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The cohesion protein ORD is required for homologue bias during meiotic recombination

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During meiosis, sister chromatid cohesion is required for normal levels of homologous recombination, although how cohesion regulates exchange is not understood. Null mutations in *orientation disruptor* (*ord*) ablate arm and centromeric cohesion during *Drosophila* meiosis and severely reduce homologous crossovers in mutant oocytes. We show that ORD protein localizes along oocyte chromosomes during the stages in which recombination occurs. Although synaptonemal complex (SC) components initially associate with synapsed homologues in *ord* mutants, their localization is severely disrupted during pachytene progression, and normal tripartite SC is not visible by electron microscopy. In *ord* germaria, meiotic double strand breaks appear and disappear with frequency and timing indistinguishable from wild type. However, Ring chromosome recovery is dramatically reduced in *ord* oocytes compared with wild type, which is consistent with the model that defects in meiotic cohesion remove the constraints that normally limit recombination between sisters. We conclude that ORD activity suppresses sister chromatid exchange and stimulates inter-homologue crossovers, thereby promoting homologue bias during meiotic recombination in *Drosophila*.

Introduction

Sister chromatid cohesion is a prerequisite for accurate chromosome segregation during mitosis and meiosis (Lee and Orr-Weaver, 2001; Nasmyth, 2001). In both types of cell division, the association between sister chromatids depends on the cohesin complex. However, several meiosis-specific cohesin subunits have been characterized, and in contrast to mitosis, meiotic cohesion must be released in a step-wise manner.

Meiotic cohesion not only ensures proper segregation of the sister chromatids during anaphase II but also functions to direct the proper behavior of homologous chromosomes during meiosis I. Arm cohesion distal to a chiasma is required for the stable association of recombinant homologues until anaphase I (Buonomo et al., 2000; Bickel et al., 2002). In addition, homologous recombination during meiosis is also dependent on sister chromatid cohesion. In yeast, flies, worms, and plants, mutations that disrupt meiotic cohesion severely reduce the number of crossovers (Mason, 1976; Broverman and Meneely, 1994; Klein et al., 1999; Parisi et al., 1999; Chan et al., 2003; Mercier et al., 2003). However, despite its essential role, the mechanism by which cohesion regulates recombination is not understood.

During meiotic prophase, recombination takes place within the context of a specialized structure called the synaptonemal complex (SC; Zickler and Kleckner, 1999; Page and Hawley, 2003). Like the proteins that mediate sister chromatid cohesion, SC axial/lateral element (AE/LE) components also assemble between sister chromatids (van Heemst and Heyting, 2000). As homologous chromosomes achieve synapsis, the AE/LEs become connected by evenly spaced transverse filaments that hold homologous chromosomes in close juxtaposition along their entire length. Because SC assembly depends in part on normal cohesion between sister chromatids (van Heemst and Heyting, 2000), mutations that disrupt cohesion may reduce meiotic exchange because the SC is defective.

After the induction of double strand breaks (DSBs) during prophase I of meiosis, only strand invasion into the homologous chromosome can produce a chiasma that will stabilize homologue association until anaphase I. Although meiotic recombination occurs preferentially between homologous chromosomes, not between sister chromatids (Schwacha and Kleckner, 1994; Petes and Pukkila, 1995), the mechanisms underlying homologue bias are largely unknown. One possibility is that meiosis-specific cohesion and/or SC components inhibit inter-sister recombination

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Abbreviations used in this paper: AE/LE, axial/lateral element; CE, central element; DSB, double strand break; γ-H2Av, phosphorylated H2Av; *ord*, *orientation disruptor*; SC, synaptonemal complex.

events. In support of this model, disruption of SC AE/LEs has been shown to increase the proportion of inter-sister recombination intermediates during meiosis in *Saccharomyces cerevisiae* (Schwacha and Kleckner, 1997). These data suggest that AE/LEs provide a meiosis-specific mechanism to suppress sister chromatid exchange, and thereby promote crossovers between homologues. Although this mechanism may be conserved evolutionarily, evidence that inter-homologue bias in metazoans is achieved by SC-mediated suppression of sister chromatid exchange is lacking. In addition, whether meiotic sister chromatid cohesion contributes directly to homologue bias has not been investigated in any organism.

Despite the lack of cohesin mutants in *Drosophila melanogaster*, analysis of the regulation of meiotic cohesion is afforded by mutations in the *orientation disruptor* (*ord*) gene. ORD protein is required for meiotic sister chromatid cohesion as well as normal levels of homologous recombination (Mason, 1976; Miyazaki and Orr-Weaver, 1992; Bickel et al., 1997). In both males and females, null *ord* alleles result in random segregation of sister chromatids during both meiotic divisions (Bickel et al., 1997). Genetic data, in addition to FISH analysis, indicate that in the absence of ORD activity, cohesion is abolished before prometaphase I when microtubule/kinetochore attachments are established (Balicky et al., 2002; Bickel et al., 2002). In addition, crossovers are reduced but not eliminated in *Drosophila* females that completely lack ORD activity (Bickel et al., 1997). At present, no alleles have been identified that separate the cohesion and recombination phenotypes. Therefore, we have proposed that meiotic exchange is reduced in *ord* females because defects in cohesion disrupt inter-homologue crossing over (Bickel et al., 1997). Our previous localization of ORD protein in *Drosophila* testes indicated that ORD associates with the meiotic chromosomes during the extended G2 phase of spermatogenesis and remains at the centromeres until cohesion is released at anaphase II (Balicky et al., 2002). However, *Drosophila* males do not undergo meiotic recombination (Morgan, 1912), and the regulation of arm cohesion in primary spermatocytes appears to be distinct from other organisms (Vazquez et al., 2002). Therefore, to investigate how cohesion and recombination are coordinately regulated, we turned our attention to the analysis of ORD function during female meiosis.

Here, we provide key insights into the mechanism by which sister chromatid cohesion promotes crossovers between homologous chromosomes during meiosis. We examine the localization of ORD protein during prophase I in *Drosophila* females and demonstrate that ORD is found along the entire length of oocyte chromosomes during the time that meiotic recombination takes place. Our data indicate that homologous chromosomes achieve synapsis in the absence of ORD activity and that the frequency and timing of DSBs are normal. In *ord* mutants, although SC components appear to load normally onto meiotic chromosomes, their association deteriorates during the progression of pachytene. Furthermore, we observe pronounced defects in SC ultrastructure. Decreased meiotic transmission of a Ring chromosome in *ord* females argues that ORD is required to suppress inter-sister crossovers during meiosis. Together, our data support the model that ORD is required for homologue bias during meiotic recombination. We propose that in *ord* oocytes, defects in sister chromatid cohesion and SC AE/LEs lead to decreased numbers of inter-homologue crossovers because the constraints that limit sister exchange are lifted. In addition, inter-homologue events may be inhibited by destabilization of the SC central element (CE) in *ord* mutants.

Results

ORD associates with arms and centromeres of oocyte chromosomes

In the *Drosophila* ovary, meiosis is initiated within the germarium, the most anterior portion of each ovariole (Spradling et al., 1997). Based on morphological criteria, the germarium can be divided into four regions (Fig. 1 A). In region 1, germline mitotic divisions produce cysts composed of 16 cells that remain interconnected by cytoplasmic bridges. A branched structure called the fusome connects the cells during the mitotic divisions and can be used as a marker to identify two-, four-, and eight-cell cysts (de Cuevas et al., 1997). The meiotic program initiates shortly after formation of the 16-cell cyst, and extensive SC assembly occurs in up to four cells per cyst in region 2A (Carpenter, 1975). DSBs, identified as phosphorylated H2Av (γ-H2Av) foci, are first apparent in region 2A (Jang et al., 2003), indicating that initiation of meiotic recombination also occurs in region 2A. As the 16-cell cysts mature, they move posteriorly. As early as region 2B but no later than region 3, oocyte determination is completed and full-length SC becomes restricted to the oocyte, which assumes a posterior position within the cyst.

To better define the role of ORD in meiotic sister chromatid cohesion and recombination, we used GFP antibodies to examine the localization of GFP-ORD in ovaries from transgenic flies. We have previously demonstrated that GFP-ORD is fully functional (Balicky et al., 2002) and that no GFP immunostaining is detectable in flies lacking the GFP-ORD fusion protein (Balicky et al., 2002; unpublished data). Fig. 1 B shows a full projection of GFP-ORD localization in a single germarium. ORD signal is visible within mitotic and meiotic germline cysts, but not in the somatically derived follicle cells that surround the germarium and older 16-cell cysts. In addition to bright ORD foci that are present in germline nuclei throughout the germarium (Fig. 1 B), diffuse ORD staining is detectable throughout the nuclei of some 8-cell cysts in region 1 (Fig. 1, C and D, arrows) and within 16-cell cysts at all stages (Fig. 1, B–D). Although ORD staining is not restricted to the oocyte, a few linear stretches of ORD signal are visible in the oocyte nucleus in region 3 (Fig. 1 B, arrowhead) and appear to overlap with the threadlike C(3)G staining that marks the SC (Fig. 1 C, arrowhead).

