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Heterochromatin-Mediated Association of Achiasmate Homologs Declines With Age When Cohesion Is Compromised

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ABSTRACT

Normally, meiotic crossovers in conjunction with sister-chromatid cohesion establish a physical connection between homologs that is required for their accurate segregation during the first meiotic division. However, in some organisms an alternative mechanism ensures the proper segregation of bivalents that fail to recombine. In Drosophila oocytes, accurate segregation of achiasmate homologs depends on pairing that is mediated by their centromere-proximal heterochromatin. Our previous work uncovered an unexpected link between sister-chromatid cohesion and the fidelity of achiasmate segregation when Drosophila oocytes are experimentally aged. Here we show that a weak mutation in the meiotic cohesion protein ORD coupled with a reduction in centromere-proximal heterochromatin causes achiasmate chromosomes to missegregate with increased frequency when oocytes undergo aging. If ORD activity is more severely disrupted, achiasmate chromosomes with the normal amount of pericentric heterochromatin exhibit increased nondisjunction when oocytes age. Significantly, even in the absence of aging, a weak *ord* allele reduces heterochromatin-mediated pairing of achiasmate chromosomes. Our data suggest that sister-chromatid cohesion proteins not only maintain the association of chiasmate homologs but also play a role in promoting the physical association of achiasmate homologs in Drosophila oocytes. In addition, our data support the model that deterioration of meiotic cohesion during the aging process compromises the segregation of achiasmate as well as chiasmate bivalents.

IN both mitotic and meiotic cells, cohesion between sister chromatids is essential for accurate chromosome segregation (Lee and Orr-Weaver 2001). Cohesion depends on the evolutionarily conserved cohesin complex that consists of two structural maintenance of chromosomes (SMC) and two non-SMC proteins (Uhlmann 2001). In addition to holding sisters together, meiotic cohesion is also required to keep recombinant homologs physically associated prior to their segregation (Buonomo *et al.* 2000; Bickel *et al.* 2002; Hodges *et al.* 2005). Chiasma maintenance relies on cohesion between the arms of sister chromatids (see Figure 1) and separase-mediated release of arm cohesion is necessary for homolog disjunction at anaphase I (Buonomo *et al.* 2000; Pasierbek *et al.* 2001; Siomos *et al.* 2001; Kudo *et al.* 2006).

Although proper segregation of homologous chromosomes during meiosis I generally requires formation and maintenance of chiasmata, several organisms harbor an alternate mechanism that ensures faithful disjunction of bivalents that fail to recombine (Wolf 1994). Accurate segregation of achiasmate bivalents has been reported in *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, and *Drosophila melanogaster* (Dawson *et al.* 1986; Guacci and Kaback 1991; Hawley *et al.* 1993; Ross *et al.* 1996; Molnar *et al.* 2001; Meneely *et al.* 2002; Davis and Smith 2005; Doll *et al.* 2005). In budding yeast, centromere pairing of achiasmate chromosomes facilitates their accurate disjunction in meiosis I (MI) (Dawson *et al.* 1986; Guacci and Kaback 1991; Ross *et al.* 1996; Kemp *et al.* 2004). In addition, the checkpoint protein Mad3 (orthologous to BubR1 in metazoans) also plays a role in directing the proper segregation of the nonrecombinant bivalents in *S. cerevisiae*, although the exact mechanism by which Mad3 facilitates achiasmate segregation is not clear (Cheslock *et al.* 2005). In fission yeast, the microtubule motor dynein promotes pairing between homologous chromosomes during prophase I, and mutations in *dlc1* (dynein light chain) cause missegregation of achiasmate bivalents (Molnar *et al.* 2001; Davis and Smith 2005). In addition, achiasmate homologs in *S. pombe* also exhibit centromere pairing (Ding *et al.* 2004). Although accurate disjunction of achiasmate autosomes has been observed during *C. elegans* spermatogenesis, the molecular mechanisms underlying this pathway have not been explored (Meneely *et al.* 2002).

The molecules and mechanisms governing achiasmate chromosome segregation have been most thor-

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oughly investigated in fruit flies. In Drosophila oocytes, accurate segregation of achiasmate bivalents depends upon homology-based association of homologs within their centromere-proximal heterochromatin (see Figure 1). Minichromosomes carrying duplications of pericentric heterochromatin compete for pairing sites on bivalents and cause MI nondisjunction (NDJ) if the bivalents are achiasmate (Hawley *et al.* 1993). Furthermore, there is a linear relationship between the amount of pericentric heterochromatin and the fidelity of achiasmate segregation (Karpen *et al.* 1996). Cytological analyses of Drosophila oocytes indicate that the centromere-proximal heterochromatin of homologous chromosomes remains associated from pachytene until anaphase I (Dernburg *et al.* 1996; Gilliland *et al.* 2009; Hughes *et al.* 2009). However, whether or how specific heterochromatin proteins promote this association has not been investigated.

In Drosophila, orientation disruptor (ORD) is essential for sister chromatid cohesion during meiosis in both sexes (Mason 1976; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1996; Balicky *et al.* 2002; Webber *et al.* 2004). In the absence of ORD activity, chromosomes segregate randomly during both meiosis I and meiosis II, consistent with complete absence of both arm and centromere cohesion (Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1996, 1997). ORD function is also required for normal levels of homologous exchange in Drosophila oocytes (Mason 1976; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1997; Webber *et al.* 2004). Additional studies have shown that the recombination defect in *ord* mutant oocytes arises because sister-chromatid exchange is elevated, resulting in a lower number of crossovers between homologous chromosomes (Webber *et al.* 2004). In addition, consistent with its role in arm cohesion, ORD is also required for chiasma maintenance until anaphase I (Bickel *et al.* 2002). Although some crossovers occur between homologous chromosomes in *ord* mutant oocytes, these bivalents still missegregate during meiosis I because chiasmata are not maintained (Mason 1976; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1997).

In Drosophila oocytes, ORD colocalizes extensively with cohesin subunits along the chromosome arms and like cohesin, ORD is enriched at the centric/pericentric regions of meiotic chromosomes (Webber *et al.* 2004; Khetani and Bickel 2007). In support of its essential role in meiotic cohesion, ORD activity is required for localization of SMC1 and SMC3 at the centromeres of meiotic chromosomes and their enrichment at the pericentric heterochromatin (Khetani and Bickel 2007). In addition, previous findings have revealed an unexpected link between ORD and achiasmate chromosome segregation during meiosis I in Drosophila oocytes (Jeffreys *et al.* 2003). When Drosophila oocytes are subjected to aging and ORD function is compromised by a weak mutation, meiotic NDJ of achiasmate bivalents is significantly higher in "aged oocytes" than in "nonaged oocytes" (Jeffreys *et al.* 2003). Although it is well established that cohesion proteins along the chromatid arms are required to hold chiasmate homologs together (see Figure 1), the finding that a sister-chromatid cohesion protein is required for accurate segregation of achiasmate homologs is novel. One possibility consistent with these data is that in addition to holding sister centromeres together, cohesion proteins enriched at the pericentric heterochromatin also play a role in holding achiasmate homologs together (see Figure 1).

In this article, we further explore the mechanism by which a sister-chromatid cohesion protein contributes to the accurate segregation of achiasmate bivalents. We show that when ORD function is compromised by a weak mutation and centromere-proximal heterochromatin also is reduced, achiasmate chromosomes become more susceptible to NDJ when oocytes undergo aging. Furthermore, in the presence of a stronger *ord* allele, achiasmate chromosomes with the normal amount of pericentric heterochromatin exhibit increased nondisjunction when oocytes age. Significantly, even in the absence of aging, a weak ORD allele disrupts heterochromatin-mediated pairing of achiasmate chromosomes. Our results indicate that the cohesion protein ORD promotes the physical association of achiasmate homologs. In addition, our data provide further support for the model that meiotic cohesion declines with age and argue that as cohesion proteins deteriorate with age, so does the fidelity of achiasmate segregation.

MATERIALS AND METHODS

Drosophila stocks and genetics: Flies were reared at 25° on standard cornmeal molasses media. The ord^4, ord^8, and ord^{10} alleles used in this study have been characterized previously (Miyazaki and Orr-Weaver 1994; Bickel *et al.* 1996, 1997). The ord^4 (A424V) and ord^8 (H366Y) mutations reside in the last quarter of the ORD open reading frame and result in low and moderate NDJ levels (respectively) in mutant oocytes. ord^{10} is a nonsense allele (L24STOP) that behaves as a genetic null. Descriptions of the other genetic markers and chromosomes used can be found at http://www.flybase.org.

Recombination analysis: To assay *X* chromosome crossover frequency and distribution in *ord* oocytes, 7–10 females were crossed to 5 *yw* males per vial. Crossover frequency and distribution were measured between the "*y sc cv v f car*" and "*y*" *X* homologs in experimental females by assaying *sc, cv, v, f, car* markers in their male progeny. The recombination data were used to estimate tetrad exchange ranks (Weinstein 1936).

