are present in all cells, scoring accuracy can be confirmed by the corroboration of results for the oocyte and the polar body.

To assess the question of nonrandom X chromosome segregation during MI, we first reanalyzed data from a previously published data set of 148 oocytes (LeMaire-Adkins *et al.* 1997). To our surprise, when the 45 oocytes that exhibited an equational segregation pattern were excluded, a significant departure from random segregation was observed in the remaining group of 103 oocytes: The intact X chromosome was retained in the oocyte in approximately two-thirds (69/103, or 67%) of cells and segregated to the polar body in the remaining one-third (34/104, or 33%). This observation prompted more detailed studies.

The results of additional studies of X chromosome segregation in over 500 oocytes obtained from XO females produced on two different inbred strain backgrounds are remarkable in two respects: First, we observed a significant difference in the propensity for equational division of the X chromosome at MI on the two genetic backgrounds. Second, despite this difference, the segregation distortion effect for intact X chromosome segregants observed in our original data set was confirmed on both genetic backgrounds.

What is the basis of the difference in "intact" *vs.* "equational" MI segregation of the X chromosome between inbred strains? Based on studies in other species, at least two explanations for the strain-specific difference in X chromosome segregation seem plausible: First, the difference may reflect structural differences between the two X chromosomes. In the budding yeast, *Saccharomycies cerevisiae*, centromeric sequences influence meiotic segregation (reviewed in Simchen and Hugerat 1993). Specifically, in mutants in which the first meiotic division is bypassed, there is significant variation among chromosomes in the likelihood of reductional *vs.* equational segregation at the anomalous MII division. Hence it is possible that, in our situation, centromeric or pericentromeric sequence differences are responsible for the strain-specific difference in the propensity for equational segregation at MI.

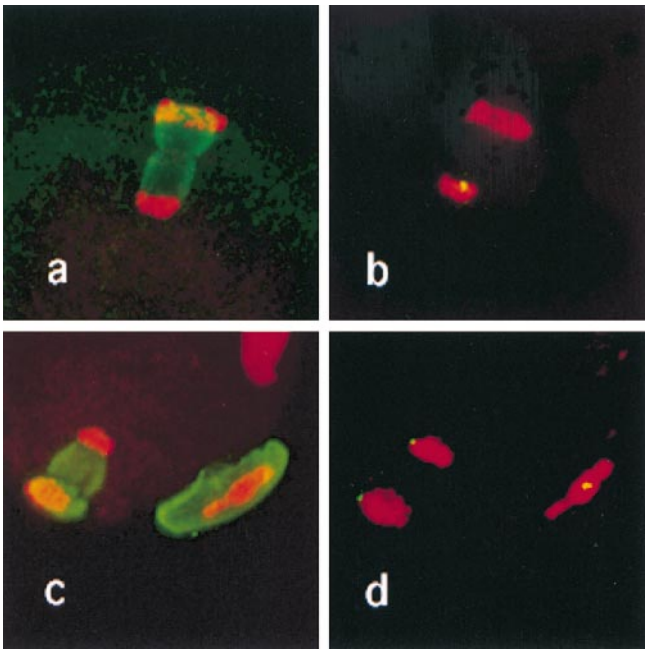

Figure 3.—Combined immunofluorescence and FISH analysis of oocytes at MII telophase. Confocal images of oocytes artificially stimulated to induce resumption of MII. (a, c) Immunofluorescence staining with an antibody to β-tubulin (green) and chromatin staining with propidium iodide (red) allow visualization of the MII spindle at telophase. (b, d) Images of the chromatin (red) from the same oocytes following FISH with an X chromosome-specific probe (yellow). (a and b) An oocyte from an XO female. Following equational segregation at MI, the single chromatid is observed at one spindle pole at MII telophase. Based on spindle orientation (not apparent in image) the single chromatid will remain in the oocyte. (c and d) Oocyte from a control XX female. Products of both first and second meiotic divisions are evident: Polar body (right) shows a doublet signal, indicating the presence of two chromatids (*e.g.*, one X homolog). The X homolog in the oocyte is undergoing equational segregation of chromatids, evident as a single signal at the edge of both chromatin clumps on the telophase spindle (left).