To better visualize ORD localization within female germ cells, we developed a chromosome spread protocol for germarial cells and early previtellogenic stages of the *Drosophila* ovary. In some instances, we obtain semi-intact cysts that allow us to observe all 16 germ cells (Fig. 1, E–G). In such cysts, ORD is clearly associated with the chromatin of all the germ cells. In addition, distinct ribbonlike ORD signal is visible within those cells that exhibit localization of the SC component C(3)G (Fig. 1 F). Although long threads of

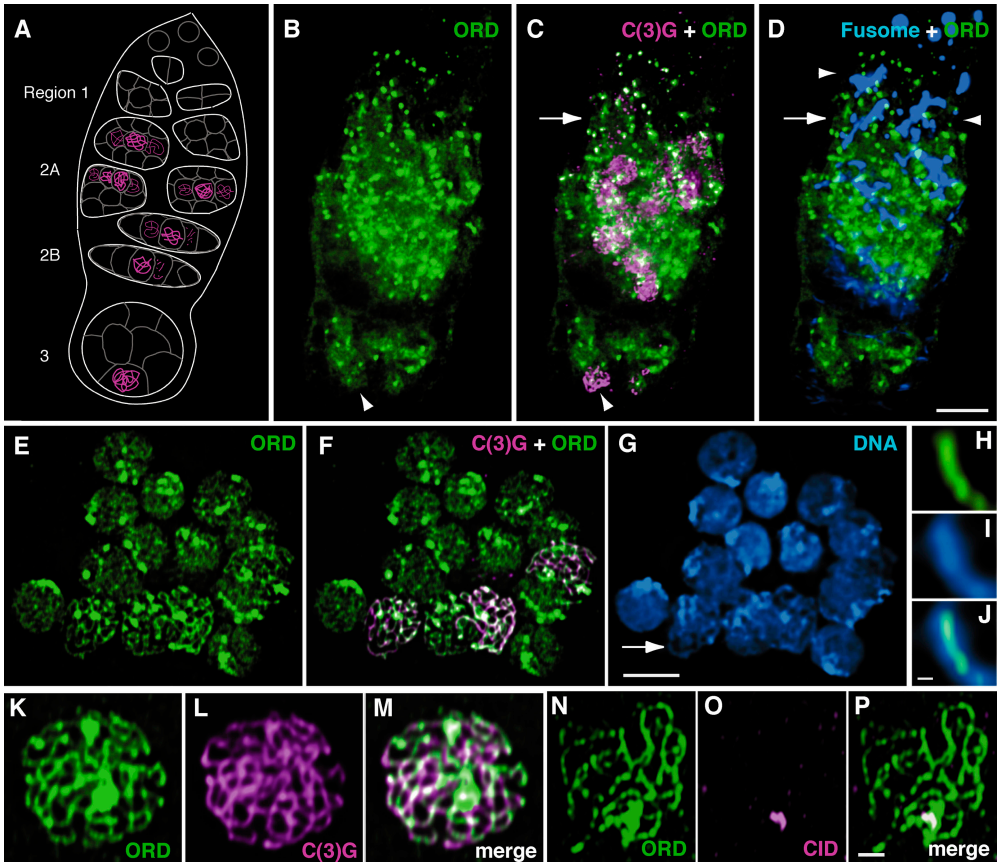

Figure 1. **Localization of ORD protein in wild-type germaria.** (A) Schematic depiction of germline cysts and SC in different regions of the germarium. (B–D) Full projection of deconvolved z-series shows ORD localization in single germarium. Bar, 10 μm. (B) Linear stretches of ORD signal are visible in region 3 oocyte nucleus (arrowhead). (C) ORD foci and whole cyst staining are visible in region 1 before C(3)G localization (arrow). Threadlike ORD staining colocalizes with C(3)G in the oocyte nucleus (arrowhead in B and C). (D) ORD foci in a four-cell cyst and an early eight-cell cyst (arrowheads). In addition to bright ORD foci, diffuse ORD signal becomes visible throughout an older 8-cell cyst (arrow) and persists during the development of 16-cell germarial cysts. (E–G) Chromosome spread of semi-intact region 2A cyst showing single optical section from a deconvolved z-series. Bar, 10 μm. (E) Bright foci and linear stretches of ORD are visible within all 16 nuclei of the cyst. (F) Threadlike ORD staining (green) is more pronounced within nuclei that also stain for C(3)G (magenta). (G) DAPI-stained DNA. (H–J) Enlarged view from G (arrow) shows ORD (green) concentrated along the center of the bivalent (blue). Bar, 500 nm. (K–M) Extensive colocalization of ORD and C(3)G in a single nucleus. (N–P) Bright focus of ORD staining overlaps with CID signal. (K–P) Each image represents a single optical section from a deconvolved z-series of a chromosome spread. Bar, 2 μm.

ORD staining are most pronounced within nuclei that contain C(3)G, less distinct linear elements are also visible within other cells of the cyst that do not form SC.

Within isolated nuclei of well-spread preparations (Fig. 1, K–M), ORD colocalizes with C(3)G along the five euchromatic arms that form continuous SC (Page and Hawley 2001). Furthermore, localization of ORD on DAPI-stained chromosomes (Fig. 1, H–J) reveals that ORD staining is restricted to a narrow region within the wider DNA signal of the bivalent. These data suggest that ORD may colocalize with the AE/LEs that connect meiotic sister chromatids.

Within each nucleus, one to four bright foci of ORD staining are prominent and often correspond to the most DAPI-intense regions of the chromatin (Fig. 1, compare E with G). We tested the possibility that ORD is enriched in centromeric heterochromatin by asking whether or not ORD colocalizes with CID, the *Drosophila* CENP-A orthologue that replaces H3 within the nucleosomes of centromeric heterochromatin (Henikoff et al., 2000; Blower and Karpen, 2001; Blower et al., 2002). We often observed one CID signal per nuclear spread (Fig. 1 O), confirming that the centromeres of pro-oocytes are often clustered (Carpenter, 1975; Dernburg et al., 1996). In colocalization experiments, bright ORD foci overlap with the CID signal (Fig. 1, N–P). However, CID staining is limited to a small region within the much larger bright ORD signal (Fig. 1 P). These data support the hypothesis that ORD is enriched within the heterochromatin of meiotic chromosomes but is not restricted to the specialized heterochromatin of the centromere.

Our localization experiments demonstrate that ORD protein is found along the entire length of oocyte chromosomes, which is consistent with its essential role in promoting both arm and centromeric cohesion during female meiosis. Moreover, extensive colocalization of ORD with the SC component C(3)G during pachytene indicates that ORD is associated with meiotic chromosomes during the stages when crossovers normally occur.