Aging regimen and generation of 24-hr broods: We have previously described an aging regimen that causes Drosophila oocytes to halt in development within the ovariole and "age" (Jeffreys *et al.* 2003). This experimentally induced aging mimics the normal aging process that human oocytes undergo during the lifetime of the female. In this study, we modified the aging regimen described by Jeffreys *et al.* (2003) such that the glucose agar media was prepared without the addition of fungal inhibitors (methyl paraben and ethyl acetate). We have

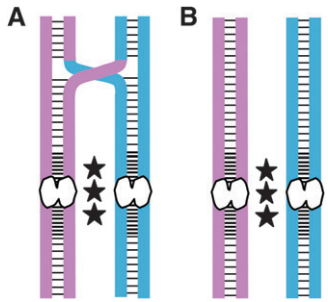


Figure 1.—Mechanisms that ensure the association of homologous chromosomes in Drosophila oocytes. Pink and blue are used to differentiate the homologous chromosomes (each composed of two sister chromatids). Sister-chromatid cohesion proteins are depicted as black horizontal lines. In Drosophila oocytes, cohesion proteins (SMC1, SMC3, and ORD) are highly enriched near the centromeres (white bilobed structures). Black stars represent heterochromatin-mediated pairing of homologous chromosomes that is required for accurate segregation of achiasmate bivalents. (A) Cohesion along the arms of sister chromatids provides an evolutionarily conserved mechanism to maintain the association of recombinant homologs until anaphase I. (B) In Drosophila oocytes, bivalents that lack a crossover rely on the achiasmate pathway to keep homologous chromosomes associated and thereby ensure their proper segregation. (Note that heterochromatin-mediated pairing of chiasmate homologs also occurs, but is not required for their accurate disjunction unless chiasmata are not maintained.)

found that omission of fungal inhibitors in the media during the aging regimen reduces the absolute level of NDJ in our assay; therefore, NDJ values in this study are lower than previously reported (Jeffreys *et al.* 2003). The glucose agar media contained 2% agar (Fisher) and 5% dextrose (Fisher) and was prepared with milli-Q grade water. Yeast paste was prepared by dissolving 30 g of active dry yeast (Red Star) in 50 ml milli-Q grade water.

A schematic of the aging regimen and the NDJ assay are shown in Figure 2, A and B. Approximately 200 virgin females of the desired genotype were collected within an 8-hr period and the females were fed yeast overnight in vials with cornmeal molasses media to promote yolk deposition and maturation of oocytes. This ensured that the ovaries contained a complete complement of oocytes at the different stages. The following day, females were split into two groups and placed in separate plexiglass laying bottles containing a glucose agar plate with a smear of yeast paste. Control and experimental flies were held in the laying bottles for 4 days with fresh yeast paste/glucose-agar plates supplied each day. The control group of females was supplied with an equal number of male flies and laid their eggs continuously. Their oocytes were "nonaged." The experimental group of females was deprived of males. Because oviposition is suppressed in these females, the majority of the oocytes (stages 1–8) halt in developmental progression and age within the female abdomen (Subramanian and Bickel 2008). Stages 9–13 continue to progress through oogenesis even when oviposition is suppressed, but then arrest at stage 14, resulting in a large excess of mature oocytes that also age for the remainder of the regimen (King 1957; Subramanian and Bickel 2008). Figure 2C depicts the relative distribution of different oocyte stages in nonaged *vs.* aged ovarioles.

At the end of the 4-day aging regimen, the experimental females (with aged oocytes) and the control females (with nonaged oocytes) were crossed to *X^Y, v f B* males to measure meiotic nondisjunction in the oocytes (see Figure 2B). To generate 24-hr broods, 10 female flies were mated with 5 *X^Y, v f B* males (per vial). The parents were transferred to new vials every 24-hr and three broods of progeny were analyzed for NDJ.

Nondisjunction assay: Because Drosophila can tolerate certain sex chromosome aneuploidies, segregation errors during meiosis can be monitored in the viable progeny by using differentially marked sex chromosomes (Figure 2B). To compensate for the fact that only half of the exceptional progeny survive, total NDJ was calculated as $[2 \times \text{exceptional progeny}/(2 \times \text{exceptional progeny} + \text{normal progeny})] \times 100$.

For some NDJ tests, we performed an additional cross to genotype the *X* chromosomes in the diplo-*X* progeny (exceptional progeny that received two *X* chromosomes from the mother) as previously described (Subramanian and Bickel 2008). This allowed us to determine the recombinational history of missegregating chromosomes and/or determine whether missegregation events were reductional or equational.

Generation of probes for *in situ* and FISH analysis in whole mount ovaries: A portion of the centromere proximal heterochromatin on the *X* chromosome consists of a 359-bp repeat sequence that spans 11 Mb. PCR amplification of the 359-bp repeat from wild-type Drosophila genomic DNA was performed using primers previously described (Dernburg *et al.* 1996) and the PCR product was digested overnight with Tsp509I restriction enzyme at 65° in a thermocycler. The 100-bp fragment was labeled with dUTP-fluorogreen using terminal transferase to generate the probe as described in Bickel *et al.* (2002).

FM7a/y w and *FM7a/y;ord^{4} bw/cn ord^{10} bw* oocytes were hybridized with the 359-bp probe. Ovaries from 15 females per sample were hand-dissected in 1× modified Robb's buffer (Theurkauf and Hawley 1992). The ovaries were fixed for 4 min in 4% formaldehyde/100 mm Na cacodylate (pH 7.2)/100 mm sucrose/40 mm K acetate/10 mm Na acetate/10 mm EGTA, prewarmed at 37°. *In situ* hybridization of the probes (mentioned above) was performed using the published protocol from Dernburg *et al.* (1996).

Microscopy and image analysis: Epifluorescence imaging was carried out using a Zeiss Axioimager M1 microscope equipped with a Hamamatsu ORCA-ER camera. Images of ovarioles were captured using a 100× Plan-Apochromat (NA 1.4) objective and Openlab software (Improvision, version 4.0.4), whereas a 63× Plan-Apochromat (NA 1.4) was used for scoring the samples. Image stacks (0.1 μm step size) were deconvolved and cropped using Volocity software from Improvision. Openlab software was used to pseudocolor the images (projection of *z*-stacks).

Data analysis and statistical significance: The frequency of missegregation is plotted as % NDJ. The error bars represent 95% confidence limits that were calculated using the extended Wald method (Agresti and Coull 1998). All statistical analyses were performed using a 2×2 χ^2 contingency test. To compare aged *vs.* nonaged NDJ values, nonadjusted data were used for the statistical tests. For all tests, a two-tailed *P*-value of <0.05 was considered statistically significant (rejection of the null hypothesis that the two groups are the same).

RESULTS

Different *ord* alleles disrupt achiasmate segregation in aged oocytes: Cohesion proteins not only provide a mechanism to hold sister chromatids together but also are required to maintain the association of chiasmate homologs during meiosis (Buonomo *et al.* 2000; Bickel *et al.* 2002; Hodges *et al.* 2005). Moreover, our recent