Alternatively, the meiotic segregation may reflect genetic factors that influence sister chromatid cohesion. During mitotic cell division, cohesion along the length of the chromosome arms is released at anaphase, allowing sister chromatids to move to opposite poles (reviewed in Rieder and Cole 1999). The cohesion between sister chromatids during meiotic cell divisions is more complex, since orderly chromosome segregation during both meiotic cell divisions requires the sequential loss of cohesion. That is, at anaphase I loss of cohesion along the length of the chromosome arms is necessary to allow homologs to segregate; however, cohesion at sister centromeres must be retained until anaphase II to facilitate the alignment and segregation of sister chromatids during MII. Both defects in homolog synapsis and the absence of a homologous partner are associated with an increased frequency of premature separation of sister chromatids at MI (reviewed in

Simchen and Hugerat 1993). Moreover, recent data suggest that proteins involved in homolog synapsis facilitate the appropriate segregation behavior of sister chromatids during the meiotic divisions (Watanabe and Nurse 1999; and reviewed in Stoop-Myer and Amon 1999). Thus, a logical explanation for the differences in X chromosome segregation that we have observed is genetic variation in the synaptic behavior of the chromosome. Studies are currently underway in our laboratory to determine whether either of these two factors—centromeric or synaptic differences—are responsible for the differences in X chromosome segregation behavior.

What is the basis of the segregation distortion effect? The term "meiotic drive" was first coined by Sandler and Novitski in 1957 (Sandler and Novitski 1957), who defined it as a situation in which "heterozygotes of certain constitutions fail to produce the two kinds of gametes with equal frequency." Subsequently it has become clear that failure to observe Mendelian ratios can derive from a variety of mechanisms, including differential viability of zygotes or gametes, in addition to processes acting directly on meiosis. In the laboratory mouse, transmission ratio distortion effects have been reported in a variety of situations, *e.g.*, in backcross matings involving F_1 interspecific animals (*e.g.*, Biddle 1987; Justice *et al.* 1990; Siracusa *et al.* 1991; Montagutelli *et al.* 1996), in crosses involving female heterozygotes for some Robertsonian translocations (reviewed in Gropp and Winking 1981; Ruvinsky *et al.* 1987), in crosses involving female heterozygotes for HSRs in wild mouse populations (Agulnik *et al.* 1990, 1993a,b), and in association with certain mutations (*e.g.*, Pardo-Manual de Villena *et al.* 1996). For most of these putative meiotic segregation distortion effects, however, data demonstrating distorted meiotic segregation, rather than a postmeiotic selection effect, are lacking. Data from studies of HSR heterozygotes (reviewed in Ruvinsky 1995) and carriers of the *Om* mutation (Pardo-Manuel de Villena *et al.* 2000) provide the best evidence for meiotic disturbances; however, in both cases the magnitude of the distortion effect is dependent upon the genetic background of the sire, raising the possibility that both meiotic and postmeiotic selection effects may be involved.

The segregation distortion effect that we have observed in oocytes from XO female mice is neither the result of postmeiotic selection nor is it a true case of genetically controlled meiotic drive. By analyzing intact MII arrested oocytes we have been able to unequivocally demonstrate nonrandom segregation at the first meiotic division; however, this meiotic disturbance is not a genetic effect mediated by specific elements on the X chromosome because (1) segregation distortion was observed on an inbred genetic background; (2) the effect was reproducable on a second inbred background; and (3) the segregation disturbance involves a univalent chromosome rather than a segregation distortion effect resulting from competitive segregation between different alleles. Indeed, this particular segregation distortion effect is similar only to the segregation behavior described for B chromosomes in several species (reviewed in Jones 1991).

Several different meiotic drive models have been proposed to explain the high rate of meiotic nondisjunction in the human female (Axelrod and Hamilton 1981; Day and Taylor 1998; Zwick *et al.* 1999). These attempts to provide an evolutionary explanation for human age-related nondisjunction have a common theme: the recognition that the assymetrical nature of the female meiotic divisions, leading to the inclusion of only one of the four chromatids of a bivalent in the mature female gamete, makes the process distinctly different from male meiosis. Indeed, Zwick *et al.* (1999) suggest that this feature makes female meiosis uniquely vulnerable to meiotic drive mechanisms and postulate that the evolution of such mechanisms is dependent upon chromosome-specific structures such as centromere or telomere sequences.