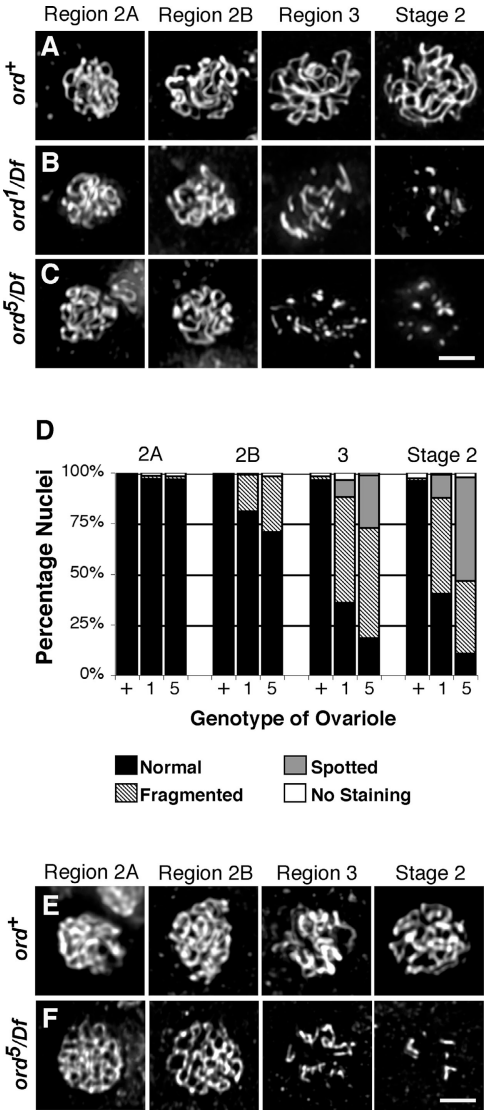

Figure 2. ***ord* mutations disrupt C(3)G and C(2)M localization.** (A–C) C(3)G localization within a single nucleus is shown for each region of the germarium and a vitellarial stage 2 egg chamber. Each image represents a full projection from a deconvolved z-series. Staining was performed on intact ovarioles (not spreads). ORB staining (not depicted) was used to identify oocytes in *ord* mutants. (A) In ord^+ germaria, extensive ribbonlike C(3)G is visible at each stage. (B) In region 2A of ord^1/Df mutant germaria, normal ribbonlike C(3)G staining is detected, but C(3)G staining becomes fragmented in the majority of ord^1/Df oocytes by region 3. (C) ord^5/Df mutant germaria also contain normal C(3)G staining in region 2A. However, the C(3)G signal is severely fragmented and spotted by region 3. Bar, 3 μm. (D) Quantification of the C(3)G defects in *ord* mutants shows the percentage of nuclei with normal, fragmented, spotted, or no C(3)G staining. Over 170 cells corresponding to each stage were examined. (E) Localization of C(2)M in ord^+ ovarioles. (F) C(2)M localization defects in ord^5/Df mutant germaria are similar to those observed for C(3)G. Although normal C(2)M signal is visible in region 2A, only fragmented and spotty staining is visible in region 3. Bar, 3 μm.

Destabilization of SC components in the absence of ORD protein

Colocalization of ORD with C(3)G, a putative transverse filament component of the SC (Page and Hawley, 2001), raises the possibility that homologous recombination is decreased in *ord* mutant females because ORD activity is required for normal assembly and/or function of the SC. Mutations that abolish SC in *Drosophila* females also abolish recombination (Gowen and Gowen, 1922; Gowen, 1933; Carpenter and Sandler, 1974; Page and Hawley, 2001). Therefore, we examined C(3)G localization in whole-mount preparations of *Drosophila* ovaries from *ord* mutants to determine whether ORD activity is required for the formation and/or maintenance of the SC (Fig. 2, A–C).

Examination of intact ovarioles allowed us to monitor the temporal progression of C(3)G staining within germaria of both wild-type and *ord* mutants. In wild-type ovaries, threadlike C(3)G staining becomes visible in up to four cells of each cyst in region 2A (Figs. 1 C and 2 A). As cysts mature during their progression through the germarium, the C(3)G signal becomes restricted to the oocyte and remains visible throughout pachytene (Figs. 1 C and 2 A).

We examined C(3)G staining in the ovarioles of two mutant *ord* backgrounds. The ord^5 mutation causes premature truncation of ORD protein and genetically behaves as a null (Bickel et al., 1996, 1997). In contrast, ord^1 contains a missense mutation that does not completely abolish ORD activity (Mason, 1976; Miyazaki and Orr-Weaver, 1992; Bickel et al., 1996). In both mutants, the onset of C(3)G staining appears normal in zygotene/early pachytene pro-oocytes in region 2A (Fig. 2, B and C). However, the C(3)G signal deteriorates as *ord* oocytes progress through the germarium. By region 3, only foci or short stretches of C(3)G are visible in most *ord* oocytes (Fig. 2, B and C).

Because the staining pattern of C(3)G varied somewhat in different ovarioles, we quantified defects within the germarium as well as in vitellarial stage 2 oocytes, the first stage after exit from the germarium (Fig. 2 D). Our analysis demonstrates a clear temporal progression in the deterioration of C(3)G signal in *ord* oocytes. In most cells, C(3)G appears to load normally onto meiotic chromosomes in region 2A, but staining progressively degenerates as the cysts mature. Defects appear less severe in ord^1 oocytes, indicating that the strength of the *ord* allele dictates the severity of C(3)G disruption. Our data argue that ORD activity is required to maintain the normal localization of C(3)G during pachytene and suggest that ORD plays a role in stabilizing the SC.

We also examined the localization of C(2)M, another putative *Drosophila* SC component (Manheim and McKim, 2003). Immunostaining experiments with C(2)M antibodies have demonstrated that, like C(3)G, threadlike C(2)M staining becomes visible within wild-type pro-oocytes in region 2A. Moreover, C(3)G staining is severely disrupted or absent in *c(2)M* mutants, which is consistent with the model that C(2)M is required for synapsis and assembly of the CE of the SC. Interestingly, computer analysis of the C(2)M sequence has identified a kleisin domain within the protein, suggesting that C(2)M may associate with SMC subunits (Schleiffer et al., 2003).

In ord^5/Df ovarioles, we observe normal threadlike C(2)M staining in region 2A (Fig. 2 F). However, in contrast to ord^+ germaria (Fig. 2 E), C(2)M signal is severely fragmented or absent in older *ord* mutant cysts (Fig. 2 F). The C(2)M and C(3)G defects that we observe in *ord* germaria are indistinguishable. Although the initial association of two different SC

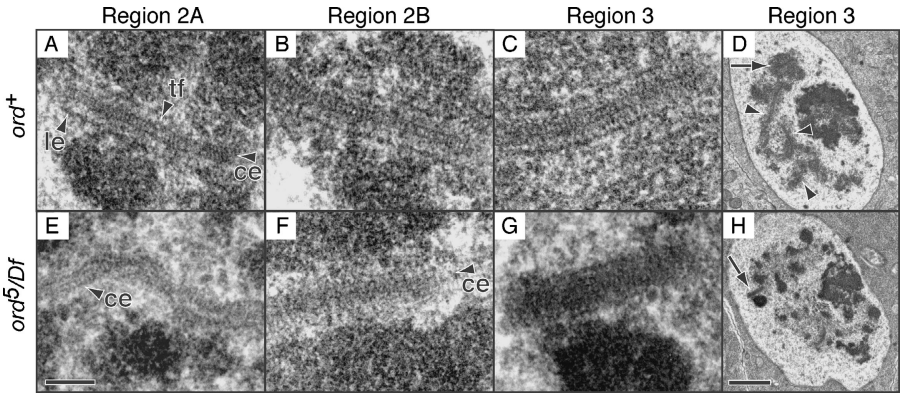

Figure 3. **Abnormal SC ultrastructure in *ord* mutants.** Transmission electron micrographs of germaria from ord^+ and ord^5/Df females. (A–C) Single sections showing examples of normal tripartite SC observed in each region of an ord^+ germarium. Lateral elements (le), transverse filaments (tf), and central elements (ce) are well defined and clearly visible in wild type. (D) Lower magnification of the entire region 3 nucleus showing multiple stretches of SC (arrowheads). The SC shown in C is indicated by the arrow. (E–G) Examples of abnormal SC-like structures from each region of ord^5/Df germaria. In some instances, (E and F) a central element (ce) is visible without obvious lateral elements. (H) Lower magnification of the entire region 3 nucleus with the area shown in G indicated by the arrow. Bars: (A–C and E–G) 200 nm; (D and H) 1 μm.

components with meiotic chromosomes appears normal in ovaries that lack ORD activity, ORD is required to maintain the normal localization of both C(2)M and C(3)G during pachytene. Because the SC is required for meiotic exchange in *Drosophila*, reduced levels of homologous recombination in *ord* mutants could be caused by destabilization of the SC.

Disruption of SC ultrastructure in *ord* oocytes

Deterioration of C(3)G and C(2)M localization during pachytene in *ord* mutants suggests that the SC may form normally in the absence of ORD but that its stabilization requires ORD activity. To test this hypothesis, we performed EM analysis to directly visualize the ultrastructure of the SC in *ord* mutants. Both wild-type and ord^5/Df ovarioles were fixed and processed for EM.

In wild type, stretches of SC were easily identified in the region 3 oocyte as well as the pro-oocytes of region 2A and 2B (Fig. 3, A–D). Distinct transverse filaments within the CE and well-defined lateral elements were clearly discernible (Fig. 3 A). We identified numerous examples of normal tripartite SC in all three regions of the wild-type germarium (136 examples in 380 longitudinal sections of a single germarium).

In two ord^5/Df germaria that were completely sectioned, structures that resembled SC were rare (18 instances in 740 longitudinal sections) and in all cases appeared abnormal (Fig. 3, E–G). In most cases, distinct lateral elements were not apparent (Fig. 3, E and F). We observed a few examples of what appeared to be a twisted CE containing organized transverse filaments in the absence of well-formed lateral elements (Fig. 3 E). In addition, some SC-like structures lacked definition between the central region and what could be lateral elements (Fig. 3 G). Their somewhat "fused" ultrastructure could represent frontal views of abnormal tripartite SC in which the central and lateral elements lack definition. Alternatively, such structures might also represent a side view of a saggital section through a CE.