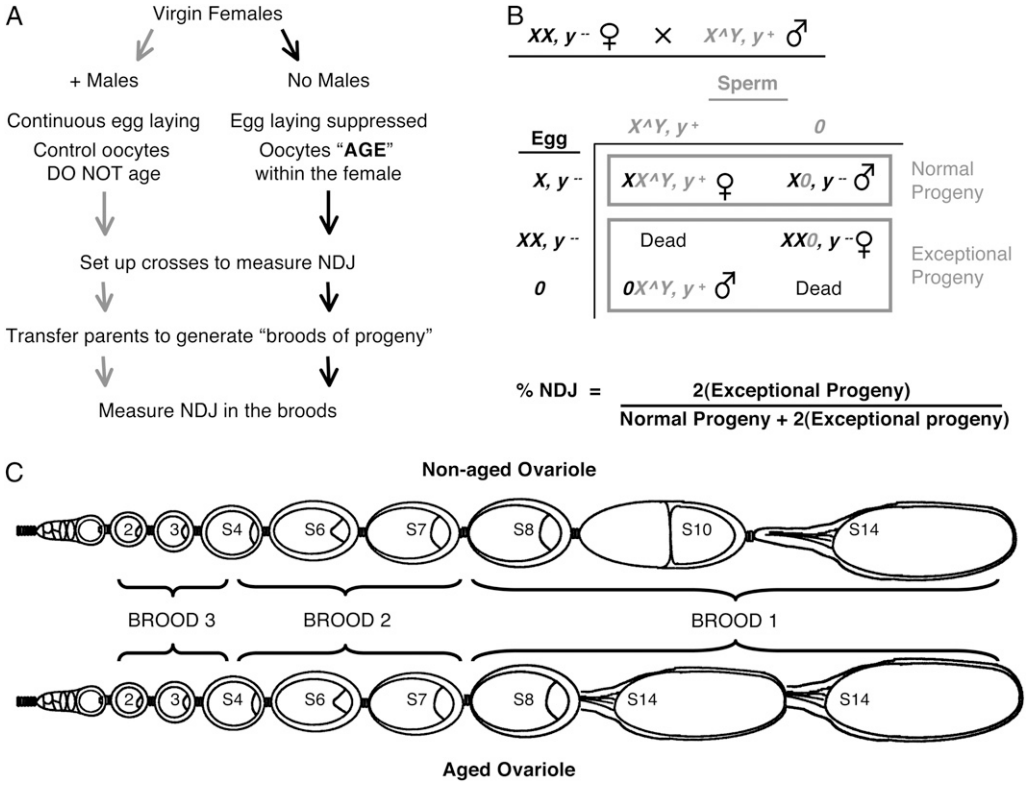


FIGURE 2.—Scheme to age oocytes and measure NDJ (A) To age oocytes in Drosophila, egg laying is suppressed in virgin females. Virgin females of the same age and genotype are split into two groups. The control group of females is allowed to mate and their oocytes do not age. In contrast, the experimental group of females is not allowed to mate; in these females egg laying is suppressed and the oocytes age within the female. After females are subjected to the aging regimen for 4 days, crosses are set up to measure NDJ (see B). The parents are transferred at 24-hr intervals to generate broods of progeny that are assayed for NDJ during maternal meiosis. (B) To measure *X* chromosome NDJ during female meiosis, we used the recessive body color mutation, *yellow* (*y*). Experimental females that are y^- are crossed to males whose *X* and *Y* chromosomes are physically linked (*X^Y*). The sperm from these males will have either an *X^Y* chromosome marked with y^+ or no sex chromosomes (designated "*0*"). If meiotic chromosome segregation is normal, the oocyte will contain one *X* chromosome. Missegregation of *X* chromosomes may result in an oocyte with two *X* chromosomes (diplo *X*, y^-) or no *X* chromosomes (Nullo, "*0*"). The exceptional progeny that arise due to meiotic NDJ can be distinguished from the progeny that arise from normal meiosis on the basis of their sex and body color. In this NDJ test, only half of the exceptional progeny survive; therefore, the number of exceptional progeny is doubled and the total number of progeny is similarly adjusted to calculate % NDJ. (C) The Drosophila ovary is composed of 15–30 ovarioles each containing a linear array of oocytes at progressive stages of development (stages 1–14). Oocytes at different stages can be distinguished on the basis of their size and morphology. Meiosis is initiated at the anterior end of the ovariole (left) and the oocyte moves posteriorly as it develops. Not all oocyte stages are present in the ovariole at any given time. The schematic illustrates which oocyte stages in aged and nonaged ovarioles give rise to each 24-hr brood of progeny after completion of the aging regimen. When egg laying is suppressed (aged ovariole), stage 14 oocytes accumulate at the expense of stages 9–13 (SUBRAMANIAN and BICKEL 2008). The schematic is adapted from ROBINSON *et al.* (1994).

work indicates that when Drosophila oocytes undergo aging, deterioration of meiotic cohesion causes loss of chiasmata and missegregation of recombinant homologs during meiosis I (SUBRAMANIAN and BICKEL 2008).

The aging regimen that we have developed for Drosophila oocytes mimics the normal aging process of human oocytes as women grow older (JEFFREYS *et al.* 2003; SUBRAMANIAN and BICKEL 2008). Briefly, when virgin females are deprived of males, the developmental progression of their oocytes halts and the oocytes age within the females (see Figure 2). Although the age of the female fly does not dictate the age of her oocytes, for simplicity we will refer to a significant increase in NDJ when the oocytes are experimentally aged as "age-dependent NDJ." Because meiotic cohesion weakens as oocytes age, we have been able to detect meiotic segregation defects that are not apparent under normal conditions (no aging) (JEFFREYS *et al.* 2003; SUBRAMANIAN and BICKEL 2008). Notably, this approach led to the surprising finding that when oocytes undergo aging, mutation of the cohesion protein ORD disrupts the segregation of obligate achiasmate homologs (JEFFREYS *et al.* 2003).

To further investigate the role of ORD in the segregation of achiasmate chromosomes, we reevaluated and extended our analysis of meiotic NDJ in females with *ord* mutations that have a weak or moderate effect on meiotic cohesion. Under normal conditions (no aging regimen) the weakest *ord* allele, ord^4, results in 2.2% sex chromosome NDJ in oocytes and 0.6% in spermatocytes when placed in *trans* to an ord^{null} allele (MIYAZAKI and ORR-WEAVER 1992; BICKEL *et al.* 1996). The slightly stronger ord^8 allele when placed over a null allele results in 10.9% sex chromosome NDJ in oocytes and 4.0% in spermatocytes (BICKEL *et al.* 1997). Because in wild-type fruit flies, crossovers do not occur during male meiosis and arm cohesion is released prior to spindle assembly (VAZQUEZ *et al.* 2002), the NDJ observed in *ord* mutant

spermatocytes can be attributed to defects in centromeric cohesion. Therefore, the meiotic NDJ in males indicate that the centromeric function of ORD is more severely compromised by the *ord*^{8} mutation than by the *ord*^{4} mutation. For the *ord*^{null} allele in the experiments described below, we utilized *ord*^{10}, which truncates the ORD open reading frame early in the coding region and behaves as a null in genetic tests (Bickel *et al.* 1997). Because of a slight modification of our aging regimen (see materials and methods), we repeated our previous analysis of age-dependent NDJ in *X/X; ord*^{4}*/ord*^{10} and *FM7a/X;ord*^{4}*/ord*^{10} oocytes (Jeffreys *et al.* 2003) for this study. These two genotypes provide the foundation for additional experiments described below.

When we subjected *ord*^{4}*/ord*^{10} female flies with normal *X* chromosomes to our 4-day aging regimen (see materials and methods), sex chromosome NDJ was not significantly greater in aged oocytes than in nonaged oocytes (supplemental Figure S1A and Table S1). These data confirm our previous results that the weak *ord*^{4} mutation does not cause normal *X* chromosomes to become more vulnerable to missegregation during the aging process.

To specifically examine the role of ORD activity in the segregation of achiasmate chromosomes, we subjected *FM7a/X;ord*^{4}*/ord*^{10} females to the aging regimen. *FM7a* is an *X* chromosome balancer that contains multiple inversions along its length (compare Figure 3, A and B). Apart from inversions in the euchromatin that suppress recombination, the pericentric heterochromatin of *FM7a* is rearranged such that a large portion is displaced to the distal end of the chromosome (Figure 3B). In a female fly with a normal *X* chromosome and an *FM7a* balancer, meiotic crossovers on the *X* chromosome are suppressed, and the *X* bivalents (*FM7a/X*) depend entirely on the achiasmate pathway for segregation in meiosis I. Following the aging regimen, *FM7a/X;ord*^{4}*/ord*^{10} aged oocytes exhibited a significant increase in NDJ that lasted for 48 hr (two 24-hr broods) (supplemental Figure S1B and Table S2). Our analysis of diplo-*X* females (exceptional progeny that received two *X* chromosomes from the mother) indicated that reductional NDJ events predominated in both aged and nonaged oocytes (*FM7a/X* diplos: 107/107 for aged, 43/44 for nonaged). Therefore, although the *ord*^{4} mutation weakens sister-chromatid cohesion, it rarely results in complete separation of sister chromatids even when oocytes undergo aging (as evidenced by the low number of equational exceptions). Together, these data confirm our previous findings that when *ord*^{4}*/ord*^{10} oocytes undergo aging, obligate achiasmate chromosomes become more susceptible to missegregation during the first meiotic division.

We next analyzed the effect of aging on the segregation of normal *X/X* bivalents in *ord*^{8}*/ord*^{10} oocytes. For this genotype, we observed significant age-dependent NDJ even in the absence of an *FM7a* balancer chromosome (Figure 4, supplemental Table S3). In addition, normal *X* chromosomes in *ord*^{8}*/ord*^{10} flies exhibited significantly higher NDJ than nonaged oocytes for at least 72 hr (all three 24-hr broods tested). For these experiments, we performed an additional cross with the recovered diplo-*X* females that allowed us to determine the recombinational history of missegregating chromosomes. Of the 45 diplo-*X* progeny from aged *ord*^{8}*/ord*^{10} oocytes that we were able to genotype, only 2 arose from missegregation of recombinant chromosomes. In nonaged oocytes, recombinant chromosomes missegregated at a similar frequency (3/47). Therefore, the *ord*^{8} allele disrupts the fidelity of achiasmate chromosome segregation, and this effect is enhanced by the aging regimen.

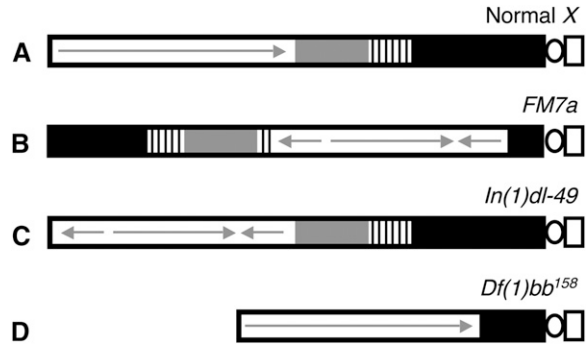


Figure 3.—Diagram illustrating the different *X* chromosomes used in NDJ tests. (A) A normal *X* chromosome is shown with the centromeric constriction on the right. Euchromatin is depicted as a white rectangle, with a continuous arrow indicating a lack of rearrangements. The shaded and striped regions represent heterochromatin with the 11-Mb satellite DNA (359-bp repeat) (solid) and rDNA (stripes). (B) The *FM7a* balancer chromosome contains multiple inversions within the euchromatin (represented with arrows) as well as a rearrangement that places a large region of centromere-proximal heterochromatin at the distal end of the chromosome. (C) The *In(1)dl-49* chromosome contains an inversion in the euchromatin that reduces recombination to ~20% of wild type. However, centromere-proximal heterochromatin is unaffected. (D) The *Df(1)bb*^{158} contains a deletion that removes ~80% of the centromere-proximal heterochromatin but the euchromatic region of the chromosome is normal.