Our observations suggest that at least one form of meiotic drive may not be mediated by specific chromosome structures, but may have a mechanical basis stemming from the asymmetrical nature of the female meiotic divisions. That is, despite the striking background effects on equational *vs.* intact segregation of the univalent X chromosome, the magnitude of the segregation distortion effect was virtually identical on two different inbred genetic backgrounds. Thus, we hypothesize that the effect that renders the intact X chromosome twice as likely to remain in the oocyte as segregate to the polar body at MI is not chromosome mediated but rather spindle mediated. Specifically, we propose that, in the event of a difference in pole "weight" (either as a result of the number of centromeres attached to a given pole or due to differences in the size of chromosomes attached to either pole), the "heavier" or dominant pole will be that directing chromosomes to remain in the oocyte cytoplasm. We suggest that, at least in mammals, a mechanism has evolved such that, in the event of a deviation from the normal process that results in an unequal number of centromeres, there will be strong pressure to retain more genetic material in the oocyte.

The predictions and implications of the dominant pole hypothesis: If a difference in pole strength exists during mammalian female meiosis, it seems likely that a similar segregation distortion effect should act at both meiotic divisions. Our limited studies of the MII segregation behavior of single X chromatids provide little support for this model; however, as only 16 MII preparations were informative and as the presence of a single chromatid at MII is a highly aberrant situation, our data may be an inappropriate test of the model.

Other data from mammals, however, are consistent

with our model. For example, previous studies of females heterozygous for chromosomes with HSRs in wild populations of mice suggest that preferential segregation of the HSR-containing chromosomes occurs at both meiotic divisions (reviewed in Ruvinsky 1995). At MI, the HSR-containing homolog preferentially segregates to the oocyte; at MII, dyads consisting of one HSR-containing chromatid and one normal chromatid (due to recombination between the centromere and the HSR) segregate nonrandomly, with the HSR-containing chromatid preferentially segregating to the oocyte.

Our hypothesis also predicts that structural aberrations that grossly alter chromosome size (*e.g.*, some reciprocal translocations; HSR-containing chromosomes) or result in an unequal number of centromeres (*e.g.*, Robertsonian translocations) should be subject to segregation distortion effects. In addition to HSR-associated segregation disturbances (Agulnik *et al.* 1990, 1993b), female carriers of many, but not all, Robertsonian translocations in the mouse and in the common shrew also exhibit segregation distortion, with a tendency for the two structurally normal homologs to be maintained in the oocyte while the Robertsonian fusion is segregated to the polar body (*e.g.*, Gropp and Winking 1981; Tease and Fisher 1991; Pacchierotti *et al.* 1995).

Although many structural abnormalities appear to conform to our model, many others show Mendelian segregation. Segregation in structural abnormalities, however, is almost certainly complicated by additional factors including the location of the breakpoints, the synaptic and recombinational behaviors of the chromosomes, and, in the case of Robertsonian translocations, the potential for one *vs.* two active centromeres. Additionally, since most mouse structural abnormalities are not maintained on an inbred background, the action of specific alleles that exert a drive effect is also possible. By analyzing the behavior of a univalent X chromosome on an inbred genetic background, we have eliminated all of these variables. Thus, we suggest that our studies have allowed us to detect a naturally occurring meiotic drive effect that is a by-product of the unique mechanism of spindle formation and the asymmetrical nature of the divisions in the mammalian female.

The dominant pole hypothesis has important implications for the origin of human aneuploidy. Direct analysis of human oocytes has been limited by the difficulty of obtaining suitable study material; hence, estimates of the rate of human nondisjunction are based largely on data on trisomies from clinically recognized pregnancies (*i.e.*, spontaneous abortions, stillbirths, and livebirths). Since autosomal monosomies are eliminated very early in pregnancy (before the time of clinical recognition), the overall rate of nondisjunction is estimated by doubling the values for trisomy. However, this assumes that monosomy and trisomy occur in equal frequency. The dominant pole hypothesis raises the possibility that this assumption is not valid. The question is, of course, complex; segregation distortion effects will almost certainly (1) differ for individual human chromosomes, (2) be influenced by recombination or the effect of modifying loci, and (3) differ for age-dependent and age-independent nondisjunctional events. The methodology that we have used to analyze intact MII-arrested oocytes from XO female mice provides a means of assessing the question of the role of nonrandom segregation in human aneuploidy. In the human, however, these studies are daunting, since the accumulation of sufficient data to assess a segregation distortion effect requires large numbers of human oocytes.

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