Our EM analysis indicates that the ultrastructure of the SC is severely perturbed in *ord* germaria. Normal threadlike C(3)G and C(2)M immunofluorescent signal is visible in all *ord* germaria in region 2A, indicating that some SC components assemble in the absence of ORD activity. However, lack of normal electron-dense tripartite SC at any stage within the germarium suggests that ORD is required for proper formation of the SC. The discrepancy between the severity of defects observed in region 2A with the two visualization techniques could arise if thin and/or disorganized SC in *ord* mutants were sufficient for an immunofluorescent signal but not the highly ordered ultrastructure required for detection by EM. Alternatively, we cannot rule out the possibility that tripartite SC forms in *ord* mutants but is unstable and therefore more vulnerable than wild-type SC to disruption by EM sample preparation.

Homologous chromosomes synapse in *ord* oocytes

We were surprised that C(3)G staining in region 2A appeared normal in the absence of ORD activity even though SC ultrastructure was clearly disrupted at this stage. Although long threads of C(3)G signal represent synapsed homologues in wild-type oocytes (Page and Hawley, 2001), we reasoned that it was formally possible that in *ord* mutants, C(3)G was loading onto individual homologues in region 2A in the absence of synapsis. In addition, failure of homologues to achieve complete synapsis might be responsible for the destabilization of C(3)G localization that we observe in *ord* oocytes.

We used two methods to ask if synapsis depends on ORD activity. First, we measured the combined length of C(3)G threads within individual nuclei of wild-type and ord^5/Df chromosome spreads. If the linear segments of C(3)G staining observed in *ord* mutants represent entirely unsynapsed homologues, the total length of C(3)G signal per *ord* nucleus should be twice that of wild type. However, as shown in Fig. 4 A, lengths of C(3)G signal in wild-type and *ord* nuclei were quite similar and consistent with those previously reported (Page and Hawley, 2001). In addition, FISH analysis using X chromosome arm and pericentromeric probes (Fig. 4, B and C) indicates that homologous chromosomes pair in *ord* mutants and rules out the possibility that C(3)G threadlike signal represents nonhomologous synapsis. These results lead us to conclude that homologous chromosomes synapse normally in the absence of ORD protein.

Normal frequency and timing of DSBs in *ord* oocytes

In addition to SC defects, a reduction in the number of meiotic DSBs might also contribute to decreased numbers of crossovers in *ord* oocytes. Therefore, we monitored the tem-

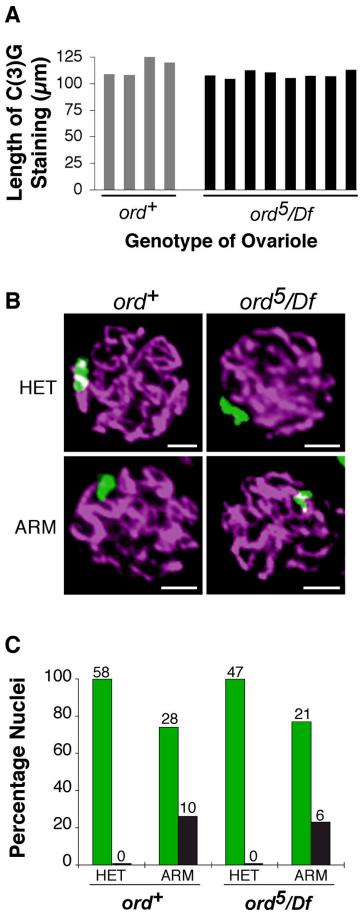

Figure 4. **Homologues synapse in the absence of ORD activity.** (A) Measured length of C(3)G staining in chromosome spread preparations from region 2B of wild-type and *ord*^{5}/*Df* mutant germaria. Mean C(3)G length is 114.6 ± 8.4 μm (n = 4) in *ord*$^{+}$ and 107.5 ± 3.2 μm (n = 8) in *ord*^{5}/*Df*. (B) Examples of chromosome spreads stained for C(3)G (magenta) and hybridized with probes (green) corresponding to pericentromeric heterochromatin or single copy sequences on the X chromosome arm (green). Bars, 2 μm. (C) Quantification of FISH analysis shows percentage of nuclei containing a single hybridization signal (green) or two to four signals (black) separated by <1 μm. n values are noted for each bar.

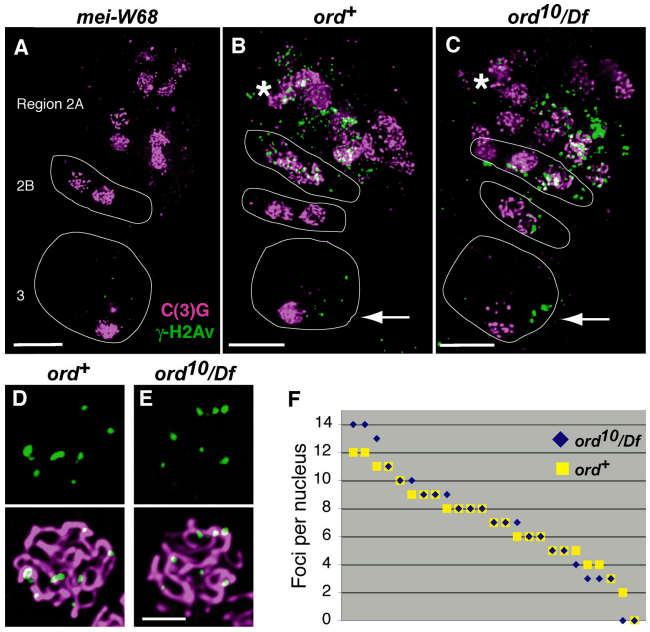

Figure 5. **Normal numbers of DSBs appear and disappear in *ord* oocytes.** (A–C) Whole germaria costained for C(3)G (magenta) and γ-H2Av (green). Images represent a full projection of a deconvolved z-series. Bars, 10 μm. Region 2B and region 3 cysts are outlined in white. (A) No γ-H2Av foci are visible in a *mei-W68* germarium. (B) In wild type, γ-H2Av foci appear in region 2A (asterisk) and disappear in the oocyte by region 3 (arrow). (C) In *ord*^{10}/*Df*, γ-H2Av foci appear and disappear with normal kinetics. Asterisk marks region 2A and arrow points to region 3 cyst. (D and E) Examples of region 2B nuclei used to generate the graph in F. Bar, 3 μm. (F) Scatter plot representing the number of γ-H2Av foci per C(3)G staining nucleus in region 2B. Mean number of γ-H2Av foci in *ord*$^{+}$ is 7.0 (n = 25) and for *ord*^{10}/*Df* mutant is 7.2 (n = 25). Late 2B cysts in which γ-H2Av foci were absent in both pro-oocytes were not included in this analysis.

poral progression of DSB appearance and disappearance in *ord*^{10}/*Df* germaria. Like *ord*^{5}, *ord*^{10} is a nonsense mutation that truncates the ORD open reading frame and genetically behaves as a null (Bickel et al., 1997). We chose *ord*^{10} for this analysis because it is the only null *ord* allele for which recombination levels have been measured (Bickel et al., 1997). Homologous recombination is severely reduced, but not eliminated in *ord*^{10}/*Df* females. As a marker for DSBs, we used an affinity-purified polyclonal antibody that recognizes the *Drosophila* H2Av histone variant when it is phosphorylated at serine 137 (Madigan et al., 2002). In *Drosophila* somatic cells, phosphorylation of H2Av has been shown to occur within 1 min after DNA damage (Madigan et al., 2002). Moreover, Jang et al. (2003) have recently demonstrated that γ-H2Av foci provide a useful marker for DSBs induced during meiotic prophase in *Drosophila* ovaries.

In wild-type germaria we observe a large number of γ-H2Av foci in region 2A nuclei (Fig. 5 B, asterisk). Foci are absent in *mei-W68* mutants that lack the *Drosophila* SPO11 protein (McKim and Hayashi-Hagihara, 1998), confirming that the staining we observe in wild-type germaria is dependent on DSB formation (Fig. 5 A). Consistent with the results of Jang et al. (2003), we observe that the number of γ-H2Av foci in wild-type germaria declines as the cysts move posteriorly (Fig. 5 B). Oocytes in region 3 rarely contain a detectable signal, suggesting that repair has progressed beyond the DSB stage (Fig. 5 B, arrow).

The γ-H2Av staining that we observe in *ord*^{10}/*Df* germaria is indistinguishable from wild type (Fig. 5, compare B with C). Foci are most numerous in region 2A nuclei (Fig. 5 C, asterisk) but are almost always absent in region 3 oocytes (Fig. 5 C, arrow). Therefore, DSBs are not only formed at the correct time in *ord* pro-oocytes, but their repair seems to occur with normal kinetics. Occasionally in both mutant and wild-type region 3 cysts, we observe residual γ-H2Av foci in the pro-oocyte that does not continue along the meiotic pathway.