By monitoring a centromere-linked marker (*car*), we also determined that the majority of NDJ events were reductional in both aged and nonaged *ord*^{8}*/ord*^{10} oocytes. However, compared to *ord*^{4}*/ord*^{10} oocytes, equational exceptions were more frequent in *ord*^{8}*/ord*^{10} oocytes (12/45 for aged, 5/47 for nonaged). These data indicate that the centromeric function of ORD^{8} protein is more severely disrupted than that of ORD^{4} and that complete loss of centromeric cohesion between sister chromatids in *ord*^{8}*/ord*^{10} oocytes increases with age. However, the majority of NDJ events in *ord*^{8}*/ord*^{10} oocytes arise because achiasmate homologs missegregate during the first meiotic division.

Homologous exchange is reduced to a similar degree by *ord*^{4} and *ord*^{8} mutations: Why are normal *X* chromo-

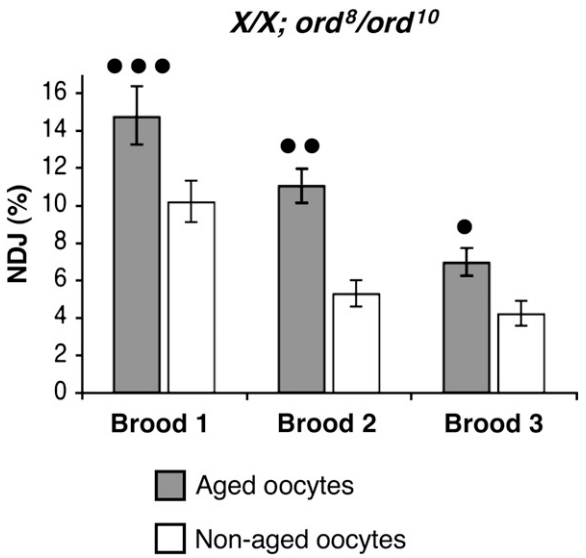


Figure 4.—Age-dependent NDJ in *ord*^{8}/*ord*^{10} oocytes. Each bar denotes the percentage of *X* chromosome NDJ in aged (shaded) or nonaged (open) oocytes. Error bars represent 95% confidence intervals. In *ord*^{8}/*ord*^{10} oocytes, normal *X* chromosomes exhibit age-dependent NDJ in all three broods tested (●●●$P = 0.0004$, ●●$P = 0.0001$, ●$P = 0.0002$; $N > 1900$ for each brood). The raw data is presented in supplemental Table S3. These data contrast sharply with those observed for *ord*^{4}/*ord*^{10} oocytes (see supplemental Figure S1A).

somes vulnerable to age-dependent NDJ in *ord*^{8}/*ord*^{10} oocytes but not *ord*^{4}/*ord*^{10} oocytes? Because ORD function is required for normal levels of interhomolog exchange (Mason 1976; Miyazaki and Orr-Weaver 1992; Bickel *et al.* 1997; Webber *et al.* 2004), we reasoned that the stronger *ord*^{8} mutation might suppress crossovers to a greater extent than *ord*^{4}. If this were the case, *ord*^{8}/*ord*^{10} oocytes would contain more achiasmate *X* chromosomes than *ord*^{4}/*ord*^{10} oocytes and these would be vulnerable to age-dependent NDJ even in the absence of the *FM7a* balancer. Similarly, if crossovers between normal *X* chromosomes were more abundant in *ord*^{4}/*ord*^{10} oocytes than in *ord*^{8}/*ord*^{10} oocytes, we might require a balancer chromosome (that completely suppresses recombination) to elicit age-dependent NDJ of achiasmate chromosomes in *ord*^{4}/*ord*^{10} oocytes. Therefore, we measured *X* chromosome exchange in the two genotypes to determine if achiasmate bivalents were more prevalent in *ord*^{8}/*ord*^{10} oocytes.

Interestingly, our analysis of *X* chromosome recombination revealed that crossovers are similarly and substantially reduced in *ord*^{4}/*ord*^{10} and *ord*^{8}/*ord*^{10} oocytes (Table 1). In both genotypes, the total map distance for the *X* chromosome was reduced to ≤20% of wild type (Table 1). The tetrad exchange rank of bivalents can be inferred from the recombinant and nonrecombinant meiotic products (Weinstein 1936). In wild-type Drosophila oocytes, 6–12% of normal *X* chromosome bivalents fail to recombine and therefore belong to the E_0 tetrad exchange rank (Ashburner 1989; Hawley *et al.* 1993; Zwick *et al.* 1999). From our recombination analysis, we estimate the achiasmate *X* chromosome tetrads (E_0) to be ≥79% in both *ord*^{4}/*ord*^{10} and *ord*^{8}/*ord*^{10} oocytes. A smaller scale analysis of recombination on the left arm of chromosome 3 indicated that both alleles also depress autosomal exchange (data not shown).

Although the majority of the *X* chromosomes are achiasmate in both *ord*^{4}/*ord*^{10} and *ord*^{8}/*ord*^{10} oocytes, age-dependent NDJ depends on the presence of the *FM7a* balancer in *ord*^{4}/*ord*^{10} oocytes but not *ord*^{8}/*ord*^{10} oocytes. This suggests that *FM7a/X* bivalents in *ord*^{4}/*ord*^{10} oocytes are prone to higher levels of NDJ after aging not because *FM7a* suppresses crossovers, but because of some other attribute of the *FM7a* chromosome.

Reduced centromere-proximal heterochromatin contributes to age-dependent NDJ of achiasmate chromosomes in *ord*^{4}/*ord*^{10} oocytes: In addition to promoting arm cohesion and meiotic exchange, ORD also functions at the centromere and is highly enriched at pericentric heterochromatin (see Figure 1). Homologous pairing of centromere proximal heterochromatin is essential for proper achiasmate chromosome disjunction in Drosophila oocytes (Hawley *et al.* 1993; Hawley and Theurkauf 1993; Karpen *et al.* 1996) and this pairing is maintained until anaphase I (Dernburg *et al.* 1996; Gilliland *et al.* 2009; Hughes *et al.* 2009). One possibility is that the *FM7a* chromosome is more vulnerable to increased NDJ in aged *ord*^{4}/*ord*^{10} oocytes because a large portion of the pericentric heterochromatin is displaced to the distal end of the chromosome (Figure 3B). Although *FM7a* associates efficiently with its partner under normal conditions (Dernburg *et al.* 1996), the small proximal region of heterochromatin on *FM7a* may result in homologous pairing that is less resistant to the effects of aging when ORD activity is compromised.

To test the hypothesis that *FM7a* alters disjunction patterns in aged *ord*^{4}/*ord*^{10} oocytes because centromere-proximal heterochromatin is reduced, we examined the effect of aging on the meiotic segregation of two different *X* chromosomes in *ord* oocytes. One *X* chromosome [*In(1)dl-49*] contains a large inversion in the euchromatin that significantly suppresses meiotic exchange whereas the other *X* chromosome [*Df(1)bb*^{158}] harbors a deletion that removes a large portion of centromere-proximal heterochromatin (see Figure 3, C and D). We subjected *ord*^{4}/*ord*^{10} females containing one normal *X* chromosome and one of the above "tester" chromosomes to the aging regimen and measured meiotic nondisjunction.

The large inversion on the *In(1)dl-49* chromosome (Figure 3C) strongly suppresses recombination with a normal *X* chromosome (<22% of wild type), and 81% of the tetrads in *In(1)dl-49/X* oocytes are estimated to be achiasmate (Sturtevant and Beadle 1936; Grell 1962; Roberts 1962). Exchange between *In(1)dl-49* and a normal *X* chromosome will be reduced even further in an *ord*^{4}/*ord*^{10} background, such that the

TABLE 1

***ord*^{4} and *ord*^{8} depress meiotic exchange to a similar degree**

Genotype	*n*	Crossovers (cM)				Total map distance (cM)	Exchange rank			
		sc-cv	*cv-v*	*v-f*	*f-car*		E_0	E_1	E_2	E_3
+/+	971	11.8	20.3	18.3	6.3	56.8	9.6	69.0	19.7	1.7
ord$^{4/10}$	610	0.7	1.6	4.6	1.8	8.7	84.5	13.5	2.0	0.0
ord$^{8/10}$	1287	1.6	2.0	4.8	2.6	11.0	79.3	19.5	1.2	0.0

number of achiasmate *X* chromosomes will approach that achieved with the *FM7a* balancer. Despite this euchromatic rearrangement, centromere-proximal heterochromatin is unaffected in *In(1)dl-49* (Xiang and Hawley 2006). Therefore, this chromosome can be utilized to test whether achiasmate chromosomes with normal heterochromatin are sensitive to age effects in *ord*^{4}/*ord*^{10} oocytes. When *In(1)dl-49/X; ord*^{4}/*ord*^{10} females were subjected to the aging regimen, age-dependent NDJ was not observed (Figure 5A, supplemental Table S4). This result argues that a further reduction of meiotic exchange caused by euchromatic aberrations on the *FM7a* balancer chromosome is not responsible for the age-dependent NDJ in *FM7a/X; ord*^{4}/*ord*^{10} oocytes.