To confirm our qualitative assessment that meiotic DSBs occur at wild-type levels in *ord* mutant germaria, we counted the number of foci in individual pro-oocytes within region 2B (Fig. 5, D–F). Although the number of foci varied from cell to cell in both genotypes, we observe a similar distribution for both wild-type and *ord* nuclei (Fig. 5 F). Moreover,

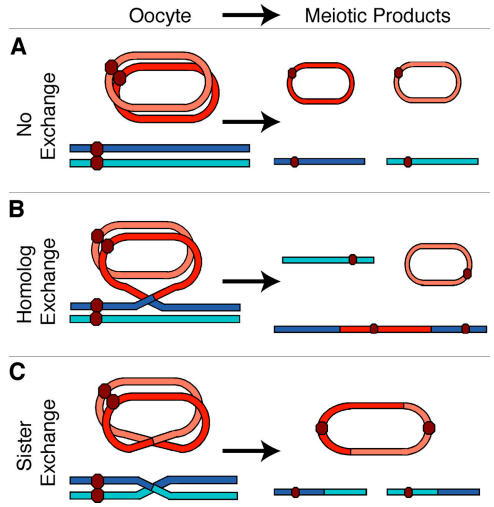

Figure 6. **Possible meiotic outcomes for females carrying one Ring X chromosome and one normal Rod X chromosome.** (A) Absence of inter-homologue or inter-sister exchange should yield equal numbers of meiotic products/progeny carrying either the Ring X or the Rod X chromatid. (B) Although a single crossover between homologous chromosomes will generate a dicentric chromosome that will not be transmitted, the number of progeny inheriting a nonrecombinant Ring or Rod chromosome should still be relatively equal. (C) Sister chromatid exchange between Rod X chromosomes should not affect their segregation. In contrast, a single crossover between the two Ring X sister chromatids will yield a large dicentric Ring chromosome that will not be transmitted to progeny. Therefore, high levels of sister chromatid exchange should cause Rod-containing progeny to greatly outnumber the Ring-containing progeny.

the average number of DSBs/nucleus in region 2B is almost identical in wild type (7.0) and mutant (7.2). Interestingly, the average number of DSBs that persist at this stage is slightly greater than the average number of crossovers per wild-type oocyte (Σ[map length for each arm/50 cM] = 5.68; Lindsley and Zimm, 1992), which is consistent with the possibility that late γ-H2Av foci represent DSBs that will be processed as crossover events.

Ring chromosome assay suggests that sister chromatid exchange is elevated in *ord* mutants

If DSBs are formed and repaired with normal frequency and timing in the absence of ORD protein, why is the number of crossovers reduced? One possibility is that recombination defects arise in *ord* oocytes because normal sister chromatid cohesion is essential to limit inter-sister repair of DSBs. Therefore, in the absence of ORD, sister exchange might be favored and the number of crossovers between homologues reduced.

To test the hypothesis that ORD inhibits sister exchange, we monitored the transmission of a Ring X chromosome during meiosis in *R(1)2*/+; *ord*/*ord* females. A single crossover between two Ring sister chromatids will create a dicentric Ring chromosome that will not be transmitted efficiently (Fig. 6 C). In contrast, recombination between normal "Rod" sister chromatids will not impair their transmission (Fig. 6 C). Therefore, if lack of ORD activity causes significant elevation of sister chromatid exchange in females that contain one Ring X and one normal X chromosome, progeny containing the normal X chromosome should greatly outnumber those that inherit the Ring chromosome.

Table I. **Decreased Ring chromosome transmission during meiosis in *ord* oocytes**

Genotype	Ring progeny	Rod progeny	Ring/Rod
R(1)2/+; +/+	1,859	2,790	0.7
R(1)2/+; ord^6/ord^{10}	697	1,692	0.4
R(1)2/+; ord^5/ord^{10}	400	1,665	0.2

We monitored meiotic transmission of the *R(1)2* chromosome in wild-type, ord^6/ord^{10}, and ord^5/ord^{10} females. Unlike ord^5 and ord^{10}, which behave as nulls, ord^6 is a missense allele that codes for an altered protein with residual activity (Bickel et al., 1996). As shown in Table I, recovery of the *R(1)2* chromosome relative to a normal Rod X chromosome is significantly lowered in *ord* oocytes when compared with wild type. The strongest effect is observed in ord^5/ord^{10} females in which ORD activity is completely absent. These data argue that sister chromatid exchange is elevated in *ord* oocytes and support the model that ORD activity is required to suppress sister chromatid exchange in *Drosophila* oocytes.

Discussion

Although sister chromatid cohesion during meiosis is essential for normal levels of homologous recombination, the mechanism by which cohesion regulates meiotic recombination has remained elusive. Here, we provide evidence that meiotic cohesion is required to suppress sister chromatid exchange and thereby promote inter-homologue recombination during meiosis. Moreover, we further define the link between meiotic cohesion and the formation/stabilization of the SC by focusing our analysis on a cohesion protein that is distinct from the cohesin complex.

Localization of ORD during pachytene

The localization of ORD protein along the entire length of oocyte chromosomes is consistent with previous genetic and cytological evidence that ORD is essential for arm and centromeric cohesion during female meiosis. Bright centromeric ORD foci in the premeiotic cysts of region 1 are similar to those detected in the mitotic cysts within the testis (unpublished data), and diffuse ORD signal throughout the nuclei of some 8-cell cysts and all 16-cell cysts suggests that ORD accumulates along the length of chromosomes before premeiotic S phase. Unlike SC components, ORD protein is not restricted to a subset of nuclei within the cyst. In chromosome spreads, ORD linear elements are visible in all of the cells within the cyst. However, distinct stretches of ribbonlike ORD staining are more pronounced within nuclei that are assembling SC, perhaps because of the high degree of chromatin structure/organization within the context of the SC. In addition, SC compaction and shortening during pachytene (Carpenter, 1975) probably enhance the ribbonlike nature of the ORD signal in the pro-oocytes. Like the SC components C(3)G and C(2)M, ORD remains along the entire length of oocyte bivalents throughout pachytene.

These data are consistent with the model that ORD promotes arm and centromeric cohesion during the stages in which meiotic recombination occurs.

ORD may direct assembly of SC lateral elements

Our work indicates that ORD activity is required for normal SC. Although C(3)G and C(2)M appear to localize normally during early prophase in *ord* mutants, their association with bivalents is severely disrupted during pachytene progression. By EM analysis, we observe SC-like structures in ord^{null} germaria that appear to be composed of CEs without distinct AE/LEs. These results, coupled with our fluorescent immunodetection of C(3)G in *ord* mutants, suggest that some CE components can assemble in the absence of normal AE/LEs. Although somewhat unexpected, similar results have been obtained in yeast and mice (Smith and Roeder, 1997; Peltttari et al., 2001) and have led to the proposal that CEs form in the absence of AE/LEs by using the cohesin complex core as a scaffold for assembly (Pelttari et al., 2001). Interestingly, although cohesin subunits initially associate with chromatid arms in *ord* germaria, their localization starts to deteriorate before the time that we first observe C(3)G localization defects (unpublished results). Therefore, assembly of CEs in *ord* mutants may rely on the cohesin complex and disintegrate when cohesin localization is destabilized.

Our results are consistent with the hypothesis that ORD activity is required for the formation of AE/LEs during meiosis in *Drosophila*. One possibility is that ORD is a structural component of the AE/LEs. However, we think this unlikely because ORD is essential for meiotic sister chromatid cohesion in both males and females, and *Drosophila* spermatocytes do not undergo meiotic recombination or form SC (Morgan, 1912; Rasmussen, 1973). In addition, our observation that ribbonlike ORD signal is visible in all 16 cells of germarial cysts demonstrates that chromatin association of ORD occurs in cells that never form extensive SC. Therefore, we propose that ORD activity is a prerequisite for formation of the AE/LE and may colocalize with this structure, but that the primary function of ORD protein is to promote sister chromatid cohesion.

Sister chromatid exchange in *ord* oocytes

During meiosis, only crossovers between homologous chromosomes can generate a stable chiasma that will promote proper segregation during meiosis I (Fig. 7 A). After the formation of a DSB, strand invasion into the sister chromatid will not be productive in maintaining the association of homologous chromosomes. Schwacha and Kleckner (1994) have provided physical evidence that recombination is biased in *S. cerevisiae* meiosis to favor recombination between homologues. Although crossovers between sisters are not completely inhibited, homologues are the preferred partners and inter-homologue intermediates represent ~70% of the joint molecules that form during strand invasion. When the AE/LE component Red1p is missing/mutated, the percentage of inter-sister events increases, indicating that in yeast, normal AE/LEs suppress sister chromatid exchange (Schwacha and Kleckner, 1997).

Although we cannot analyze recombination intermediates at the molecular level in *Drosophila*, our Ring chromosome genetic assay argues that mutations in *ord* disrupt homologue bias during *Drosophila* meiosis. Similar to other investigators (Sandler et al., 1974; Hall, 1977; McKim et al., 1998; Manheim and McKim, 2003), we obtain a Ring/Rod ratio in wild type that is <1 and probably reflects the level of sister chromatid exchange that normally occurs in *Drosophila* oocytes (Hawley and Walker, 2003). However, the recovery of Ring chromosome–containing progeny from ord^{6}/ord^{10} and ord^{5}/ord^{10} females is significantly lower than from ord^{+} females, and ord^{null} females exhibit lower transmission of the Ring chromosome than those with partial ORD activity.

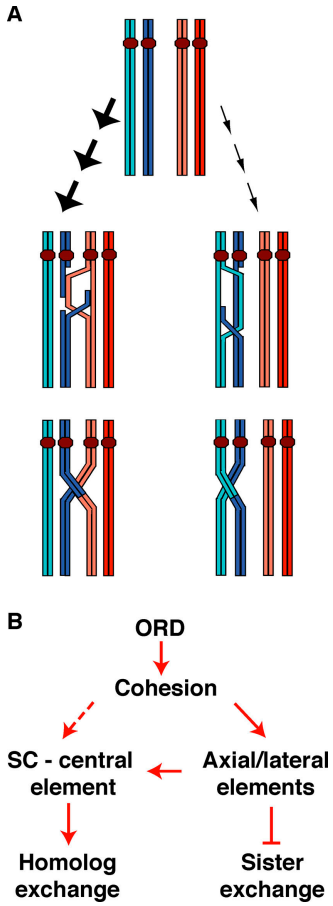

Figure 7. **Homologue bias during meiotic recombination.** (A) After premeiotic S phase, the oocyte enters meiotic prophase with two homologous chromosomes (blue and orange) each composed of a pair of sister chromatids (top). Different hues are used for each sister chromatid and both strands of DNA are shown. After the formation of a DSB, strand invasion can occur between homologues (middle left) or between sisters (middle right). Only a crossover between homologous chromosomes can generate a stable chiasma and the preferred pathway for strand invasion and recombination is between homologues (large arrows). Sister chromatid strand invasion and exchange occur much less frequently (small arrows). (B) Model for how ORD promotes homologue bias. ORD maintains meiotic sister chromatid cohesion, which is required for normal AE/LEs and stabilization of the CE of the SC. AE/LEs are required to suppress recombination between sister chromatids and stabilize CEs. In *Drosophila*, the SC is required for crossovers between homologous chromosomes. High levels of sister chromatid exchange occur in *ord* mutants because DSBs are preferentially repaired by strand invasion into the sister chromatid. ORD promotes homologue bias both by limiting crossovers between sister chromatids and by promoting exchange between homologous chromosomes.

Our results are most consistent with the model that disruption of ORD activity allows increased levels of sister chromatid exchange during meiosis. Null mutations in *ord* cause random segregation of normal Rod chromosomes due to complete loss of centromeric cohesion. Therefore, reduced recovery of the Ring chromosome in ord^5/ord^{10} females cannot arise because the Ring chromosome is more sensitive to cohesion defects than the Rod chromosome. Moreover, nearly equivalent recovery of Ring and Rod chromosomes from *c(3)G* oocytes (Sandler et al., 1974) argues that cis-acting sequences on the *R(1)2* chromosome are capable of mediating proper centromeric cohesion and kinetochore function during meiosis. Although failure to decatenate interlocked Ring chromosomes could result in their reduced recovery, chromosome bridges have not been observed for normal chromosomes in *ord* mutants. Therefore, we propose that ORD activity is required during *Drosophila* meiosis to limit exchange between sisters and thereby promote inter-homologue crossovers. To our knowledge, these data represent the first evidence in metazoans of a gene product that suppresses recombination between sister chromatids during meiosis.

Homologue bias in *Drosophila* meiosis

An increase in sister exchange in *ord* mutants cannot be explained by the disruption of C(3)G localization that occurs during pachytene progression. Although inter-homologue crossovers are abolished in *c(3)G* mutant females (Gowen and Gowen, 1922; Gowen, 1933; Page and Hawley, 2001), sister exchange is not elevated (Sandler et al., 1974; Hall, 1977). In contrast to Jang et al. (2003), we find that DSBs are not significantly reduced in *c(3)G* oocytes (unpublished data). Therefore, absence of the SC CE is not sufficient to lift the constraints that limit inter-sister events. Colaiacovo et al. (2003) have recently made a similar argument for *Caenorhabditis elegans* meiosis.

Homologue bias during meiotic recombination in *Drosophila* most likely arises from mechanisms that promote homologous recombination as well as those that suppress inter-sister events. Recombination defects in *ord* mutants may result from disruption of both pathways (Fig. 7 B). We propose that the absence of ORD activity disrupts the formation and/or stability of AE/LEs which normally limit inter-sister strand invasion after the induction of DSBs. Although meiotic DSBs appear and disappear with normal frequency and timing in ord^{null} germaria, the preferred pathway for DSB repair in *ord* oocytes is strand invasion into the sister chromatid. In addition, ORD is required to maintain C(3)G localization and presumably the integrity of the CE. Elimination of crossovers in *c(3)G* mutants indicates that the SC is absolutely required for repair of DSBs as inter-homologue crossovers in *Drosophila*. Although crossovers are decreased in oocytes completely lacking ORD activity, they are not abolished. Transient localization of C(3)G in *ord* mutants may allow the few crossovers that do occur, but subsequent disruption of C(3)G localization most likely prevents normal numbers of inter-homologue events. We conclude that ORD activity promotes homologue bias during *Drosophila* meiosis by suppressing inter-sister events as well as promoting inter-homologue events and that disruption of both pathways leads to decreased numbers of crossovers in *ord* mutants.

Meiosis-specific controls that direct partner choice during recombination are essential for accurate chromosome segregation of homologous chromosomes during meiosis I. Our results provide critical answers about how sister chromatid cohesion ensures that crossovers occur between homologous chromosomes during meiosis in *Drosophila*. Because cohesion is required for normal levels of meiotic exchange in several species, we predict this mechanism to be highly conserved among eukaryotes.

Materials and methods

Fly strains

Flies were reared at 25°C on standard cornmeal molasses media. GFP-ORD immunolocalization was performed on ovaries from *yw/yw*; ord^{10} *bw/*ord^{10} *bw*; *P{gfp::ord}/P{gfp::ord}* (Balicky et al., 2002) adult females. ord^+ oocytes were obtained from *y/y; cn bw sp* females, and ord^1, ord^5, ord^{10}, and *Df(2R)WI370* stocks have been previously described (Mason, 1976; Miyazaki and Orr-Weaver, 1992; Bickel et al., 1996, 1997). For γ-H2Av analyses, *y/*y^+*Y;* $mei\text{-}W68^1$ homozygotes were selected from a *y/*y^+*Y;* $mei\text{-}W68^1$*/CyO* stock (McKim et al., 1998). For the Ring X chromosome assay, a *Dp(1;Y)* $B^S$$y^+$*/C(1)DX,* y^1 f^1*/R(1)2,* y^1 $w^{hd80k17}$ f^1 stock (BL-3957; Bloomington) was used to generate females containing the *R(1)2* chromosome over a normal Rod X chromosome (see Genetic assay for sister chromatid exchange).

Immunolocalization in whole-mount ovaries

Before dissection, newly eclosed females were fattened overnight in vials with yeast and males. Ovaries were dissected in 1× Modified Robb's Medium (Matthies et al., 2000) for 15 min, rinsed three times in PBS (130 mM NaCl, 7 mM Na_2HPO_4, and 3 mM NaH_2PO_4) containing 0.2% Tween 20, and fixed and processed for immunofluorescence as described by Page and Hawley (2001). After staining, ovaries were separated into individual ovarioles, transferred to an 18-mm poly-L-lysine–treated coverslip, and mounted in 18–20 μl of Prolong Antifade reagent (Molecular Probes).