Unlike *In(1)dl-49*, the *Df(1)bb*^{158} chromosome lacks ~80% of the pericentric heterochromatin (including the rDNA cluster) (Figure 3D), but contains no aberrations in the euchromatin (Yamamoto and Miklos 1977, 1978). *X* chromosome meiotic exchange and segregation in *Df(1)bb*^{158}/*X* females are similar to wild type (Yamamoto and Miklos 1977, 1978). However, in *ord*^{4}/*ord*^{10} oocytes, a significant percentage of *Df(1)bb*^{158}/*X* bivalents will be achiasmate. Following the aging regimen, the *Df(1)bb*^{158}/*X* bivalents exhibit significantly higher missegregation in aged *ord*^{4}/*ord*^{10} oocytes compared to the nonaged oocytes of the same genotype (Figure 5B, supplemental Table S5). However, when ORD function was wild type, we did not observe age-dependent NDJ of *Df(1)bb*^{158}/*X* chromosomes (supplemental Table S6). The above data indicate that the combination of compromised ORD activity and reduced levels of centromere-proximal heterochromatin act together to increase missegregation of achiasmate chromosomes in aged oocytes.

ORD promotes heterochromatin-mediated pairing: The finding that mutations in *ord* sensitize achiasmate chromosomes to missegregate in aged oocytes led us to test the hypothesis that ORD activity promotes heterochromatin-mediated association of homologous chromosomes. ORD protein is highly enriched within the pericentric heterochromatin of oocyte chromosomes during prophase I (Webber *et al.* 2004; Khetani and Bickel 2007) and therefore is in the right place at the right time to assist in the pairing of homologs via pericentric heterochromatin (see Figure 1). To test this possibility, we utilized FISH (fluorescence *in situ* hybridization) to determine if the association of *FM7a* with a normal *X* chromosome is affected in *ord* mutant oocytes. Cytological analysis has indicated that in wild-type oocytes, both the small region of pericentric heterochromatin and the distal heterochromatin on the *FM7a* chromosome are able to pair with the centromere-proximal heterochromatin on the normal *X* chromosome (Dernburg *et al.* 1996).

For the FISH analysis, we used a probe that recognizes a 359-bp repeat sequence within the pericentric heterochromatin of the *X* chromosome. In *FM7a/X* oocytes, hybridization with this probe will result in one spot if the distal and proximal heterochromatin of *FM7a* both pair with the homologous pericentric sequence on the normal *X* (Figure 6A) (Dernburg *et al.* 1996). Con-

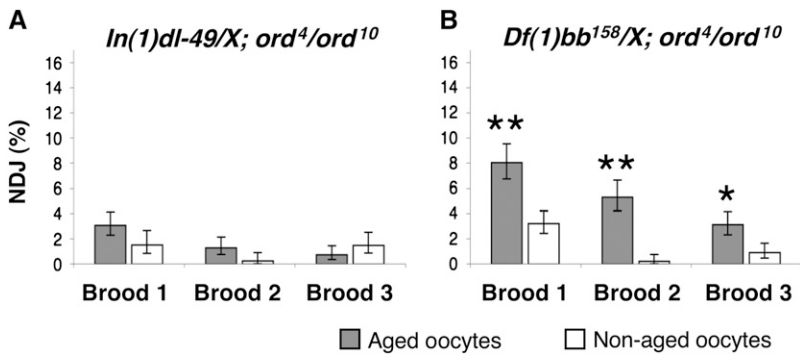
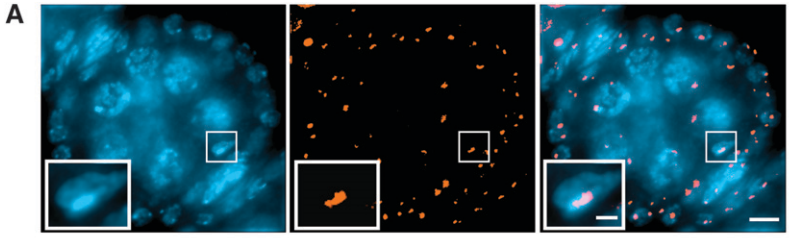


Figure 5.—Reduction of centromere-proximal heterochromatin renders *X* chromosomes vulnerable to age-dependent NDJ in *ord*^{4}/*ord*^{10} oocytes The percentage of *X* chromosome NDJ is shown for aged (shaded bars) and nonaged (open bars) oocytes. Error bars represent 95% confidence intervals. (A) Aging does not increase *X* chromosome NDJ *In(1)dl-49/y;ord*$^{4/10}$ oocytes ($P \geq 0.0656$; $N > 790$ for each brood). (B) *Df(1)bb*^{158}*/y;ord*$^{4/10}$ oocytes exhibit age dependent in all three broods tested (**$P = 0.0001$, *$P = 0.0059$; $N > 1030$ for each brood). The raw data for A and B are presented in supplemental Tables S4 and S5, respectively.



C

Genotype	FISH Signal			% Apart	*P* value
	O	OO	Oo		
FM7a/X	98	8	1	8.4	0.0069
FM7a/X;ord^{4}/ord^{10}	161	18	23*	20.3	

FIGURE 6.—Disruption of *ord* activity weakens *FM7a/X* heterochromatic pairing. (A and B) Drosophila ovaries from females reared under normal conditions (no aging regimen) were hybridized with the 359-bp repeat pericentric heterochromatin probe (orange) and stained with DAPI (blue). Single egg chambers are shown with the oocyte nucleus enlarged in the inset. Bars, 5 μm in the panels; 1 μm in the insets. (A) Pairing of the distal and the proximal heterochromatin of *FM7a* with the pericentric heterochromatin of the normal *X* chromosome results in a single focus within the oocyte nucleus. A stage 3 oocyte is shown. (B) Separated FISH signals result if the interrupted heterochromatin of *FM7a* is not completely paired with its homolog. A stage 5 oocyte is shown. (C) Heterochromatin pairing was quantified in *FM7a/X*; *ord*$^{+}$ and *FM7a/X*; *ord^{4}/ord^{10}* oocytes using the 359-bp FISH probe. The data represent tabulated results for oocyte stages 2–11 (see supplemental Table S7). "O" denotes a single FISH signal (as seen in A). "OO" denotes separated FISH signals of equal size that occur when the distal heterochromatin of *FM7a* is not paired with the normal *X* chromosome. "Oo" denotes separated FISH signals of different sizes (as shown in B) that occur when the centromere-proximal heterochromatin of *FM7a* fails to pair with the normal *X* chromosome. (*) 2/23 oocytes contained three FISH signals. Pairing between the heterochromatic regions of the *FM7a* balancer and a normal *X* chromosome is disrupted more often in *ord^{4}/ord^{10}* than in *ord*$^{+}$ oocytes ($P = 0.0069$).

versely, more than one spot will be observed (Figure 6B) if either the distal or the proximal heterochromatin of *FM7a* is not paired with the normal *X* homolog (Dernburg *et al.* 1996). Furthermore, because the centromere proximal heterochromatin of *FM7a* is much smaller than the distal heterochromatin, a small FISH signal separated from a large FISH signal indicates that the centromeres of the two *X* chromosomes are no longer paired (see Figure 6B).

For these experiments, we examined prophase I oocytes spanning ovariole stages 2–11 (see Figure 2C). Stages 2–6 represent pachytene oocytes and stages 7–11 correspond to oocytes after synaptonemal complex disassembly but before nuclear envelope breakdown or spindle formation (Ashburner 1989). Although pairing between homologous euchromatic regions is lost at the end of pachytene (Dernburg *et al.* 1996), the pericentric heterochromatin of homologs remains in contact until anaphase I (Dernburg *et al.* 1996; Gilliland *et al.* 2009; Hughes *et al.* 2009). Therefore, analysis of *FM7a/X* heterochromatin pairing during stages 2–11 allowed us to examine the behavior of this achiasmate bivalent during pachytene and postpachytene stages.