Chromosome spreads

To develop a procedure to spread *Drosophila* oocyte chromosomes, we adapted protocols used successfully for mammalian meiotic cells (Peters et al., 1997; Koehler et al., 2002). Seven sets of ovaries from newly eclosed females fattened overnight with yeast and males were dissected in PBS and rinsed once in freshly made hypo buffer containing 50 mM sucrose, 17 mM trisodium citrate dihydrate, 5 mM EDTA, 0.5 mM DTT, 30 mM Tris, pH 8.2, and 0.5 mM Pefabloc. Ovaries were immersed in 500 μl hypo buffer, incubated ~20–30 min, and transferred to a drop of hypo buffer. Tungsten needles were used to isolate the transparent tips from the ovaries, and all egg chambers containing yolk were discarded. Ovary tips were transferred to a single 25-μl drop of 100 mM sucrose and minced with tungsten needles leaving no intact ovarioles. The ovary mixture was pipetted up and down several times through a P-2 pipet tip that had been coated in BSA. A Superfrost Plus slide (VWR) was dipped for ~15 s into fixative (0.25 g PFA dissolved in 22.5 ml of water containing one drop of 1 N NaOH, and subsequently adjusted to pH 9.2 using 50 mM of boric acid and supplemented with 350 μl Triton X-100). The slide was held at an angle, and 10 μl of the ovary mixture was applied to the middle of the slide and rolled around to spread out the cells. A second slide was prepared with the remaining 10 μl of ovary mixture and slides were immediately placed in a humidified chamber at RT. After ~14 h, the chamber was opened slightly, allowing slides to dry completely (~6 h). Dry slides were placed in a 0.4% solution of photoflo (Kodak) in water for 2 min. The edges were dabbed dry and the slides were allowed to air dry in a dry coplin jar ~2 h and stored overnight at −20°C. Longer storage resulted in weaker immunofluorescent signal.

Tissue was rehydrated in PBS for 15 min and blocked for 1 h in a humidified chamber at RT in 5% donkey serum, 2% BSA, 0.1% Triton X-100, and 0.01% sodium azide in PBS. All antibodies were diluted in PBS containing 0.1% BSA and 0.1% Triton X-100. For incubations, 100 μl of solution was added to the slide and covered with a parafilm coverslip for 1 h in a humidified chamber at RT. After each incubation, slides were rinsed three times in 0.1% Triton X-100 in PBS followed by three additional 10-min washes. Tissue was stained with DAPI at 1 μg/ml in PBS for 10 min followed by one rinse with PBS and mounted under a 24 × 50–mm coverslip with 40–50 μl of Prolong.

Antibodies

Rabbit anti-GFP antibodies (Molecular Probes) diluted 1:2,000 and Alexa 488–conjugated anti–rabbit antibodies (Molecular Probes) were used to detect GFP-ORD. Guinea pig anti-C(3)G serum (provided by S. Page and R.S. Hawley, Stowers Institute for Medical Research, Kansas City, MO; Page and Hawley, 2001) was used at a final dilution of 1:1,000 and visualized with either CY3- or CY5-conjugated anti–guinea pig antibodies (Jackson ImmunoResearch Laboratories). For ORB staining, 4H8 and 6H4 mouse mAbs (Developmental Studies Hybridoma Bank [DSHB]; Lantz et al., 1994) were used together, each at a final concentration of 1:30 and detected with Alexa 488–conjugated anti–mouse antibodies (Molecular Probes). Rabbit anti-C(2)M antibodies (provided by K. McKim, Waksman Institute, Rutgers University, Piscataway, NJ; Manheim and McKim, 2003) were diluted 1:1,000 and detected with CY3 anti–rabbit (Jackson ImmunoResearch Laboratories). For CID staining, affinity-purified chicken anti-CID antibodies (provided by G. Karpen, Lawrence Berkeley National Lab, Berkeley, CA; Blower and Karpen, 2001) were used at 1:200 followed by CY3 anti–chicken secondary (Jackson ImmunoResearch Laboratories). 1B1 mouse mAbs (DSHB; Zaccai and Lipshitz, 1996) were used at 1:20 to detect the fusome with CY5-conjugated anti–mouse antibodies (Jackson ImmunoResearch Laboratories). Affinity-purified rabbit anti-H2Av-ser137-PO4 antibodies (provided by R. Glaser, Wadsworth Center, Albany, NY; Madigan et al., 2002) were diluted 1:2,000 and visualized with Alexa 488–conjugated anti–rabbit antibodies (Molecular Probes). All secondary antibodies were diluted 1:400.

FISH analysis

For FISH experiments, chromosome spreads were processed for immunolocalization as described in Chromosome spreads section with a final C(3)G antibody dilution of 1:500 and secondary antibody at 1:200. After antibody incubations, slides were postfixed in 1% formaldehyde in PBS for 5 min. FISH was performed on chromosome spreads using X chromosome arm and pericentromeric (359 bp satellite) probes as described by Balicky et al. (2002).

Microscopy and image analysis

Epifluorescence microscopy was performed on a microscope (model Axioplan2; Carl Zeiss MicroImaging, Inc.) equipped with a camera (model ORCA-ER; Hamamatsu) and a 100× Plan-APOCHROMAT objective (NA 1.4). Openlab 3.1.5 (Improvision) was used to capture, overlay, and crop z-series image stacks (0.1 μm step size). Volocity 2.0.1 (Improvision) was used to deconvolve image stacks. Total length of C(3)G staining was calculated using the Volocity measurements function. Tetraspeck fluorescent beads (Molecular Probes) and the Openlab registration module were used to resolve registration differences.

EM

Fixation and embedding were performed as described by McKim et al. (1998) with modifications. Ovaries were dissected in PBS from *y; cn bw sp (ord$^+$)* and *ord^5/Df* females that had been fattened overnight with yeast and males. Fixation in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.4, was performed for 3 h at RT. Ovaries were rinsed in 0.1 M sodium Cacodylate buffer, pH 7.4, and placed in 2% OsO_4 in 0.1 M sodium Cacodylate, pH 7.4, for 1 h at RT. En-bloc staining in 2% aqueous uranyl acetate was performed for 1 h in the dark at RT and dehydrated through a graded series of ethanol to 70%. Single ovarioles were separated with tungsten needles and dehydrated to 100% ethanol followed by propylene oxide and conventionally flat-embedded in epon (LX112).

Two complete germaria were consecutively sectioned from two different *ord^5/Df* females, and one germarium from a *y; cn bw sp* female was sectioned as a control. Each germarium was oriented with the long axis parallel to the plane of sectioning, and 350–400 sections were required to cut through each entire germarium. 80–100-nm sections were collected on uncoated 400HH grids and stained for 20 min with 2% uranyl acetate in methanol, followed by 5 min in Reynold's lead citrate. Every germarial cyst from every section was examined on a transmission electron microscope (model 2000FX or 100CX; JEOL). Nuclei with SC were photographed at a magnification of 8,300. High magnification (50,000) photographs were taken of every structure that resembled SC in the two *ord^5/Df* germaria as well as numerous stretches of SC in the control germarium. Negatives were digitized with a UMAX optical scanner.

Genetic assay for sister chromatid exchange

Sister chromatid exchange in wild-type and *ord* oocytes was indirectly assayed by monitoring the recovery of a Ring X chromosome relative to a normal (Rod) X chromosome. Because exchange between Ring sister chromatids will generate a dicentric Ring chromosome that is not transmitted to progeny, reduced recovery of the Ring chromosome from mutant females is diagnostic of increased levels of sister chromatid exchange in their oocytes.

R(1)2, y^1 w^{hd80k17} f^1/y$^+$Y males were crossed with *C(1)DX, y^1 f^1/y$^+$Y; ord^{10} cn bw sp If/+* virgins to generate males that were *R(1)2, y^1 w^{hd80k17} f^1/y$^+$Y; ord^{10} cn bw sp If/+*. Then, these males were crossed with either *y/y; ord^5 bw/SM1; pol* or *y/y; ord^6 px bw/SM1; pol* virgins. Progeny virgins that were *R(1)2, y^1 w^{hd80k17} f^1/y; ord^{10} cn bw sp If/ord^x* were selected and crossed with *yw/y$^+$Y* males. As an *ord$^+$* control, *R(1)2, y^1 w^{hd80k17} f^1/y; cn bw sp* virgins were crossed to *yw/y$^+$Y* males. To look at sister exchange in its normal context we chose to perform these experiments in the absence of an X chromosome balancer so that inter-homologue and inter-sister pathways were both available in wild type and mutants. Eye color was used to differentiate female progeny containing the maternal *R(1)2* Ring chromosome (white eyes) from those that inherited the Rod chromosome (red eyes). Male progeny containing the maternal Ring chromosome (white$^-$ forked$^-$) could be differentiated from those carrying the Rod chromosome (white$^+$ forked$^+$). XO male progeny that did not inherit any maternal X chromosome could also be distinguished (white$^-$ forked$^+$).

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References

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.

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.

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.

Blower, M.D., and G.H. Karpen. 2001. The role of *Drosophila* CID in kinetochore formation, cell-cycle progression and heterochromatin interactions. *Nat. Cell Biol.* 3:730–739.