FISH analysis demonstrated that heterochromatin pairing between the *FM7a* balancer and a normal *X* chromosome is significantly disrupted in *ord* oocytes. In *ord*$^{+}$ oocytes, the 359-bp satellite sequences were apart (two spots) in 8.4% of the oocytes examined (Figure 6C, supplemental Table S7). In addition, only one out of the nine instances observed corresponded to separated centromeres (Oo). In the *ord^{4}/ord^{10}* mutant oocytes, disruption of heterochromatin pairing was significantly more frequent ($P = 0.0069$). Separated FISH signals were observed in 20.3% of the *ord* oocytes (Figure 6C, supplemental Table S7) and detectable at every stage examined (stages 2–11). Moreover, centromeres of *FM7a* and the normal *X* chromosome were apart in 23 out of the 202 oocytes examined, more than 10 times the incidence that we observed for wild type. The separated FISH signals in *FM7a/X;ord^{4}/ord^{10}* oocytes do not represent individual sister chromatids because in genetic tests, this same genotype results in very few diplo-*X* progeny that arise from an equational NDJ event (see above). Therefore, our FISH data indicate that pairing at the centromere-proximal heterochromatin in *FM7a/X* bivalents is compromised in *ord* mutants (even in the absence of aging) and supports the model that the cohesion protein ORD promotes heterochromatin-mediated association of achiasmate bivalents.

DISCUSSION

In general, proper segregation of homologous chromosomes during meiosis requires the formation and maintenance of chiasmata (Buonomo *et al.* 2000; Bickel *et al.* 2002; Hodges *et al.* 2005). Cohesion along the arms of sister chromatids is required to hold recombinant homologs together until anaphase I (see Figure 1). In some organisms, the presence of an

achiasmate pathway ensures that bivalents that lack a crossover still segregate accurately (Wolf 1994). In Drosophila oocytes, accurate segregation of achiasmate chromosomes relies on homologous pairing of centromere-proximal heterochromatin (see Figure 1). Although heterochromatin-mediated association of all homologs occurs (Dernburg *et al.* 1996), only achiasmate bivalents require this mechanism to segregate properly (see Figure 1). Our previous work suggested an unexpected link between sister-chromatid cohesion and the achiasmate segregation pathway (Jeffreys *et al.* 2003) but how cohesion proteins are able to influence the disjunction of achiasmate chromosomes was not readily apparent. In this study we have used mutations that disrupt the cohesion protein ORD and *X* chromosomes with different amounts of centromere proximal heterochromatin to better understand how sister-chromatid cohesion contributes to achiasmate segregation.

In all organisms examined, defects in meiotic sister-chromatid cohesion also have a severe effect on meiotic exchange (van Heemst and Heyting 2000). This holds true for ord^{null} oocytes in which meiotic crossing over on the *X* chromosome is reduced to ~16% of wild type (Bickel *et al.* 1997). Surprisingly, we have found that *ord* mutations that only mildly affect segregation (ord^{4} and ord^{8}), exhibit a severe defect in meiotic recombination; nonexchange tetrads in ord^{4}/ord^{10} and ord^{8}/ord^{10} oocytes are as frequent as when ORD activity is absent. This differential effect on meiotic exchange and segregation may arise because arm cohesion is disrupted to a greater degree than centromeric cohesion in both ord^{4} and ord^{8} mutant oocytes. Alternatively, it is also possible that a small defect in arm cohesion has a substantial impact on meiotic exchange.

Studies using both flies and mice support the hypothesis that sister-chromatid cohesion deteriorates as oocytes age and results in the missegregation of recombinant chromosomes (Hodges *et al.* 2005; Subramanian and Bickel 2008). In addition, we find that aging in Drosophila oocytes is accompanied by a significant increase in the missegregation of achiasmate bivalents when oocytes begin with meiotic cohesion that is slightly compromised (Jeffreys *et al.* 2003; this study). Our ability to examine the effect of age on the fidelity of meiotic chromosome segregation in Drosophila oocytes has provided us with a sensitive assay that has uncovered a functional link between meiotic cohesion and pericentric heterochromatin in the pairing and disjunction of achiasmate homologs. We observe age-dependent NDJ of achiasmate chromosomes in ord^{4}/ord^{10} as well as ord^{8}/ord^{10} mutant oocytes. However, in the presence of the weaker ord^{4} allele, achiasmate bivalents are only susceptible to age effects when the centromere-proximal heterochromatin of one of the *X* chromosomes is also reduced. The effect we observe depends on reduction of ORD activity; age-dependent NDJ of *FM7a/X* achiasmate bivalents does not occur in ord^{+} oocytes (Jeffreys *et al.* 2003).

Using *in situ* hybridization, we have shown that pairing between the heterochromatin of *FM7a* and a normal *X* chromosome is destabilized in *ord* mutant oocytes. In our previous FISH analyses of ord^{null} oocytes we did not detect pairing defects between two normal *X* chromosomes during pachytene (Webber *et al.* 2004) or prior to nuclear envelope breakdown in late prophase (Bickel *et al.* 2002). However, the reduction of centromere proximal heterochromatin on *FM7a* provides a sensitized system that has revealed a role for ORD in promoting the continued physical association between the centromere-proximal heterochromatin of homologous chromosomes.

Consistent with a role for cohesion proteins in heterochromatin-mediated homolog pairing, ORD, SMC1, and SMC3 are enriched at the centric/pericentric regions of meiotic chromosomes in Drosophila oocytes (Webber *et al.* 2004; Khetani and Bickel 2007). This localization pattern is not unique to Drosophila meiosis. Strong pericentric localization of cohesin subunits has been observed during both mitosis and meiosis in several organisms (Watanabe and Kitajima 2005). Moreover, in *S. pombe*, recruitment of cohesins to the pericentric heterochromatin depends on Swi6 (HP1 ortholog) and therefore on heterochromatin structure (Bernard *et al.* 2001; Bernard and Allshire 2002; Nonaka *et al.* 2002). However, whether such a mechanism operates universally is controversial (Koch *et al.* 2008).

Here we show that the sister-chromatid cohesion protein ORD plays a role in maintaining heterochromatin-mediated associations between achiasmate homologs and that these associations weaken with age. Moreover, our recent work also indicates that when the dosage of the cohesin subunit SMC1 is reduced, missegregation of nonrecombinant chromosomes increases with age (Subramanian and Bickel 2008). Therefore, two different proteins required for meiotic cohesion in Drosophila oocytes participate in the accurate segregation of achiasmate chromosomes. These data argue that ORD and cohesin proteins function similarly to ensure the proper segregation of achiasmate homologs.

We propose that cohesion proteins within pericentric heterochromatin provide a structural framework necessary for the physical association of homologous chromosomes (Figure 7). One possibility is that additional (as yet unidentified) proteins interact with cohesion proteins and heterochromatin proteins to form a bridge between the homologous chromosomes (Figure 7A). A more conservative possibility is that a heterochromatin protein on one homolog interacts directly with a cohesion protein on the other homolog. Such models are consistent with the recent report of Hawley and colleagues describing heterochromatic threads connecting achiasmate bivalents during prometaphase I oscillation (Hughes *et al.* 2009). We propose that when oocytes age under normal conditions (*i.e.*, wild-type

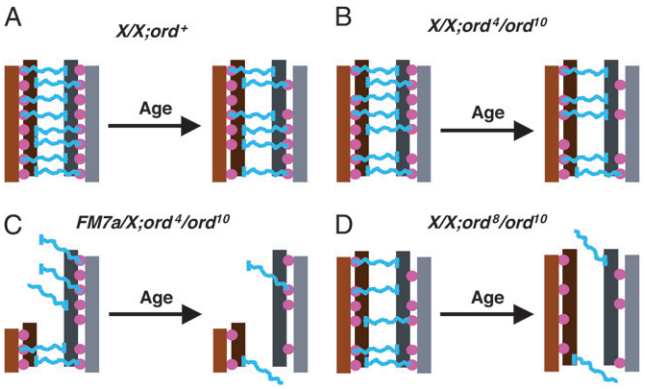


Figure 7.—Model for how cohesion proteins and pericentric heterochromatin might cooperate to maintain the association of achiasmate homologs. This schematic provides one possible model to explain why heterochromatin-mediated attachments between achiasmate chromosomes depend on sister-chromatid cohesion proteins and how decline of cohesion with age contributes to achiasmate nondisunction. The pericentric heterochromatin of a set of achiasmate homologs is depicted in shades of brown and gray, with sister chromatids shown in different shades. Pink filled circles represent sister-chromatid cohesion proteins. In this model, hypothetical linker proteins that physically connect the two homologs are depicted in blue. Each blue "linker" physically interacts with heterochromatin of one homolog and cohesion proteins that join the two sisters of the other homolog. For simplicity, interaction of the linkers with heterochromatin (vertical blue bars) is shown for only one of the sisters. A more conservative possibility is that the blue "linker" represents a heterochromatin protein on one homolog that interacts with a cohesion protein on the other homolog. (A) In wild-type flies with two normal *X* chromosomes, even though some deterioration of cohesion occurs during the aging process, the association of achiasmate chromosomes remains intact. (B) In ord^4/ord^{10} oocytes, the centromeric function of ORD is slightly compromised even in young oocytes (fewer filled circles). However, weakening of cohesion with age does not significantly affect the association between homologs when normal amounts of heterochromatin reside near the centromere. (C) In contrast, when centromere-proximal heterochromatin is reduced (*FM7a*) in ord^4/ord^{10} oocytes, the combination of reduced ORD activity at the centromere, fewer heterochromatin attachment sites and deterioration of cohesion with age causes achiasmate homologs to lose their association. (For ease of illustration, the reduction of heterochromain in *FM7a* is not drawn to scale). (D) In ord^8/ord^{10} oocytes, the centromeric function of ORD is more severely compromised and age-dependent deterioration of cohesion significantly affects homolog association even when pericentric heterochromatin is normal. Moreover, sister chromatid NDJ is also observed with the stronger ord^8 mutation and the frequency of equational NDJ increases when oocytes undergo aging. Note that the loss of homolog association shown in C and D is meant to represent a significant increase in achiasmate missegregation with age, not complete dissociation of the bivalents in 100% of the oocytes.

sister-chromatid cohesion as well as a normal amount of centromere-proximal heterochromatin), achiasmate segregation errors are not observed because the association of homologs remains intact even though some cohesion is lost with age (Figure 7A). Similarly, even when cohesion is weakened by the ord^4 mutation, if a large region of heterochromatin resides close to the centromere (normal *X* chromosome), the compromised activity of ORD^4 protein is still sufficient to support pairing between homologous chromosomes when oocytes undergo aging (Figure 7B). However, when the number of heterochromatin pairing sites near the centromere is reduced [*FM7a* or $Df(1)bb^{158}$] and the strength of these homolog connections is also compromised by the partial loss-of-function ord^4 mutation, loss of cohesion with age causes achiasmate chromosomes to lose their associations and exhibit age-dependent NDJ (Figure 7C). In ord^8/ord^{10} mutant flies, ORD activity at the centromere is more severely compromised than in ord^4/ord^{10} oocytes (Figure 7D) and we observe age-dependent NDJ of *X* chromosomes that contain the normal amount of centromere-proximal heterochromatin. Our data suggest that even though the amount of heterochromatin is not reduced, the physical connections holding achiasmate homologs together in ord^8/ord^{10} oocytes are weaker and exhibit a greater susceptibility to age. In some cases, centromeric cohesion is completely lost in ord^8/ord^{10} oocytes (not depicted), resulting in equational NDJ events and these also become more frequent as oocytes age.

In the model presented in Figure 7, cohesion proteins and heterochromatin both interact directly with hypothetical proteins that bridge the homologous chromosomes. Another possibility (not depicted) is that cohesion proteins within the pericentric heterochromatin on the two homologs physically interact with each other and form the bridge themselves. Such a scenario would be consistent with the "hand-cuff" model recently described in which individual cohesin rings on two chromatids are held together by a common Scc3/SA subunit (Zhang *et al.* 2008). A third alternative (also not depicted) is that cohesion proteins associated with the centromere-proximal heterochromatin play a more indirect role in which they help maintain proper heterochromatin structure, which is required for achiasmate associations but cohesion proteins do not interact directly with the proteins that physically hold the achiasmate homologs.

Although at this time we cannot distinguish between the above scenarios, our findings clearly indicate that cohesion proteins play an important role in heterochromatin-mediated pairing and accurate segregation of achiasmate chromosomes in Drosophila oocytes. Notably, our data argue that meiotic cohesion proteins not only function to keep sister chromatids associated and chiasmate bivalents intact, but also collaborate with heterochromatin to keep achiasmate chromosomes physically connected, thereby ensuring their proper segregation. Although each set of homologs exhibits heterochromatin-mediated association during prophase I (Dernburg *et al.* 1996), only achiasmate bivalents require this mechanism to segregate properly (see Figure 1). Because the majority of bivalents are achias-

mate in *ord* mutant oocytes, our analysis and model are restricted to their behavior in this article. However, given its essential role in arm cohesion and chiasma maintenance (Bickel *et al.* 2002), we expect that deterioration of ORD activity during the aging process most likely also reduces chiasmata stability.

Our work with Drosophila oocytes that have been subjected to aging indicates that both chiasmate and achiasmate bivalents are impacted by the aging process (Jeffreys *et al.* 2003; Subramanian and Bickel 2008; this study). In our studies, the unifying factor for age-dependent NDJ of chiasmate and achiasmate chromosomes is loss of cohesion with age. Deterioration of arm cohesion with age leads to destabilization of chiasmata, which allows recombinant homologs to missegregate more frequently in aged oocytes (Subramanian and Bickel 2008). In addition, the activity of cohesion proteins enriched at pericentric heterochromatin also appears to decline with age (this article) and this leads to increased missegregation of achiasmate chromosomes.

Chromosome segregation errors in human oocytes increase dramatically as women age and much work has focused on understanding the mechanisms that lead to age-dependent NDJ of recombinant chromosomes (Sherman *et al.* 1994; Lamb *et al.* 1997, 2005; Hassold and Hunt 2001; Hodges *et al.* 2005). Given that chiasmata must remain intact for decades in human oocytes, loss of cohesion with age could account, at least in part, for the high incidence of NDJ of chiasmate bivalents observed in older women and studies in mice and Drosophila support this conclusion (Hodges *et al.* 2005; Subramanian and Bickel 2008). Whether an achiasmate chromosome segregation pathway exists in human oocytes remains controversial (Koehler and Hassold 1998). However, recent analysis of the recombinational history of chromosome 21 in human oocytes indicates that achiasmate chromosomes segregate accurately more often than expected; 20% of the normal segregation events analyzed originated from achiasmate chromosome 21 tetrads (Oliver *et al.* 2008). Moreover, the data of Sherman and colleagues also suggest that a greater proportion of E_0 bivalents missegregate in older oocytes than in the younger age groups (Oliver *et al.* 2008). Although still speculative, such evidence supports the hypothesis that an achiasmate pathway may indeed operate during female meiosis in humans and that its effectiveness deteriorates with age. While additional evidence will be necessary to support this claim, the link between sister-chromatid cohesion and achiasmate segregation that we have uncovered in Drosophila oocytes provides an intriguing framework within which to consider the maternal age effect in humans.

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LITERATURE CITED

Agresti, A., and B. A. Coull, 1998 Approximate is better than "exact" for interval estimation of binomial proportions. Am. Stat. **52:** 119–126.

Ashburner, M., 1989 *Drosophila: A Laboratory Handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Balicky, E. M., M. W. Endres, C. Lai and S. E. Bickel, 2002 Meiotic cohesion requires accumulation of ORD on chromosomes prior to condensation. Mol. Biol. Cell **21:** 3890–3900.

Bernard, P., and R. Allshire, 2002 Centromeres become unstuck without heterochromatin. Trends Cell Biol. **12:** 419–424.

Bernard, P., J. F. Maure, J. F. Partridge, S. Genier, J. P. Javerzat *et al.*, 2001 Requirement of heterochromatin for cohesion at centromeres. Science **294:** 2539–2542.

Bickel, S. E., T. Orr-Weaver and E. M. Balicky, 2002 The sister-chromatid cohesion protein ORD is required for chiasma maintenance in Drosophila oocytes. Curr. Biol. **12:** 925–929.

Bickel, S. E., D. W. Wyman, W. Y. Miyazaki, D. P. Moore and T. L. Orr-Weaver, 1996 Identification of ORD, a Drosophila protein essential for sister-chromatid cohesion. EMBO J. **15:** 1451–1459.

Bickel, S. E., D. W. Wyman and T. L. Orr-Weaver, 1997 Mutational analysis of the Drosophila sister-chromatid cohesion protein ORD and its role in the maintenance of centromeric cohesion. Genetics **146:** 1319–1331.

Buonomo, S. B., R. K. Clyne, J. Fuchs, J. Loidl, F. Uhlmann *et al.*, 2000 Disjunction of homologous chromosomes in meiosis I depends on proteolytic cleavage of the meiotic cohesin Rec8 by separin. Cell **103:** 387–398.

Cheslock, P. S., B. J. Kemp, R. M. Boumil and D. S. Dawson, 2005 The roles of MAD1, MAD2 and MAD3 in meiotic progression and the segregation of nonexchange chromosomes. Nat. Genet. **37:** 756–760.

Davis, L., and G. R. Smith, 2005 Dynein promotes achiasmate segregation in *Schizosaccharomyces pombe*. Genetics **170:** 581–590.

Dawson, D. S., A. W. Murray and J. W. Szostak, 1986 An alternate pathway for meiotic chromosome segregation in yeast. Science **234:** 713–717.

Dernburg, A. F., J. W. Sedat and R. S. Hawley, 1996 Direct evidence of a role for heterochromatin in meiotic chromosome segregation. Cell **86:** 135–146.

Ding, D. Q., A. Yamamoto, T. Haraguchi and Y. Hiraoka, 2004 Dynamics of homologous chromosome pairing during meiotic prophase in fission yeast. Dev. Cell **6:** 329–341.

Doll, E., M. Molnar, Y. Hiraoka and J. Kohli, 2005 Characterization of rec15, an early meiotic recombination gene in *Schizosaccharomyces pombe*. Curr. Genet. **48:** 323–333.

Gilliland, W. D., S. F. Hughes, D. R. Vietti and R. S. Hawley, 2009 Congression of achiasmate chromosomes to the metaphase plate in *Drosophila melanogaster* oocytes. Dev. Biol. **325:** 122–128.

Grell, R. F., 1962 A new model for secondary nondisjunction: the role of distributive pairing. Genetics **47:** 1737–1754.