Blower, M.D., B.A. Sullivan, and G.H. Karpen. 2002. Conserved organization of centromeric chromatin in flies and humans. *Dev. Cell.* 2:319–330.

Broverman, S.A., and P.M. Meneely. 1994. Meiotic mutants that cause a polar decrease in recombination on the *X* chromosome in *Caenorhabditis elegans*. *Genetics.* 136:119–127.

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

Carpenter, A.T.C. 1975. Electron microscopy of meiosis in *Drosophila melanogaster* females. *Chromosoma.* 51:157–182.

Carpenter, A.T.C., and L. Sandler. 1974. On recombination-defective meiotic mutants in *Drosophila melanogaster*. *Genetics.* 76:453–475.

Chan, R.C., A. Chan, M. Jeon, T.F. Wu, D. Pasqualone, A.E. Rougvie, and B.J. Meyer. 2003. Chromosome cohesion is regulated by a clock gene paralogue TIM-1. *Nature.* 424:1002–1009.

Colaiacovo, M.P., A.J. MacQueen, E. Martinez-Perez, K. McDonald, A. Adamo, A. La Volpe, and A.M. Villeneuve. 2003. Synaptonemal complex assembly

in *C. elegans* is dispensable for loading strand-exchange proteins but critical for proper completion of recombination. *Dev. Cell.* 5:463–474.

de Cuevas, M., M.A. Lilly, and A.C. Spradling. 1997. Germline cyst formation in *Drosophila*. *Annu. Rev. Genet.* 31:405–428.

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.

Gowen, J.W. 1933. Meiosis as a genetic character in *Drosophila melanogaster*. *J. Exp. Zool.* 65:83–106.

Gowen, M.S., and J.W. Gowen. 1922. Complete linkage in *Drosophila melanogaster*. *Am. Naturalist.* 56:286–288.

Hall, J.C. 1977. Recombination influenced by two alleles of the meiotic mutant c(3)g. *Drosoph. Inf. Serv.* 52:143–144.

Hawley, R.S., and M.Y. Walker. 2003. Advanced Genetic Analysis: Finding Meaning in a Genome. Blackwell Science, Ltd., Malden, MA. 239 pp.

Henikoff, S., K. Ahmad, J.S. Platero, and B. van Steensel. 2000. Heterochromatic deposition of centromeric histone H3-like proteins. *Proc. Natl. Acad. Sci. USA.* 97:716–721.

Jang, J.K., D.E. Sherizen, R. Bhagat, E.A. Manheim, and K.S. McKim. 2003. Relationship of DNA double-strand breaks to synapsis in *Drosophila*. *J. Cell Sci.* 116:3069–3077.

Klein, F., P. Mahr, M. Galova, S.B. Buonomo, C. Michaelis, K. Nairz, and K. Nasmyth. 1999. A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis. *Cell.* 98: 91–103.

Koehler, K.E., J.P. Cherry, A. Lynn, P.A. Hunt, and T.J. Hassold. 2002. Genetic control of mammalian meiotic recombination. I. Variation in exchange frequencies among males from inbred mouse strains. *Genetics.* 162:297–306.

Lantz, V., J.S. Chang, J.I. Horabin, D. Bopp, and P. Schedl. 1994. The *Drosophila* orb RNA-binding protein is required for the formation of the egg chamber and establishment of polarity. *Genes Dev.* 8:598–613.

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

Lindsley, D., and G. Zimm. 1992. The Genome of *Drosophila melanogaster*. Academic Press, Inc., New York. 1133 pp.

Madigan, J.P., H.L. Chotkowski, and R.L. Glaser. 2002. DNA double-strand break-induced phosphorylation of *Drosophila* histone variant H2Av helps prevent radiation-induced apoptosis. *Nucleic Acids Res.* 30:3698–3705.

Manheim, E.A., and K.S. McKim. 2003. The synaptonemal complex component C(2)M regulates meiotic crossing over in *Drosophila*. *Curr. Biol.* 13:276–285.

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

Matthies, H.J.G., M. Clarkson, R.B. Saint, R. Namba, and R.S. Hawley. 2000. Analysis of meiosis in fixed and live oocytes by light microscopy. *In Drosophila* Protocols. W. Sullivan, M. Ashburner, and R.S. Hawley, editors. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York. 67–85.

McKim, K.S., and A. Hayashi-Hagihara. 1998. mei-W68 in *Drosophila melanogaster* encodes a Spo11 homolog: evidence that the mechanism for initiating meiotic recombination is conserved. *Genes Dev.* 12:2932–2942.

McKim, K.S., B.L. Green-Marroquin, J.J. Sekelsky, G. Chin, C. Steinberg, R. Khodosh, and R.S. Hawley. 1998. Meiotic synapsis in the absence of recombination. *Science.* 279:876–878.

Mercier, R., S.J. Armstrong, C. Horlow, N.P. Jackson, C.A. Makaroff, D. Vezon, G. Pelletier, G.H. Jones, and F.C. Franklin. 2003. The meiotic protein SWI1 is required for axial element formation and recombination initiation in Arabidopsis. *Development.* 130:3309–3318.

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

Morgan, T.H. 1912. Complete linkage in the second chromosome in the male of *Drosophila melanogaster*. *Science.* 36:719–720.

Nasmyth, K. 2001. Disseminating the genome: joining, resolving, and separating sister chromatids during mitosis and meiosis. *Annu. Rev. Genet.* 35:673–745.

Page, S.L., and R.S. Hawley. 2001. c(3)G encodes a *Drosophila* synaptonemal complex protein. *Genes Dev.* 15:3130–3143.

Page, S.L., and R.S. Hawley. 2003. Chromosome choreography: the meiotic ballet. *Science.* 301:785–789.

Parisi, S., M.J. McKay, M. Molnar, M.A. Thompson, P.J. van der Spek, E. van Drunen-Schoenmaker, R. Kanaar, E. Lehmann, J.H. Hoeijmakers, and J. Kohli. 1999. Rec8p, a meiotic recombination and sister chromatid cohesion phosphoprotein of the Rad21p family conserved from fission yeast to humans. *Mol. Cell. Biol.* 19:3515–3528.

Pelttari, J., M.R. Hoja, L. Yuan, J.G. Liu, E. Brundell, P. Moens, S. Santucci-Darmanin, R. Jessberger, J.L. Barbero, C. Heyting, and C. Hoog. 2001. A meiotic chromosomal core consisting of cohesin complex proteins recruits DNA recombination proteins and promotes synapsis in the absence of an axial element in mammalian meiotic cells. *Mol. Cell. Biol.* 21:5667–5677.

Peters, A.H., A.W. Plug, M.J. van Vugt, and P. de Boer. 1997. A drying-down technique for the spreading of mammalian meiocytes from the male and female germline. *Chromosome Res.* 5:66–68.

Petes, T.D., and P.J. Pukkila. 1995. Meiotic sister chromatid recombination. *Adv. Genet.* 33:41–62.

Rasmussen, S.W. 1973. Ultrastructural studies of spermatogenesis in *Drosophila melanogaster* Meigen. *Z. Zellforsch. Mikrosk. Anat.* 140:125–44.

Sandler, L., P. Romans, and J. Figenshow. 1974. An effect of centromere function on the behavior of ring-X chromosomes in *Drosophila melanogaster*. *Genetics.* 77:299–307.

Schleiffer, A., S. Kaitna, S. Maurer-Stroh, M. Glotzer, K. Nasmyth, and F. Eisenhaber. 2003. Kleisins: a superfamily of bacterial and eukaryotic SMC protein partners. *Mol. Cell.* 11:571–575.

Schwacha, A., and N. Kleckner. 1994. Identification of joint molecules that form frequently between homologs but rarely between sister chromatids during yeast meiosis. *Cell.* 76:51–63.

Schwacha, A., and N. Kleckner. 1997. Interhomolog bias during meiotic recombination: meiotic functions promote a highly differentiated interhomolog-only pathway. *Cell.* 90:1123–1135.

Smith, A.V., and G.S. Roeder. 1997. The yeast Red1 protein localizes to the cores of meiotic chromosomes. *J. Cell Biol.* 136:957–967.

Spradling, A.C., M. de Cuevas, D. Drummond-Barbosa, L. Keyes, M. Lilly, M. Pepling, and T. Xie. 1997. The *Drosophila* germarium: stem cells, germ line cysts, and oocytes. *Cold Spring Harb. Symp. Quant. Biol.* 62:25–34.

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.

Zaccai, M., and H.D. Lipshitz. 1996. Differential distributions of two adducin-like protein isoforms in the *Drosophila* ovary and early embryo. *Zygote.* 4:159–166.

Zickler, D., and N. Kleckner. 1999. Meiotic chromosomes: integrating structure and function. *Annu. Rev. Genet.* 33:603–754.