Guacci, V., and D. B. Kaback, 1991 Distributive disjunction of authentic chromosomes in *Saccharomyces cerevisiae*. Genetics **127:** 475–488.

Hassold, T., and P. Hunt, 2001 To err (meiotically) is human: the genesis of human aneuploidy. Nat. Rev. Genet. **2:** 280–291.

Hawley, R. S., H. Irick, A. E. Zitron, D. A. Haddox, A. Lohe *et al.*, 1993 There are two mechanisms of achiasmate segregation in *Drosophila*, one of which requires heterochromatic homology. Dev. Genet. **13:** 440–467.

Hawley, R. S., and W. E. Theurkauf, 1993 Requiem for distributive segregation: achiasmate segregation in *Drosophila* females. Trends Genet. **9:** 310–317.

Hodges, C. A., E. Revenkova, R. Jessberger, T. J. Hassold and P. A. Hunt, 2005 SMC1beta-deficient female mice provide evidence that cohesins are a missing link in age-related nondisjunction. Nat. Genet. **37:** 1351–1355.

Hughes, S. E., W. D. Gilliland, J. L. Cotitta, S. Takeo, K. A. Collins *et al.*, 2009 Heterochromatic threads connect oscillating chromosomes during prometaphase I in Drosophila oocytes. PLoS Genet. **5:** e1000348.

Jeffreys, C. A., P. S. Burrage and S. E. Bickel, 2003 A model system for increased meiotic nondisjunction in older oocytes. Curr. Biol. **13:** 498–503.

Karpen, G. H., M. H. Le and H. Le, 1996 Centric heterochromatin and the efficiency of achiasmate disjunction in *Drosophila* female meiosis. Science **273:** 118–122.

Kemp, B., R. M. Boumil, M. N. Stewart and D. S. Dawson, 2004 A role for centromere pairing in meiotic chromosome segregation. Genes Dev. **18:** 1946–1951.

Khetani, R. S., and S. E. Bickel, 2007 Regulation of meiotic cohesion and chromosome core morphogenesis during pachytene in Drosophila oocytes. J. Cell. Sci. **120:** 3123–3137.

King, R. C., 1957 Oogenesis in adult *Drosophila melanogaster*. II. Stage distribution as a function of age. Growth **21:** 95–102.

Koch, B., S. Kueng, C. Ruckenbauer, K. S. Wendt and J. M. Peters, 2008 The Suv39h-HP1 histone methylation pathway is dispensable for enrichment and protection of cohesin at centromeres in mammalian cells. Chromosoma **117:** 199–210.

Koehler, K. E., and T. J. Hassold, 1998 Human aneuploidy: lessons from achiasmate segregation in *Drosophila melanogaster*. Ann. Hum. Genet. **62:** 467–479.

Kudo, N. R., K. Wassmann, M. Anger, M. Schuh, K. G. Wirth *et al.*, 2006 Resolution of chiasmata in oocytes requires separase-mediated proteolysis. Cell **126:** 135–146.

Lamb, N. E., E. Feingold, A. Savage, D. Avramopoulos, S. Freeman *et al.*, 1997 Characterization of susceptible chiasma configurations that increase the risk for maternal nondisjunction of chromosome 21. Hum. Mol. Genet. **6:** 1391–1399.

Lamb, N. E., S. L. Sherman and T. J. Hassold, 2005 Effect of meiotic recombination on the production of aneuploid gametes in humans. Cytogenet. Genome Res. **111:** 250–255.

Lee, J. Y., and T. L. Orr-Weaver, 2001 The molecular basis of sister-chromatid cohesion. Annu. Rev. Cell Dev. Biol. **17:** 753–777.

Mason, J. M., 1976 Orientation disruptor (*ord*): a recombination-defective and disjunction-defective meiotic mutant in *Drosophila melanogaster*. Genetics **84:** 545–572.

Meneely, P. M., A. F. Farago and T. M. Kauffman, 2002 Crossover distribution and high interference for both the X chromosome and an autosome during oogenesis and spermatogenesis in *Caenorhabditis elegans*. Genetics **162:** 1169–1177.

Miyazaki, W. Y., and T. L. Orr-Weaver, 1992 Sister-chromatid misbehavior in Drosophila *ord* mutants. Genetics **132:** 1047–1061.

Miyazaki, W. Y., and T. L. Orr-Weaver, 1994 Sister-chromatid cohesion in mitosis and meiosis. Annu. Rev. Genet. **28:** 167–187.

Molnar, M., J. Bahler, J. Kohli and Y. Hiraoka, 2001 Live observation of fission yeast meiosis in recombination-deficient mutants: a study on achiasmate chromosome segregation. J. Cell. Sci. **114:** 2843–2853.

Nonaka, N., T. Kitajima, S. Yokobayashi, G. Xiao, M. Yamamoto *et al.*, 2002 Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. Nat. Cell Biol. **4:** 89–93.

Oliver, T. R., E. Feingold, K. Yu, V. Cheung, S. Tinker *et al.*, 2008 New insights into human nondisjunction of chromosome 21 in oocytes. PLoS Genet. **4:** e1000033.

Pasierbek, P., M. Jantsch, M. Melcher, A. Schleiffer, D. Schweizer *et al.*, 2001 A *Caenorhabditis elegans* cohesion protein with functions in meiotic chromosome pairing and disjunction. Genes Dev. **15:** 1349–1360.

Roberts, P., 1962 Interchromosomal effects and the relation between crossing-over and nondisjunction. Genetics **47:** 1691–1709.

Robinson, D. N., K. Cant and L. Cooley, 1994 Morphogenesis of Drosophila ovarian ring canals. Development **120:** 2015–2025.

Ross, L. O., S. Rankin, M. F. Shuster and D. S. Dawson, 1996 Effects of homology, size and exchange on the meiotic segregation of model chromosomes in *Saccharomyces cerevisiae*. Genetics **142:** 79–89.

Sherman, S. L., M. B. Petersen, S. B. Freeman, J. Hersey, D. Pettay *et al.*, 1994 Non-disjunction of chromosome 21 in maternal meiosis I: Evidence for a maternal age-dependent mechanism involving reduced recombination. Hum. Mol. Genet. **3:** 1529–1535.

Siomos, M. F., A. Badrinath, P. Pasierbek, D. Livingstone, J. White *et al.*, 2001 Separase is required for chromosome segregation during meiosis I in *Caenorhabditis elegans*. Curr. Biol. **11:** 1825–1835.

Sturtevant, A. H., and G. W. Beadle, 1936 The relations of inversions in the X chromosome of *Drosophila melanogaster* to crossing over and disjunction. Genetics **21:** 554–604.

Subramanian, V. V., and S. E. Bickel, 2008 Aging predisposes oocytes to meiotic nondisjunction when the cohesin subunit SMC1 is reduced. PLoS Genet. **4:** e1000263.

Theurkauf, W. E., and R. S. Hawley, 1992 Meiotic spindle assembly in Drosophila females: behavior of nonexchange chromosomes and the effects of mutations in the *nod* kinesin-like protein. J. Cell Biol. **116:** 1167–1180.

Uhlmann, F., 2001 Chromosome cohesion and segregation in mitosis and meiosis. Curr. Opin. Cell Biol. **13:** 754–761.

van Heemst, D., and C. Heyting, 2000 Sister chromatid cohesion and recombination in meiosis. Chromosoma **109:** 10–26.

Vazquez, J., A. S. Belmont and J. W. Sedat, 2002 The dynamics of homologous chromosome pairing during male Drosophila meiosis. Curr. Biol. **12:** 1473–1483.

Watanabe, Y., and T. S. Kitajima, 2005 Shugoshin protects cohesin complexes at centromeres. Philos. Trans. R. Soc. Lond. B Biol. Sci. **360:** 515–521.

Webber, H. A., L. Howard and S. E. Bickel, 2004 The cohesion protein ORD is required for homologue bias during meiotic recombination. J. Cell Biol. **164:** 819–829.

Weinstein, A., 1936 The theory of multiple-strand crossing over. Genetics **21:** 155–199.

Wolf, K. W., 1994 How meiotic cells deal with non-exchange chromosomes. BioEssays **16:** 107–114.

Xiang, Y., and R. S. Hawley, 2006 The mechanism of secondary nondisjunction in *Drosophila melanogaster* females. Genetics **174:** 67–78.

Yamamoto, M., and G. L. Miklos, 1977 Genetic dissection of heterochromatin in Drosophila: the role of basal X heterochromatin in meiotic sex chromosome behaviour. Chromosoma **60:** 283–296.

Yamamoto, M., and G. L. Miklos, 1978 Genetic studies on heterochromatin in *Drosophila melanogaster* and their implications for the functions of satellite DNA. Chromosoma **66:** 71–98.

Zhang, N., S. G. Kuznetsov, S. K. Sharan, K. Li, P. H. Rao *et al.*, 2008 A handcuff model for the cohesin complex. J. Cell Biol. **183:** 1019–1031.

Zwick, M. E., D. J. Cutler and C. H. Langley, 1999 Classic Weinstein: tetrad analysis, genetic variation and achiasmate segregation in Drosophila and humans. Genetics **152:** 1615–1629.

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